

Polymorphisms in DNA Repair Genes, Medical Exposure to Ionizing Radiation, and Breast Cancer Risk

Robert C. Millikan,¹ Jon S. Player,¹ Allan Rene deCotret,¹ Chiu-Kit Tse,¹ and Temitope Keku²

¹Department of Epidemiology, School of Public Health and Lineberger Comprehensive Cancer Center, School of Medicine and ²Center for Gastrointestinal Biology and Disease, School of Medicine, University of North Carolina, Chapel Hill, North Carolina

Abstract

An epidemiologic study was conducted to determine whether polymorphisms in DNA repair genes modify the association between breast cancer risk and exposure to ionizing radiation. Self-reported exposure to ionizing radiation from medical sources was evaluated as part of a population-based, case-control study of breast cancer in African-American (894 cases and 788 controls) and White (1,417 cases and 1,234 controls) women. Genotyping was conducted for polymorphisms in four genes involved in repair of radiation-induced DNA damage, the double-strand break repair pathway: *X-ray cross-complementing group 3 (XRCC3)* codon 241 Thr/Met, *Nijmegen breakage syndrome 1 (NBS1)* codon 185 Glu/Gln, *X-ray cross-complementing group 2 (XRCC2)* codon 188 Arg/His, and *breast cancer susceptibility gene 2 (BRCA2)* codon 372 Asn/His. Allele and genotype frequencies were not significantly different in cases compared with controls for all four

genetic polymorphisms, and odds ratios for breast cancer were close to the null. Combining women with two, three, and four variant genotypes, a positive association was observed between breast cancer and number of lifetime mammograms ($P_{\text{trend}} < 0.0001$). No association was observed among women with zero or one variant genotype ($P = 0.86$). Odds ratios for radiation treatments to the chest and number of lifetime chest X-rays were slightly elevated but not statistically significant among women with two to four variant genotypes. The study has several limitations, including inability to distinguish between diagnostic and screening mammograms or reliably classify prediagnostic mammograms and chest X-rays in cases. Prospective studies are needed to address whether common polymorphisms in DNA repair genes modify the effects of low-dose radiation exposure from medical sources. (Cancer Epidemiol Biomarkers Prev 2005;14(10):2326–34)

Introduction

Exposure to ionizing radiation is a well-established risk factor for breast cancer (1-3). Epidemiologic studies consistently show elevated breast cancer risk following moderate to high-dose radiation exposure. Examples include studies of atomic bomb survivors in Japan, women treated with repeated exposure to radiation from fluoroscopic chest radiography, and women undergoing radiation treatment for a variety of medical conditions (for review, see refs. 1-3). Risk of breast cancer from exposure to very low levels of ionizing radiation, such as chest X-rays and mammograms, is controversial (1). Based on linear extrapolation from high to low dose, some researchers estimate the carcinogenic effects of very low level radiation exposure to be negligible (4). Assessing cancer risk from low-dose radiation presents several obstacles, including the difficulties in measuring lifetime exposure, the large sample sizes needed to quantify effects, and the appropriateness of linear extrapolation from high to low dose (1, 4).

One method for increasing power to detect the effects of low-level environmental exposures is to identify genetically susceptible subgroups (5). Several previous studies suggest that breast cancer patients exhibit decreased ability to repair radiation-induced DNA damage compared with unaffected controls (refs. 6-17; for review, see ref. 2). A genetic basis for radiosensitivity in breast cancer patients has been hypothe-

sized. Swift et al. (18, 19) proposed that carriers of rare, highly penetrant ataxia telangiectasia mutated mutations were at increased risk of breast cancer following exposure to low-dose radiation from mammograms and other medical procedures. Studies of ataxia telangiectasia mutated mutations and breast cancer risk have been inconsistent (1, 20, 21), and breast cancer patients who are heterozygous for ataxia telangiectasia mutated mutations do not exhibit radiosensitivity (22). Other investigators proposed that common, low-penetrance susceptibility genes exist in human populations that interact with radiation exposure to increase risk of breast cancer (1, 2, 6, 8, 23-25). Polymorphisms in DNA repair genes, particularly double-strand break (DSB) repair, were specifically proposed as candidate genes for radiosensitivity by four investigators (2, 6, 24, 25).

