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Gamma-ray-induced mutagen sensitivity and risk of sporadic breast cancer in young women: a case–control study

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Abstract

Hypersensitivity to radiation exposure has been suggested to be a risk factor for the development of breast cancer. In this case–control study of 515 young women (< 55 years) with newly diagnosed sporadic breast cancer and 402 cancer-free controls, we examined the radiosensitivity as measured by the frequency of chromatid breaks induced by gamma-radiation exposure in the G2 phase of phytohemagglutinin-stimulated and short-term cultured fresh lymphocytes. We found that the average chromatid breaks per cell from 50 well-spread metaphases were statistically significantly higher in 403 non-Hispanic White breast cancer patients (0.52 ± 0.22) than that in 281 non-Hispanic White controls (0.44 ± 0.16) (P value < 0.001), and in 60 Mexican American breast cancer patients (0.52 ± 0.19) than that in 65 Mexican American controls (0.44 ± 0.16) (P value = 0.021), but the difference was not significant in African Americans (52 cases [0.45 ± 0.16] versus 56 controls [0.47 ± 0.16], $P = 0.651$). The frequency of chromatid breaks per cell above the median of control subjects was associated with two-fold increased risk for breast cancer in non-Hispanic Whites and Mexican Americans. A dose–response relationship was evident between radiosensitivity and risk for breast cancer ($P_{\text{trend}} < 0.001$) in these two ethnic groups. We concluded that gamma-ray-induced mutagen sensitivity may play a role in susceptibility to breast cancer in young non-Hispanic White and Mexican American women.

Keywords

Radiation; Chromosomal instability; Breast neoplasm; Molecular epidemiology

Introduction

Breast cancer is the most common incident cancer and the second leading cause of death among females with an estimated 230 480 new invasive cases, 57 650 new cases of in situ breast cancer, and 39 520 deaths in the United States in 2011 [1].

Breast cancer is a complex, multifactorial disease and its etiology has been associated with genetic, environmental, and reproductive risk factors [2–5]. Although the role of genetic factors in the etiology of breast cancer is well established in familial breast cancer with germline mutations in *BRCA1* and *BRCA2* [6–10], the contribution of environmental factors and genetic factors related to sporadic breast cancer remains less understood. Ionizing radiation, such as cosmic, X-ray, and gamma-ray, is a known risk factor for breast cancer, particularly in flight attendants [11–13] and has attracted considerable research interest over the last decades [14–24].

Ionizing radiation causes a variety of DNA damage, including generation of reactive oxygen species that cause DNA base damage and DNA strand breaks [25, 26]. Impaired DNA strand breaks will lead to chromosomal instability [27]. Host response to ionizing radiation and capacity to repair DNA damage induced by ionizing radiation are important factors for cancer development. For example, an early study reported that the frequency of chromosome aberrations in lymphocytes was higher in breast cancer patients with excessive normal tissue damage after radiotherapy of a low dose-rate irradiation as than in patients treated but without such normal tissue damage and normal healthy donors [28]. The findings suggested that subsets of breast cancer patients have abnormal sensitivity to ionizing radiation. Further studies found that increased G2-phase radiosensitivity induced by X-ray or gamma-ray in lymphocytes from breast cancer patients was common and associated with increased risk of breast cancer [29–36]. However, those studies included relatively small samples.

In this study, we evaluated gamma-ray-induced mutagen sensitivity by measuring the frequency of chromatid breaks in G2-phase lymphocytes in 515 young women (< 55 years)

with newly diagnosed sporadic breast cancer and 402 cancer-free healthy control subjects who were genetically unrelated to the cases to determine the association between the G2 chromosomal radiosensitivity and risk of breast cancer.

Materials and methods

Study population

This study consisted of 515 female patients who were 55 years and younger when they were diagnosed with primary breast cancer. Patients were recruited at The University of Texas M.D. Anderson Cancer Center between January of 1998 and January of 2006. Patients with previous cancer history, multiple primary neoplasms at the time of diagnosis, or metastatic cancer from a site other than breast were excluded. All cases were histopathologically confirmed, and the blood was drawn before any treatment.

During the same time period, 402 cancer-free controls were recruited from M.D. Anderson Cancer Center visitors who were genetically unrelated to the cases of this study. All controls were female and frequency-matched to the cases by age (± 5 years) and ethnicity. Each participant donated 30 ml of blood after signing a written informed consent and completed a short questionnaire that provided information on their age, ethnicity, smoking status, and alcohol consumption. Participants who had smoked at least 100 cigarettes in their lifetime were assigned as ever-smokers and the remainder as never-smokers. Participants who had consumed alcoholic beverages at least once a week for 1 year in their lifetime were assigned as ever-drinkers and the remaining as never-drinkers. Additional information on estrogen receptor (ER)/progesterone receptor (PR) expression status and reproductive history (menarche, age at first full-term pregnancy, parity, and use of contraceptive or hormonal replacement therapy) was available for the cases only. The M.D. Anderson Cancer Center institutional review board approved this study protocol.

The gamma-ray-induced mutagen sensitivity assay

The gamma-ray-induced mutagen sensitivity assay was performed as previously described [37]. Briefly, two primary lymphocyte cultures were set up for each individual by inoculating 1 ml of blood into T-25 plastic culture flasks with 9 ml of RPMI 1640 (Invitrogen Corporation, Grand Island, NY) containing 112.5 $\mu\text{g/ml}$ phytohemagglutinin (Thermo Fisher Scientific, Remel Products, Lenexa, KS). After 67 h of incubation, one of the cultures was irradiated with 1.5 Gy incident radiations, at a dose rate of 13.18 Gy/min for 6.9 s from a ^{137}Cs source (Cesium Irradiator Mark 1, model 30; J.L. Shepherd and Associates, Glendale, CA). Afterwards, the irradiated culture was incubated for another 5 h and treated with 0.06 $\mu\text{g/ml}$ Colcemid for 1 h (Invitrogen Corporation) before harvesting to induce mitotic arrest. The cells were harvested and prepared on slides as described previously [37]. The number of simple chromatid breaks per sample was counted from 50 well-spread metaphases. Each simple chromatid break was counted as a single break, whereas each isochromatid break, exchange figure, or interstitial deletion was counted as two breaks. The mean value of chromatid breaks per cell (b/c) was then calculated.

Statistical analysis

The distribution of demographic information between cases and controls was compared by using the χ^2 test. The chromatid b/c was analyzed as a continuous variable. The student's t test was used to compare differences in the mean b/c values between cases and controls, and the analysis of variance (ANOVA) tests were used to compare differences among cases. The data were stratified by age, ethnicity, smoking status, and alcohol use. Odd ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the association between gamma-ray-induced b/c and risk of breast cancer.

We used the median (i.e., 0.43) and tertiles (i.e., 0.36, 0.37–0.49, 0.50) of b/c in the control group as the cutoff values to calculate the ORs and 95% CIs. Subjects with b/c values greater than controls' median b/c were considered to be radiosensitive. Multivariate logistic regression analyses were performed to calculate the ORs and 95% CIs with adjustment for age, smoking status, and alcohol use in the model. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant, by using the SAS/Genetics software (version 9.2; SAS Institute Inc., Cary, NC, USA).

We also conducted a meta-analysis using Review Manager (RevMan) version 5.1 (Cochrane Collaboration, Copenhagen, Denmark). By searching the term 'breast cancer and G2 radiosensitivity' in the PubMed database, nine case-control studies on G2-phase radiosensitivity with sporadic breast cancer patients and cancer-free controls were included in addition to the present study, which provided the numbers of individuals who were originally defined as sensitive or nonsensitive to ionizing radiation (e.g., X-ray, ^{60}Co , or ^{137}Cs) by assessing the chromatid breaks at G2-phase [30, 32–34, 36, 38–41]. A random-effects model was used to pool the results based on the heterogeneity between studies ($P < 0.05$) [42, 43].

Results

Characteristics of the study population

The characteristics of cases and controls are summarized in Table 1. This study included 515 female sporadic breast cancer cases and 402 female cancer-free controls aged 55 years and younger. The age distribution between cases and controls were frequency-matched ($P = 0.139$). However, there were more non-Hispanic White cases (403, 78.2%) than controls (281, 69.9%). The others included 60 (11.7%) versus 65 (16.2%) Mexican Americans and 52 (10.1%) versus 56 (13.9%) African Americans between cases and controls, respectively. There were no significant differences in the distribution of smoking status ($P = 0.824$), whereas more cases ever drank alcohol than controls (49.9% versus 39.5%, $P = 0.002$). Among the cases, 83 (16.1%) had breast carcinoma in situ and 432 (83.9%) had invasive breast carcinomas, of whom 37.7% were stage I, 35.3% stage II, and 10.9% stage III–IV. About 30% were ER negative and 42.0% were PR negative (Table 1). The confounding effect from alcohol use or any possible residual effect from age and smoking status were further adjusted for in multivariate logistic regression models to evaluate the association between gamma-ray-induced chromatid breaks and risk of sporadic breast cancer.