DSBs are the most common form of radiation-induced DNA damage (26) and are repaired by two pathways—homologous recombination repair (HRR) and nonhomologous end-joining (27-29). HRR requires an undamaged, homologous partner DNA strand, whereas nonhomologous end-joining involves direct DNA end-joining with little or no sequence homology. For the present study, we focused on HRR because it occurs during the phases of the cell cycle (G_2 or S; ref. 30) where lymphocytes from breast cancer patients exhibit maximal radiosensitivity (8). The HRR pathway consists of at least 16 protein components, including X-ray cross-complementing group 2 (XRCC2), X-ray cross-complementing group 3 (XRCC3), and breast cancer susceptibility gene 2 (BRCA2; ref. 27). In both HRR and nonhomologous end-joining, the initial step in repair is the recognition of DSBs by a complex of proteins that includes Nijmegen breakage syndrome 1 (NBS1), meiotic recombination 11 homologue, and human RAD50 homologue. Common polymorphisms have been identified in at least 12 genes involved in HRR (27). Using an algorithm based on allele frequency, potential functional effect, and results from previous epidemiologic studies, we chose to

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Requests for reprints: Robert C. Millikan, Department of Epidemiology, School of Public Health, University of North Carolina, CB 7400, Chapel Hill, NC 27599-7400. Phone: 919-966-7437; Fax: 919-966-2089. E-mail: bob_millikan@unc.edu

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evaluate polymorphisms in four DSB genes: *XRCC2*, *XRCC3*, *BRCA2*, and *NBS1*. We hypothesized that combinations of polymorphisms in these four DSB repair genes could contribute to increased risk of breast cancer among women exposed to low levels of ionizing radiation from medical procedures. We evaluated this hypothesis in the Carolina Breast Cancer Study (CBCS), a population-based, case-control study of African-American and White women in North Carolina.

Materials and Methods

Study Population. The CBCS is a population-based, case-control study of invasive and *in situ* breast cancer conducted in 24 counties of central and eastern North Carolina (31). Incident cases were identified using a Rapid Case Ascertainment System in cooperation with the North Carolina Central Cancer Registry. Controls were selected from Division of Motor Vehicles (women ages <65 years) and U.S. Health Care Financing Administration lists (women ages ≥65 years). Controls were frequency matched to cases based on age and race (±5 years) using randomized recruitment (32). Participants ranged in age from 21 to 74 years. Women with a prior history of breast cancer were excluded from the case and control groups, but cancer at other sites was not used as a basis for exclusion.

In-person interviews were conducted to obtain blood samples and information on potential breast cancer risk factors. Enrollment of participants occurred in two phases: phase 1 (1993-1996: invasive breast cancer cases and controls) and phase 2 (1996-2001: invasive breast cancer cases and controls and *in situ* breast cancer cases and controls). Overall response rates (product of contact and cooperation rates) were 76% for cases and 55% for controls. Among cases, the median time interval between date of diagnosis and date of in-person interview was 3.9 months (mean, 5.2 months), and 90% of cases were interviewed within 9 months of diagnosis. Among controls, the median time interval between date of ascertainment (sampling) and date of interview was 3.8 months (mean, 6.4 months), and 90% of interviews occurred within 15 months of ascertainment. CBCS participant characteristics were reported previously (33-35). Race was classified according to self-report. Less than 2% of participants reported Native American or other race and were classified as White. Procedures for recruiting and enrolling study participants were approved by the Institutional Review Board of the University of North Carolina (UNC) School of Medicine and informed consent was obtained from each participant.

A total of 861 invasive breast cancer cases (335 African Americans and 526 Whites) and 790 controls (332 African Americans and 458 Whites) were enrolled in phase 1. Enrollment in phase 2 included 947 invasive breast cancer cases (453 African Americans and 494 Whites) and 774 controls (386 African Americans and 388 Whites) as well as 503 *in situ* breast cancer cases (106 African Americans and 397 Whites) and 458 controls (70 African Americans and 388 Whites). A total of 170 cases and 154 controls reported a previous diagnosis of cancer. The most common sites were non-melanoma skin cancer, cervical cancer, endometrial cancer, lung cancer, melanoma, and lymphoma.

Response rates for blood draws and obtaining DNA were 89% for cases and 90% for controls. DNA samples were available for a total of 2,045 cases (781 phase 1, 809 phase 2 invasive, and 455 phase 2 *in situ*) and 1,818 controls (744 phase 1, 675 phase 2 invasive, and 399 phase 2 *in situ*). Odds ratios (ORs) for breast cancer risk factors did not differ significantly between participants with and without DNA samples (data not shown).

In-Person Interview. Information on breast cancer risk factors, including exposure to ionizing radiation, was obtained

from in-person interviews conducted by trained nurses (36). Participants were asked about exposure before the reference date, defined as the date of diagnosis in cases and the date of ascertainment in controls. Medical exposure to ionizing radiation was assessed by asking participants about previous diagnostic procedures and treatments. Participants were asked, "Have you ever had a coronary angiogram, angioplasty, or cardiac catheterization?" and "Did you ever have radiation to treat or monitor any condition?" Women were asked to identify the number of procedures and the age at which the procedures were done and to "Identify the disease that was being treated, the part of your body that was treated, and your age at the beginning and end of the treatments." Participants were allowed to list multiple procedures and treatments. Cases were asked to exclude procedures conducted as part of the diagnosis or treatment of breast cancer.

The variable "History of radiation to the chest" was defined so that the exposed group included women with coronary catheterization, chest fluoroscopy, or angioplasty (89% of exposed cases and 93% of exposed controls) and women who reported having the axilla, lungs, breast, or chest treated or monitored with radiation (11% of exposed cases and 7% of exposed controls). Conditions requiring radiation treatment included a previous diagnosis of cancer (7 cases and 4 controls); infectious lung conditions, such as tuberculosis, histoplasmosis, or pleurisy (6 cases and 3 controls); skin conditions, such as acne (4 cases and 2 controls); and benign breast disease (2 cases and 0 controls). For participants undergoing radiation treatment for cancer, the diagnoses were lung cancer (1 case, 4 controls), Hodgkin's disease (1 case), and lymphoma (5 cases). Data on "History of radiation to the chest" were available from the entire CBCS (phase 1 and phase 2 invasive and *in situ*) and missing on 1 case and 0 controls.

Chest X-rays and mammograms were assessed only in phase 2 of the CBCS (invasive and *in situ*). Information on mammograms and chest X-rays was not obtained during phase 1. Women were asked, "Have you ever had a chest X-ray?" and to specify the number of chest X-rays as a categorical variable (0, 1-5, 6-10, or ≥11), the age at first and last chest X-ray, and the reason the chest X-rays were taken. Participants were queried, "Have you ever had a mammogram?" and asked to specify the age at first mammogram, the number of mammograms (as a continuous variable) at age <40, between 40 and 50, and ≥50 years, and the number and dates of mammograms conducted during the 2 years before the reference date (date of diagnosis in cases or ascertainment in controls). Cases were asked to exclude mammograms and chest X-rays conducted as part of the diagnosis or treatment of breast cancer. Because we did not validate self-reported exposure information using medical records, we could not determine with certainty whether any of the reported mammograms or chest X-rays were conducted as part of a diagnostic work-up in cases. Number of lifetime mammograms was determined by summing over all age categories. The maximum number of total lifetime mammograms reported by study participants was 41 for cases and 47 for controls. The maximum number of mammograms at age <40 years differed according to participant age: for women ages 40 to 49 years, the maximum number was 20 for cases and 16 for controls; for women ages 50 to 59 years, 15 for cases and 20 for controls; and for women ages ≥60 years, 13 for cases and 8 for controls. Most of the mammograms at age <40 years were probably conducted as part of the work-up for benign breast disease or other breast conditions: among participants who reported ≥10 mammograms at age <40 years, 74% of cases and 87% of controls reported a previous diagnosis of benign breast disease. Data were missing on chest X-rays on 16 cases and 14 controls and on mammograms for 4 cases and 3 controls from the phase 2 invasive and *in situ* portions of the CBCS. Both variables were missing for all participants in phase 1.