Comparison of gamma-ray-induced chromatid breaks between cases and controls

Figure 1 shows the distribution of b/c in 515 cases and 402 controls, which were ranged between 0.14 and 2.46 for cases and 0.08–1.16 for controls. The mean b/c values in cases were significantly higher than that in controls (0.52 ± 0.20 versus 0.44 ± 0.16 , $P < 0.0001$). Having stratified the data by ethnic groups, we found that the differences in the b/c values were statistically significant for non-Hispanic Whites (0.52 ± 0.21 versus 0.44 ± 0.16 , $P < 0.0001$) and for Mexican Americans (0.52 ± 0.19 versus 0.44 ± 0.16 , $P = 0.021$), but not for African Americans (0.45 ± 0.16 versus 0.47 ± 0.16 , $P = 0.651$) (Table 2). Since b/c values did not follow a normal distribution, we also compared the differences between cases and controls by ethnic groups after natural log transformation. The results remained similar. Therefore, we used untransformed b/c values by ethnic groups in the remaining analyses. When participants were stratified by the variables listed in Table 1, the mean values of gamma-ray-induced b/c remained statistically significantly higher in non-Hispanic White case patients than those in the controls for all strata and in Mexican Americans for older age (46–55 years), never smoker and never drinker subgroups. However, this trend was only seen in ever-smoking African American subgroup (0.54 ± 0.20 versus 0.40 ± 0.11 , $P =$

0.030). Intriguingly, the sensitivity to gamma-radiation was significantly higher with more frequent b/c in never-smoking African American controls than the corresponding breast cancer patients (0.42 ± 0.12 versus 0.49 ± 0.16 , $P = 0.027$), which was likely a result of the small sample size in the strata. In further ANOVA tests to compare differences in gamma-ray-induced sensitivity between carcinoma in situ and invasive patients, in patients with ER or PR negative and positive, and among the AJCC stages, we did not observe any statistically significant differences in b/c in any of the ethnic groups (Table 2).

Association of gamma-ray-induced sensitivity and breast cancer risk

The association between gamma-ray-induced sensitivity measured as chromatid breaks and risk of breast cancer were estimated by calculating ORs and 95% CIs with adjustment for age, smoking and alcohol status using logistic regression analyses. The gamma-ray-induced b/c values were fitted in the logistic regression models either as continuous or categorical variables. When the chromatid b/c values were fitted as a continuous variable, we found that there was a 35% significantly increased risk of breast cancer associated with each 0.1 increment of chromatid b/c value for non-Hispanic Whites and 27% for Mexican Americans (Table 3) but not for African Americans (OR, 0.93; 95% CI, 0.73–1.20). Using the median b/c (0.43) of the controls as the cutoff point, cases with higher b/c values had more than two-fold increased risk of breast cancer in non-Hispanic Whites (OR, 2.23; 95% CI, 1.62–3.07) and Mexican Americans (OR, 2.20; 95% CI, 1.04–4.67) (Table 3). When we divided the study subjects by b/c tertiles of the controls, we found the association of radiosensitivity and risk of breast cancer in a dose-dependent manner in non-Hispanic Whites (OR, 1.70; 95% CI, 1.13–2.58 for middle tertile; OR, 3.03; 95% CI, 2.05–4.47 for upper tertile; $P_{\text{trend}} < 0.0001$) and in Mexican Americans (OR, 1.95; 95% CI, 0.71–5.36 for middle tertile; OR, 3.15; 95% CI, 1.17–8.46; $P_{\text{trend}} = 0.012$) (Table 3). However, this trend was not observed in African Americans.

We subsequently conducted a meta-analysis of ten case-control studies on G2-phase radiosensitivity with a total of 1 154 sporadic breast cancer patients and 1 057 cancer-free controls without restriction on ethnicity because of only one study on Indian and one on African American, and the others mainly on European ancestries [30, 32–34, 36, 38–41]. The studies on high-risk populations with *BRCA1/2* mutation, known family history, or from the same research group with overlapping study participants have been excluded. The OR for overall risk for breast cancer associated with sensitivity to ionizing radiation-induced G2-phase chromatid breaks was 3.99 (95% CI, 2.34–6.81; $P_{\text{heterogeneity}} < 0.0001$) by random effects model (Fig. 2).

Discussion

The mutagen sensitivity assay was first developed by Dr. T. C. Hsu using bleomycin in cultured lymphocytes of peripheral blood to measure its genotoxicity that was expressed as the average number of chromatid breaks per cell [44, 45]. It has been modified with a variety of etiologically related mutagens to evaluate cancer susceptibility in association studies [29–34, 46–50]. These studies constantly showed mutagen sensitivity as a risk factor for cancer development.