Laboratory Methods. DNA was extracted from peripheral blood lymphocytes using an automated ABI-DNA extractor (Nucleic Acid Purification System, Applied Biosystems, Foster City, CA) in the UNC Specialized Programs of Research Excellence Tissue Procurement Facility. Genotyping was conducted using the ABI 7700 Sequence Detection System or Taqman assay (Applied Biosystems). Laboratory personnel were unaware of disease status or other characteristics of study participants.

The following single-nucleotide polymorphisms (SNP) were genotyped and listed according to locus name and database for SNP reference sequence number (<http://snp500cancer.nci.nih.gov>): XRCC3 codon 241 Thr/Met (rs861539), NBS1 codon 185 Glu/Gln (rs1805794), XRCC2 codon 188 Arg/His (rs3218536), and BRCA2 codon 372 Asn/His (rs144848). The goal in selecting SNPs was to construct "pathway genotypes" for the HRR process in DSB repair as suggested by Mohrenweiser et al. (27). SNPs were chosen according to the following criteria: (a) essential role for the gene in HRR, (b) minimum frequency for the less common allele of $\geq 5\%$ in African Americans or Whites, (c) previous laboratory data and/or computer simulations suggesting significant functional effect, (d) previous epidemiologic studies showing an association with breast cancer or susceptibility to ionizing radiation, and (e) calculations that showed we had sufficient power to stratify on combinations of four genetic polymorphisms when estimating ORs for number of lifetime mammograms. Genotyping was conducted as part of larger screen of DNA repair gene polymorphisms and breast cancer, but only the four listed polymorphisms were genotyped from the list of candidate HRR genes.

Primer and probe sequences as well as annealing temperatures for each genotyping assay are listed in Table 1. Probes were labeled on the 5' end with either FAM or VIC (Applied Biosystems). Probes were labeled on the 3' end with the quencher dye TAMRA.

PCR reactions were done in 15 μ L reaction volumes. Reactions contained 0.7 \times ABI Universal Master Mix, 200 nmol/L of each allele specific probe, 900 nmol/L of each primer, and 15 ng genomic DNA. After reactions tubes were set up, amplification was done using a GeneAmp 9700 thermocycler (Perkin-Elmer, Wellesley, MA). Reaction tubes were placed into the thermocycler after the temperature had reached 50°C. The amplification was preformed using the following conditions: 50°C for 2 minutes (AmpErase UNG Activation), 95°C for 10 minutes (AmpliAq Gold Activation), and 40 cycles of 92°C for 15 seconds (denature) and 60°C or 62°C (Table 1) for 1 minute

(anneal/extend). Samples that failed to amplify were repeated and samples that failed to amplify on the second run were scored as missing. The number of missing genotypes for each locus is listed in Table 2. Genotypes were repeated on a random 10% sample for each locus, and repeats were identical to the original results. DNA samples from the Coriell Tissue Repository (Coriell Institute for Medical Research, Camden, NJ) that had been sequenced for the DSB repair loci of interest (<http://snp500cancer.nci.nih.gov>) were included with each genotyping assay as positive controls.

Statistical Methods. Departures from Hardy-Weinberg equilibrium were evaluated for each locus by calculating expected genotype frequencies among controls based on observed allele frequencies and comparing the expected frequencies to observed genotype frequencies using χ^2 tests. Differences between allele or genotype frequencies in cases and controls were estimated using χ^2 or Fisher's exact tests when expected counts were < 5 . The Cochran-Armitage test for trend was used to compare genotype frequencies when controls were not in Hardy Weinberg equilibrium (37).

Unconditional logistic regression was used to calculate ORs for breast cancer and 95% confidence intervals (95% CI). PROC GENMOD in SAS (version 8.2, SAS Institute, Cary, NC) was used to incorporate offsets derived from sampling probabilities used to identify eligible participants (32) and to adjust for race (African-American and White) and age (as an 11-level ordinal variable that reflected 5-year age categories). The main exposure variables of interest were DSB repair genotypes and radiation exposure. "History of radiation to the chest" was analyzed in two ways: (a) as a dichotomous variable (yes versus no), where "yes" included the procedures listed previously (coronary catheterization, chest fluoroscopy, or angioplasty; having the axilla, lungs, breast, or chest treated or monitored with radiation), and (b) as a four-level variable, where "yes" was divided into coronary catheterization, chest fluoroscopy, or angioplasty; radiation treatments for conditions other than cancer; radiation treatments for cancer.

Multivariable logistic regression was used to adjust for potential confounding factors. Confounding was evaluated by determining whether adding a variable to a model resulted in a change in the β coefficient of at least 10% for the main exposures of interest. Potential confounders were checked singly as well as in combination. ORs for DSB repair genotypes and breast cancer, as well as radiation exposure variables and breast cancer, were evaluated for confounding by the following variables: age at menarche, age at first full-term pregnancy, parity, breast-feeding, family history of breast cancer, smoking,

Table 1. PCR-based Taqman genotyping assay conditions for polymorphisms in DSB repair genes

Locus	SNP	Database for SNP reference sequence no.	Forward/reverse primers	Probe sequences,* VIC/FAM probes	Annealing temperature (°C)
XRCC3	Nucleotide 18067 C→T Thr ²⁴¹ Met	rs861539	CCAGGCATCTGCAGTCCC/ ACAGCACAGGGCTCTGGAA	Thr ²⁴¹ (C) VIC-TCACGCAGCgTGGCCCC/ Met ²⁴¹ (T) FAM-CACGCAGCaTGGCCCC	62.0
NBS1	Nucleotide 11122 G→C Glu ¹⁸⁵ Gln	rs1805794	TTCAATTGTGGAGGCTGCT/ GACGTCCAATTGTAAAGCCAGAATA	Gln ¹⁸⁵ (C) VIC-AGCAGTTcAGTCCAA/ Glu ¹⁸⁵ (G) FAM-AGCAGTTgAGTCCAA	60.0
XRCC2	Nucleotide 31279 G→A Arg ¹⁸⁸ His	rs3218536	TGTCTCAGTGCTTAGAGAAGCTTGT/ GCATTATAGTTGTGCTGTGCAAA	Arg ¹⁸⁸ (G) VIC-ATGACTATCgCCTGGTT/ His ¹⁸⁸ (A) FAM-ATGACTATCaCCTGGTTC	60.0
BRCA2	Nucleotide 1342 A→C Asn ³⁷² His	rs144848	AACCAAATGATACTGATCCATTAGATTC/ GAGATTTGTCACTTCCACTCTCAAA	Asn ³⁷² (A) VIC-AAATGTAGCAaATCAGAAG/ His ³⁷² (C) FAM-AAATGTAGCAcATCAGAA	60.0

*Lowercase bases indicate sequence variants. NBS1, XRCC2, and BRCA2 were minor groove binding probes designed for the antisense strand.

alcohol consumption, body mass index, income, education, oral contraceptive use, hormone replacement therapy (postmenopausal women), menopausal status, and number of prior diagnoses of benign breast disease. None of the listed variables resulted in a $\geq 10\%$ change in β coefficients for the main exposures of interest. Therefore, ORs are presented adjusting for sampling probabilities, age, and race only.

ORs for radiation exposure variables were calculated for all participants as well among subgroups defined by race, menopausal status, body mass index (<25 , 25 - 30 , >30 kg/m²), age at exposure (age <20 , 20 - 40 , or >40 years), family history of breast cancer, annual household income ($< \$15,000$, $\$15,000$ - $50,000$, or $> \$50,000$), education (less than high school, high school, college, or postgraduate), and reproductive history (parity, age at first full term pregnancy, history of breast-feeding). ORs did not differ appreciably across subgroups. Likelihood ratio tests (LRT) comparing models with and without interaction terms between the subgroup variables and radiation exposure variables did not reveal evidence for interaction. ORs for the main exposures of interest did not differ for *in situ* versus invasive breast cancer.