In the early 80s, Dr. Sanford's research group has also started to evaluate the chromatid breaks induced by X-ray irradiation in mice and human cell lines. The increased chromosomal radiosensitivity during the G2-phase of the cell cycle was reported to be associated with deficient DNA repair in diverse tumor cells and predisposed to genetic or familial disorders in skin fibroblasts and blood lymphocytes [51–54]. It is well known that radiation induces single- and double-strand DNA breaks [26]; however, how DNA breaks

convert to chromatid breaks remain unknown, though there is a clear linear relationship between radiation dose and intensity of chromatid breaks [55, 56].

Previous studies have consistently shown that increased G2-phase sensitivity to radiation is associated with risk of breast cancer from several research groups in relatively small sample sizes [29–36]. In this case–control study, we investigated the radiosensitivity induced by gamma-ray in a much larger sample size than prior studies in the setting of sporadic breast cancer patients and cancer-free controls who were 55 years and younger. We confirmed that the high radiosensitivity is associated with risk of breast cancer in non-Hispanic Whites and Mexican Americans, but not in African Americans because of a limited sample size. In the study reported by Natarajan et al. [36], the significant difference in gamma-radiation-induced mutagen sensitivity was found in 61 African American breast cancer patients and 86 healthy controls from Washington DC area without a correlation between chromatid breaks and smoking ($r = -0.0646$, $P = 0.437$); however, the age distribution of the study subjects was between 21 and 80 years, and the age was associated with breast cancer risk ($r = 0.276$, $P < 0.001$) [36].

In non-Hispanic Whites, the increased chromatid breaks per cell in cases were observed regardless of the status of selected variables, suggesting that genetic predisposition, but not environmental factors, may have contributed to radiosensitivity in cultured cells in this study population. However, in Mexican Americans, the significantly increased sensitivity to gamma-radiation was observed only in women older than 45 years, never-smokers, and never-drinkers, even though the similar trend was evident in other strata.

There are some limitations in our study. One is the relatively small numbers in minority participants. However, this is the first study to our knowledge to focus on young age of homogeneous ethnic groups with gamma-radiation-induced G2-phase radiosensitivity. Because we recruited the controls in the hospital setting, they might not represent the general population. Another limitation is that we did not have reproductive or family history data for 40% of study participants, so we could not exclude the confounding of these parameters. In the 70% patients with family history data, we found that those with family history of any cancer in first-degree relatives had non-significantly higher radio-sensitivity than those without family history. In summary, our results showed that gamma-ray-reduced mutagen sensitivity may be a risk factor for genetic susceptibility to breast cancer in young non-Hispanic White and Mexican American women, and there is a dose–response correlation between radiosensitivity expressed as chromatid breaks and breast cancer development. The retrospective meta-analysis from ten research groups further supported our notion that G2-phase radiosensitivity expressed as a high frequency of ionizing radiation-induced chromatid breaks is associated with an increased risk for development of sporadic breast cancer. More rigorous studies on various ethnic groups are needed to validate our findings and elucidate the genetic underlying mechanisms.

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References

1. DeSantis C, Siegel R, Bandi P, Jemal A. Breast cancer statistics, 2011. *CA Cancer J Clin.* 2011; 61(6):409–418. [PubMed: 21969133]
2. Martin AM, Weber BL. Genetic and hormonal risk factors in breast cancer. *J Natl Cancer Inst.* 2000; 92(14):1126–1135. [PubMed: 10904085]