Based on the availability of exposure data, ORs were calculated combining phase 1 and phase 2 invasive and *in situ* study participants for "History of radiation to the chest." ORs

combining phase 2 invasive and *in situ* study participants (excluding phase 1) were calculated for chest X-rays and mammograms. For the chest X-ray and mammogram variables, analyses were conducted excluding exposures that occurred during the 2 years before the reference date among cases and controls. These analyses were conducted to further decrease the potential for including chest X-rays or mammograms related to the diagnosis or treatment of breast cancer.

Stratified analyses were used to determine whether ORs for radiation exposure variables and breast cancer were modified by DSB repair genotypes. Each DSB repair genotype was evaluated separately, and combinations of DSB repair genotypes then were created by categorizing participants based on number of variant genotypes. "Variant genotype" was defined as the presence of one or more copies of the less common allele for a given locus: XRCC3 codon 241 Thr/Met or Met/Met ("any Met"), NBS1 codon 185 Glu/Gln or Gln/Gln ("any Gln"), XRCC2 codon 188 Arg/His or His/His ("any His"), and BRCA2 codon 372 Asn/His or His/His ("any His"). Individuals were classified as having zero variant genotypes, variant genotypes at any one of the four loci, and so forth up to four variant genotypes. The rationale for combining homozygotes for the less common allele with heterozygotes was based on the fact that substitution of a single amino acid

Table 2. Genotype frequencies, allele frequencies, and ORs for DSB repair gene polymorphisms and breast cancer in African-American and White women

Locus	African Americans			Whites		
	Cases (n = 768)	Controls (n = 681)	OR* (95% CI)	Cases (n = 1,277)	Controls (n = 1,137)	OR* (95% CI)
XRCC3 codon 241						
Thr/Thr [†]	482 (65)	421 (62)	Reference	505 (40)	435 (38)	Reference
Thr/Met	222 (30)	211 (31)	0.9 (0.7-1.0)	578 (46)	555 (49)	0.9 (0.7-1.0)
Met/Met	41 (5)	44 (7)	0.7 (0.5-1.2)	171 (14)	142 (13)	1.0 (0.8-1.4)
Missing	23	45		13	5	
Cochrane-Armitage test [‡] (P)	0.29			0.79		
Thr [§]	0.80 (0.78-0.82)	0.78 (0.76-0.80)		0.63 (0.61-0.65)	0.63 (0.61-0.65)	
Met	0.20 (0.18-0.22)	0.22 (0.20-0.23)		0.37 (0.35-0.39)	0.37 (0.35-0.39)	
χ^2 test [‡] (P)	0.26			0.79		
NBS1 codon 185						
Glu/Glu [†]	440 (57)	400 (59)	Reference	588 (46)	518 (46)	Reference
Glu/Gln	287 (38)	240 (35)	1.1 (0.9-1.4)	566 (45)	503 (44)	1.0 (0.8-1.2)
Gln/Gln	39 (5)	41 (6)	0.9 (0.5-1.4)	119 (9)	115 (10)	0.8 (0.6-1.1)
Missing	2	0		4	1	
χ^2 test [‡] (P)	0.56			0.81		
Glu [§]	0.76 (0.74-0.78)	0.76 (0.74-0.79)		0.68 (0.67-0.70)	0.68 (0.66-0.69)	
Gln	0.24 (0.22-0.26)	0.24 (0.21-0.26)		0.32 (0.30-0.33)	0.32 (0.30-0.34)	
χ^2 test [‡] (P)	0.91			0.61		
XRCC2 codon 188						
Arg/Arg [†]	744 (97)	653 (96)	Reference	1,084 (85)	982 (86)	Reference
Arg/His	21 (3)	25 (4)	0.7 (0.4-1.3)	176 (14)	145 (13)	1.0 (0.8-1.3)
His/His	0	0	ND	8 (1)	7 (1)	0.7 (0.2-1.9)
Missing	3	3	9	3		
χ^2 test [‡] (P)	0.31			0.73		
Arg [§]	0.99 (0.98-0.99)	0.98 (0.97-0.99)		0.92 (0.91-0.93)	0.93 (0.92-0.94)	
His	0.01 (0.0-0.02)	0.02 (0.01-0.03)		0.08 (0.07-0.09)	0.07 (0.06-0.08)	
χ^2 test [‡] (P)	0.31			0.46		
BRCA2 codon 372						
Asn/Asn [†]	564 (74)	510 (76)	Reference	662 (52)	579 (51)	Reference
Asn/His	183 (24)	153 (23)	1.2 (0.9-1.5)	521 (41)	467 (41)	1.0 (0.8-1.2)
His/His	15 (2)	12 (2)	1.3 (0.6-2.8)	82 (7)	89 (8)	0.8 (0.6-1.2)
Missing	6	6		12	2	
χ^2 test [‡] (P)	0.79			0.42		
Asn [§]	0.86 (0.84-0.88)	0.87 (0.85-0.89)		0.73 (0.71-0.75)	0.72 (0.70-0.73)	
His	0.14 (0.14-0.16)	0.13 (0.12-0.15)		0.27 (0.25-0.29)	0.28 (0.27-0.30)	
χ^2 test [‡] (P)	0.50			0.30		