3. Bray F, McCarron P, Parkin DM. The changing global patterns of female breast cancer incidence and mortality. *Breast Cancer Res.* 2004; 6(6):229–239. [PubMed: 15535852]
4. Kelsey JL, Gammon MD. The epidemiology of breast cancer. *CA Cancer J Clin.* 1991; 41(3):146–165. [PubMed: 1902137]
5. McPherson K, Steel CM, Dixon JM. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. *BMJ.* 2000; 321(7261):624–628. [PubMed: 10977847]
6. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science.* 1994; 266(5182):66–71. [PubMed: 7545954]
7. Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, et al. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12–13. *Science.* 1994; 265(5181):2088–2090. [PubMed: 8091231]
8. Scully R. Role of BRCA gene dysfunction in breast and ovarian cancer predisposition. *Breast Cancer Res.* 2000; 2(5):324–330. [PubMed: 11250724]
9. Newman B, Mu H, Butler LM, Millikan RC, Moorman PG, King MC. Frequency of breast cancer attributable to BRCA1 in a population-based series of American women. *JAMA.* 1998; 279(12):915–921. [PubMed: 9544765]
10. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. *Am J Hum Genet.* 1998; 62(3):676–689. [PubMed: 9497246]
11. Rafnsson V, Tulinius H, Jonasson JG, Hrafnkelsson J. Risk of breast cancer in female flight attendants: a population-based study (Iceland). *Cancer Causes Control.* 2001; 12(2):95–101. [PubMed: 11246849]
12. Tokumaru O, Haruki K, Bacal K, Katagiri T, Yamamoto T, Sakurai Y. Incidence of cancer among female flight attendants: a meta-analysis. *J Travel Med.* 2006; 13(3):127–132. [PubMed: 16706942]
13. Reynolds P, Cone J, Layefsky M, Goldberg DE, Hurley S. Cancer incidence in California flight attendants (United States). *Cancer Causes Control.* 2002; 13(4):317–324. [PubMed: 12074501]
14. Wolff MS, Collman GW, Barrett JC, Huff J. Breast cancer and environmental risk factors: epidemiological and experimental findings. *Annu Rev Pharmacol Toxicol.* 1996; 36:573–596. [PubMed: 8725402]
15. Chakraborty R, Little MP, Sankaranarayanan K. Cancer predisposition, radiosensitivity and the risk of radiation-induced cancers. III. Effects of incomplete penetrance and dose-dependent radiosensitivity on cancer risks in populations. *Radiat Res.* 1997; 147(3):309–320. [PubMed: 9052677]
16. Feig SA, Hendrick RE. Radiation risk from screening mammography of women aged 40–49 years. *J Natl Cancer Inst Monogr.* 1997; 1997(22):119–124. [PubMed: 9709287]
17. Welp EA, Weiderpass E, Boffetta P, Vainio H, Vasama-Neuvonen K, Petralia S, Partanen TJ. Environmental risk factors of breast cancer. *Scand J Work Environ Health.* 1998; 24(1):3–7. [PubMed: 9562394]
18. Baria K, Warren C, Eden OB, Roberts SA, West CM, Scott D. Chromosomal radiosensitivity in young cancer patients: possible evidence of genetic predisposition. *Int J Radiat Biol.* 2002; 78(5):341–346. [PubMed: 12020425]
19. Smith TR, Miller MS, Lohman KK, Case LD, Hu JJ. DNA damage and breast cancer risk. *Carcinogenesis.* 2003; 24(5):883–889. [PubMed: 12771032]
20. Strumylaite L, Mechonosina K, Tamasauskas S. Environmental factors and breast cancer. *Medicina (Kaunas).* 2010; 46(12):867–873. [PubMed: 21532292]
21. Ng AK, Travis LB. Radiation therapy and breast cancer risk. *J Natl Compr Cancer Netw.* 2009; 7(10):1121–1128.
22. Golubicic I, Borojevic N, Pavlovic T. Risk factors for breast cancer: is ionizing radiation among them? *J BUON.* 2008; 13(4):487–494. [PubMed: 19145669]
23. Hildreth NG, Shore RE, Dvoretzky PM. The risk of breast cancer after irradiation of the thymus in infancy. *N Engl J Med.* 1989; 321(19):1281–1284. [PubMed: 2797100]