*Adjusted for offsets and age.

[†] Genotype frequencies n (%). ND, not determined.

[‡] Comparing cases and controls.

[§] Allele frequencies (95% CI).

can influence protein-protein interactions in DNA repair (27) as well as limited power to examine interactions between radiation exposure and less common homozygous genotypes in our data set.

Multiplicative interaction between number of variant DSB repair genotypes and the radiation exposure variables was evaluated using LRTs. For each radiation variable, the LRT compared a logistic regression model that included main effects for the radiation variable and the number of variant DSB repair genotypes (0-1 and 2-4) to a model containing main effects and an interaction term between the radiation variables and number of DSB repair genotypes. For number of chest X-rays and mammograms, tests for trend were conducted by calculating P s for the β coefficient in logistic regression models with exposure coded as an ordinal variable. All P s were two sided. Additional analyses were conducted using four categories for the number of variant genotypes (0, 1, 2, and 3-4). ORs for radiation exposure variables were similar for women with zero and one variant and for women with two and three to four variants. ORs were unstable for participants with four variant genotypes. Therefore, groups were combined as zero to one and two to four variants to increase precision. t tests were used to compare the number of mammograms reported by participants with as zero to one and two to four variant genotypes.

To further investigate the effects of stage at diagnosis in cases, case-only analyses were conducted (38). Using logistic regression, case-only ORs were calculated measuring the association between DSB genotypes (0-1 and 2-4 variants) and radiation exposure variables with and without adjustment for American Joint Committee on Cancer stage at diagnosis (stage 0 or *in situ*, 1, 2, 3, and 4). ORs were unchanged after adjustment for stage (data not shown).

Results

Genotype and allele frequencies for DSB repair gene polymorphisms are presented in Table 2. There were no statistically significant differences in genotype or allele frequencies between cases and controls in African Americans or Whites. Tests for departure from Hardy-Weinberg equilibrium were statistically significant only for *XRCC3* codon 241 genotype in African American cases ($P = 0.02$) and controls ($P = 0.01$). In both groups, the frequency of Met/Met homozygotes was slightly greater than expected. Therefore, the Cochran-Armitage test was used to compare genotype frequencies for *XRCC3*. For all four DSB repair genes, 95% CIs for allele frequencies included allele frequencies from previous studies for White controls (28, 29, 39-45) and the National Cancer Institute SNP500 database (<http://snp500cancer.nci.nih.gov>) for African American and White controls.

ORs for breast cancer and DSB repair polymorphisms are presented in Table 2. ORs were close to the null in African Americans and Whites. ORs for combinations of DSB repair genotypes and breast cancer are presented in Table 3. ORs were close to the null for participants with one, two, three, or four variant genotypes compared with participants with zero variant genotypes.

ORs for radiation exposure variables and breast cancer, stratified by the number of variants in DSB repair genes, are presented in Table 4. ORs were similar in African-American and White women; therefore, only combined results are presented to increase precision. "History of radiation to the chest" (yes versus no) showed a weak positive association among participants with two to four variant genotypes. No association was observed among women with zero to one variant genotypes, and the LRT was not statistically significant.