24. Mohan AK, Hauptmann M, Linet MS, Ron E, Lubin JH, Freed-man DM, Alexander BH, Boice JD Jr, Doody MM, Matanoski GM. Breast cancer mortality among female radiologic technologists in the United States. *J Natl Cancer Inst.* 2002; 94(12):943–948. [PubMed: 12072548]
25. Lindahl T, Wood RD. Quality control by DNA repair. *Science.* 1999; 286(5446):1897–1905. [PubMed: 10583946]
26. Little JB. Radiation carcinogenesis. *Carcinogenesis.* 2000; 21(3):397–404. [PubMed: 10688860]
27. van Gent DC, Hoeijmakers JH, Kanaar R. Chromosomal stability and the DNA double-stranded break connection. *Nat Rev Genet.* 2001; 2(3):196–206. [PubMed: 11256071]
28. Jones LA, Scott D, Cowan R, Roberts SA. Abnormal radiosensitivity of lymphocytes from breast cancer patients with excessive normal tissue damage after radiotherapy: chromosome aberrations after low dose-rate irradiation. *Int J Radiat Biol.* 1995; 67(5):519–528. [PubMed: 7775827]
29. Baria K, Warren C, Roberts SA, West CM, Scott D. Chromosomal radiosensitivity as a marker of predisposition to common cancers? *Br J Cancer.* 2001; 84(7):892–896. [PubMed: 11286467]
30. Helzlsouer KJ, Harris EL, Parshad R, Perry HR, Price FM, Sanford KK. DNA repair proficiency: potential susceptibility factor for breast cancer. *J Natl Cancer Inst.* 1996; 88(11):754–755. [PubMed: 8637030]
31. Parshad R, Price FM, Bohr VA, Cowans KH, Zujewski JA, Sanford KK. Deficient DNA repair capacity, a predisposing factor in breast cancer. *Br J Cancer.* 1996; 74(1):1–5. [PubMed: 8679441]
32. Scott D. Chromosomal radiosensitivity and low penetrance predisposition to cancer. *Cytogenet Genome Res.* 2004; 104(1–4):365–370. [PubMed: 15162066]
33. Buchholz TA, Wu X. Radiation-induced chromatid breaks as a predictor of breast cancer risk. *Int J Radiat Oncol Biol Phys.* 2001; 49(2):533–537. [PubMed: 11173151]
34. Patel RK, Trivedi AH, Arora DC, Bhatavdekar JM, Patel DD. DNA repair proficiency in breast cancer patients and their first-degree relatives. *Int J Cancer.* 1997; 73(1):20–24. [PubMed: 9334804]
35. Scott D, Barber JB, Spreadborough AR, Burrill W, Roberts SA. Increased chromosomal radiosensitivity in breast cancer patients: a comparison of two assays. *Int J Radiat Biol.* 1999; 75(1):1–10. [PubMed: 9972785]
36. Natarajan TG, Ganesan N, Carter-Nolan P, Tucker CA, Shields PG, Adams-Campbell LL. 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.* 2006; 15(3):437–442. [PubMed: 16537698]
37. Wang LE, Bondy ML, de Andrade M, Strom SS, Wang X, Sigurdson A, Spitz MR, Wei Q. Gender difference in smoking effect on chromosome sensitivity to gamma radiation in a healthy population. *Radiat Res.* 2000; 154(1):20–27. [PubMed: 10856961]
38. Riches AC, Bryant PE, Steel CM, Gleig A, Robertson AJ, Preece PE, Thompson AM. Chromosomal radiosensitivity in G2-phase lymphocytes identifies breast cancer patients with distinctive tumour characteristics. *Br J Cancer.* 2001; 85(8):1157–1161. [PubMed: 11710829]
39. Howe OL, Daly PA, Seymour C, Ormiston W, Nolan C, Mothersill C. Elevated G2 chromosomal radiosensitivity in Irish breast cancer patients: a comparison with other studies. *Int J Radiat Biol.* 2005; 81(5):373–378. [PubMed: 16076752]
40. Baeyens A, Van Den Broecke R, Makar A, Thierens H, De Ridder L, Vral A. Chromosomal radiosensitivity in breast cancer patients: influence of age of onset of the disease. *Oncol Rep.* 2005; 13(2):347–353. [PubMed: 15643523]
41. Docherty Z, Georgiou A, Langman C, Kesterton I, Rose S, Camplejohn R, Ball J, Barwell J, Gilchrist R, Pangon L, et al. Is chromosome radiosensitivity and apoptotic response to irradiation correlated with cancer susceptibility? *Int J Radiat Biol.* 2007; 83(1):1–12. [PubMed: 17357435]
42. Kavvoura FK, Ioannidis JP. Methods for meta-analysis in genetic association studies: a review of their potential and pitfalls. *Hum Genet.* 2008; 123(1):1–14. [PubMed: 18026754]
43. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med.* 2002; 21(11):1539–1558. [PubMed: 12111919]
44. Cherry LM, Hsu TC. Bleomycin-induced chromosome damage in lymphocytes of medullary thyroid carcinoma patients and their family members. *Anticancer Res.* 1983; 3(6):367–372. [PubMed: 6200055]

45. Hsu TC, Johnston DA, Cherry LM, Ramkissoon D, Schantz SP, Jessup JM, Winn RJ, Shirley L, Furlong C. Sensitivity to genotoxic effects of bleomycin in humans: possible relationship to environmental carcinogenesis. *Int J Cancer*. 1989; 43(3):403–409. [PubMed: 2466800]
46. Wang LE, Hsu TC, Xiong P, Strom SS, Duvic M, Clayman GL, Weber RS, Lippman SM, Goldberg LH, Wei Q. 4-Nitro-quinoline-1-oxide-induced mutagen sensitivity and risk of non-melanoma skin cancer: a case-control analysis. *J Invest Dermatol*. 2007; 127(1):196–205. [PubMed: 16841033]
47. Wang LE, Sturgis EM, Eicher SA, Spitz MR, Hong WK, Wei Q. Mutagen sensitivity to benzo(a)pyrene diol epoxide and the risk of squamous cell carcinoma of the head and neck. *Clin Cancer Res*. 1998; 4(7):1773–1778. [PubMed: 9676854]
48. Wang LE, Xiong P, Strom SS, Goldberg LH, Lee JE, Ross MI, Mansfield PF, Gershenwald JE, Prieto VG, Cormier JN, et al. In vitro sensitivity to ultraviolet B light and skin cancer risk: a case-control analysis. *J Natl Cancer Inst*. 2005; 97(24):1822–1831. [PubMed: 16368944]
49. Bondy ML, Wang LE, El-Zein R, de Andrade M, Selvan MS, Bruner JM, Levin VA, Alfred Yung WK, Adatto P, Wei Q. Gamma-radiation sensitivity and risk of glioma. *J Natl Cancer Inst*. 2001; 93(20):1553–1557. [PubMed: 11604478]
50. Wang LE, Xiong P, Zhao H, Spitz MR, Sturgis EM, Wei Q. Chromosome instability and risk of squamous cell carcinomas of head and neck. *Cancer Res*. 2008; 68(11):4479–4485. [PubMed: 18519711]
51. Sanford KK, Parshad R, Gantt R, Tarone RE, Jones GM, Price FM. Factors affecting and significance of G2 chromatin radiosensitivity in predisposition to cancer. *Int J Radiat Biol*. 1989; 55(6):963–981. [PubMed: 2567334]
52. Parshad R, Sanford KK, Jones GM, Tarone RE. G2 chromosomal radiosensitivity of ataxia-telangiectasia heterozygotes. *Cancer Genet Cytogenet*. 1985; 14(1–2):163–168. [PubMed: 3965121]
53. Parshad R, Gantt R, Sanford KK, Jones GM. Chromosomal radiosensitivity of human tumor cells during the G2 cell cycle period. *Cancer Res*. 1984; 44(12 Pt 1):5577–5582. [PubMed: 6498819]
54. Sanford KK, Parshad R, Gantt RR, Tarone RE. A deficiency in chromatin repair, genetic instability, and predisposition to cancer. *Crit Rev Oncog*. 1989; 1(3):323–341. [PubMed: 2488136]
55. Bryant PE. The signal model: a possible explanation for the conversion of DNA double-strand breaks into chromatid breaks. *Int J Radiat Biol*. 1998; 73(3):243–251. [PubMed: 9525252]
56. Bryant PE, Riches AC, Terry SY. Mechanisms of the formation of radiation-induced chromosomal aberrations. *Mutat Res*. 2010; 701(1):23–26. [PubMed: 20348019]

Fig. 1. Distribution of chromatid breaks per cell in breast cancer patients and cancer-free controls

Fig. 2.

Forest plot of the overall risk for breast cancer associated with sensitivity to ionizing radiation-induced G2-phase chromatid breaks (Events were defined as sensitive to ionizing radiation as described in the original studies)

Table 1
Distribution of selected characteristics of breast cancer patients and cancer-free controls

Variables	Cases (<i>n</i> = 515)		Controls (<i>n</i> = 402)		<i>P</i> value ^a
	No.	%	No.	%	
Age (years)					0.139
45	236	(45.8)	204	(50.7)	
46–55	279	(54.2)	198	(49.3)	
Ethnicity					0.016
Non-Hispanic White	403	(78.2)	281	(69.9)	
Mexican American	60	(11.7)	65	(16.2)	
African American	52	(10.1)	56	(13.9)	
Smoking status ^b					0.824
Never	318	(61.7)	248	(62.5)	
Ever	197	(38.3)	149	(37.5)	
Drinking status ^b					0.002
Never	258	(50.1)	240	(60.5)	
Ever	257	(49.9)	157	(39.5)	
Histology					
In situ	83	(16.1)			
Invasive	432	(83.9)			
ER ^c					
Negative	142	(30.4)			
Positive	325	(69.6)			
PR ^c					
Negative	195	(42.0)			
Positive	269	(58.0)			
Stage					
0	83	(16.1)			
I	194	(37.7)			
II	182	(35.3)			
III–IV	56	(10.9)			

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^aTwo-sided χ^2 tests

^bFive control subjects with missing smoking status and alcohol use information

^cForty-eight patients with missing information on ER status and 51 on PR status

Table 2
Comparison of differences in chromatid breaks per cell induced by gamma-radiation between breast cancer patients and cancer-free controls

Selected variables	Non-Hispanic White			<i>p^a</i>	Mexican American			<i>p^a</i>	African American			<i>p^a</i>			
	Cases		Controls		Cases		Controls		Cases		Controls				
	No.	b/c	No.		b/c	No.	b/c		No.	b/c	No.		b/c		
Total	403	0.52 ± 0.21	281	0.44 ± 0.16	<0.0001	60	0.52 ± 0.19	65	0.44 ± 0.16	0.021	52	0.45 ± 0.16	56	0.47 ± 0.16	0.651
Age (years)															
45	177	0.54 ± 0.25	134	0.45 ± 0.17	0.0001	27	0.51 ± 0.20	34	0.45 ± 0.17	0.205	32	0.42 ± 0.13	36	0.47 ± 0.15	0.096
46–55	226	0.51 ± 0.18	147	0.43 ± 0.15	<0.0001	33	0.52 ± 0.18	31	0.44 ± 0.15	0.048	20	0.51 ± 0.19	20	0.46 ± 0.17	0.306
Smoking ^b															
Never	242	0.54 ± 0.23	156	0.44 ± 0.15	<0.0001	39	0.52 ± 0.17	52	0.45 ± 0.17	0.048	37	0.42 ± 0.12	40	0.49 ± 0.16	0.027
Ever	161	0.50 ± 0.18	121	0.43 ± 0.17	0.0007	21	0.51 ± 0.21	12	0.41 ± 0.12	0.178	15	0.54 ± 0.20	16	0.40 ± 0.11	0.030
Drinking ^b															
Never	185	0.55 ± 0.25	145	0.44 ± 0.16	<0.0001	37	0.51 ± 0.20	52	0.43 ± 0.17	0.047	36	0.46 ± 0.18	43	0.47 ± 0.14	0.735
Ever	218	0.50 ± 0.17	132	0.43 ± 0.15	0.0001	23	0.53 ± 0.16	12	0.49 ± 0.14	0.526	16	0.44 ± 0.11	13	0.46 ± 0.21	0.825
Histology					0.279					0.917					0.445
In situ	62	0.50 ± 0.17				10	0.51 ± 0.16				11	0.42 ± 0.11			
Invasive	341	0.53 ± 0.22				50	0.52 ± 0.19				41	0.46 ± 0.17			
ER ^c					0.094					0.947					0.346
Negative	108	0.55 ± 0.28				19	0.53 ± 0.18				15	0.48 ± 0.21			
Positive	256	0.51 ± 0.18				37	0.52 ± 0.20				32	0.44 ± 0.14			
PR ^c					0.178					0.335					0.951
Negative	151	0.54 ± 0.27				21	0.49 ± 0.15				23	0.45 ± 0.19			
Positive	212	0.51 ± 0.16				33	0.55 ± 0.22				24	0.45 ± 0.14			
Stage					0.232					0.556					0.860
0	62	0.50 ± 0.17				10	0.51 ± 0.16				11	0.42 ± 0.11			
I	162	0.53 ± 0.23				15	0.55 ± 0.22				17	0.45 ± 0.13			
II	135	0.51 ± 0.18				30	0.52 ± 0.18				17	0.46 ± 0.19			
III–IV	44	0.58 ± 0.27				5	0.41 ± 0.09				7	0.48 ± 0.22			

^aTwo-sided student's *t* tests for differences between cases and controls, or ANOVA tests for differences among cases

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^b Five control subjects with missing smoking status and alcohol use information

^c Forty-eight patients with missing information on ER status and 51 on PR status

The bold parts meant the statistical significance of P value < 0.05

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Table 3
Logistic regression analysis of gamma-ray-induced chromatid breaks per cell in breast cancer patients and cancer-free controls

Chromatid breaks per cell	Non-Hispanic White		Mexican American		African American	
	Cases No. (%)	Control No. (%)	OR (95% CI) ^d	Cases No. (%)	Controls No. (%)	OR (95% CI) ^d
0.1 increment ^a	403 (100%)	281 (100%)	1.35 (1.22–1.49)	60 (100%)	65 (100%)	1.27 (1.01–1.60)
By median ^b						
0.43	136 (33.7%)	146 (52.0%)	1.00 (Ref.)	20 (33.3%)	35 (53.8%)	1.00 (Ref.)
>0.43	267 (66.3%)	135 (48.0%)	2.23 (1.62–3.07)	40 (66.7%)	30 (46.2%)	2.20 (1.04–4.67)
By tertile ^c						
0.36	81 (20.1%)	102 (36.3%)	1.00 (Ref.)	10 (23.7%)	21 (32.3%)	1.00 (Ref.)
0.37–0.49	110 (27.3%)	85 (30.3%)	1.70 (1.13–2.58)	20 (8.5%)	23 (35.4%)	1.95 (0.71–5.36)
0.50	212 (52.6%)	94 (33.4%)	3.03 (2.05–4.47)	30 (20.3%)	21 (32.3%)	3.15 (1.17–8.46)
Trend test ^d			P_{trend} < 0.0001			P_{trend} = 0.012
						P_{trend} = 0.846

^aChromatid breaks per cell were fitted in logistic regression models as a continuous variable

^bMedian of the controls' values

^cTertiles of the controls' values

^dAdjusted for age, smoking, and alcohol status in logistic regression models

The bold parts meant the statistical significance of *P* value <0.05