Analyses using a four-level variable for "History of radiation to the chest" yielded highly imprecise ORs (data not shown), and the LRT was not statistically significant

Table 3. ORs for number of variant genotypes in DSB repair genes and breast cancer in African Americans and Whites

Genotype combination	Cases/controls	OR* (95% CI)
African Americans [†]		
0 variants	193/183	Reference
1 variant	343/293	1.1 (0.8-1.4)
2 variants	168/162	1.0 (0.7-1.4)
3 variants	36/30	1.1 (0.6-1.9)
4 variants	1/2	0.6 (0.1-6.4) [‡]
Whites [†]		
0 variants	111/88	Reference
1 variant	375/339	0.8 (0.6-1.2)
2 variants	494/449	0.8 (0.6-1.1)
3 variants	232/233	0.7 (0.5-1.0)
4 variants	31/20	1.1 (0.6-2.1)

*Adjusted for offsets and age.

[†]Variant genotypes: *XRCC3* codon 241 any Met, *NBS1* codon 185 any Gln, *XRCC2* codon 188 any His, and *BRCA2* codon 372 any His.

[‡]Imprecise estimate.

($P = 0.17$). ORs were unchanged when we excluded participants (170 cases and 154 controls) who reported a previous diagnosis of cancer (data not shown).

A weak positive association was observed for the highest level of chest X-rays in participants with two to four variant genotypes, but trend tests and the LRT were not statistically significant (Table 4). ORs for breast cancer increased with increasing number of mammograms among women with two to four variant genotypes, and tests for trend were statistically significant. No significant trends were observed among women with zero to one variant genotypes. LRTs were statistically significant for number of mammograms, mammograms at age <40 years, and mammograms at age >40 years. Associations were attenuated after excluding mammograms conducted with 2 years of the reference date in cases and controls, but LRTs and trend tests remained statistically significant. Trend tests for participants with two to four variant genotypes and LRTs were statistically significant for women ages <50 and ≥ 50 years and for premenopausal and postmenopausal women (data not shown).

Discussion

Moderate to high-dose exposure to ionizing radiation is a well-established risk factor for breast cancer, but relatively few studies have addressed the effects of low-dose exposure (3). We studied the effects of exposure to ionizing radiation from medical procedures in a population-based, case-control study of breast cancer in African-American and White women in North Carolina. ORs for breast cancer and exposure to ionizing radiation from mammograms were modified by inherited polymorphisms in four genes involved in DSB repair of DNA: *XRCC2*, *XRCC3*, *BRCA2*, and *NBS1*. A positive dose-response relationship was observed for number of chest X-rays and mammograms and breast cancer risk among carriers of two or more copies of the less common alleles for the four DSB repair genes but not among women with one or zero variant genotypes. ORs for radiation treatments to the chest and chest X-rays were slightly elevated for women with two to four variant genotypes, but LRTs were not statistically significant. Given the importance of the DSB repair pathway in repair of radiation-induced DNA damage (26, 27) and its role in breast carcinogenesis (46), these results are biologically plausible.

Researchers have long hypothesized that genetic susceptibility to ionizing radiation could contribute to increased risk of breast cancer (1, 2, 6, 8, 18, 19, 23-25). The DSB repair pathway includes several candidate genes. For the present study, we chose four common SNPs in DSB repair genes that result in nonconservative amino acid substitutions within conserved

regions of the encoded proteins (47). Conserved regions within proteins often mediate protein-protein interactions and regulate enzymatic activity (48, 49). The functional significance of the four polymorphisms chosen for this investigation—*XRCC2* codon 188 Arg/His, *XRCC3* codon 241 Thr/Met, *NBS1* codon 185 Glu/Gln, and *BRCA2* codon 372 Asn/His—has been addressed in several previous studies. Rafii et al. (43) reported that the *XRCC2* codon 188 His showed a slight decrease in repair of DNA damage induced by mitomycin C compared with the Arg allele. Savas et al. (48) and Zhu et al. (49) predicted that the *XRCC3* codon 241 Met allele would have significant

functional effect based on amino acid conservation analysis. Matullo et al. (50) observed a higher level of DNA adducts in healthy volunteers with *XRCC3* codon 241 Met/Met genotype compared with Thr/Thr. There are no functional studies of the *NBS1* codon 185 polymorphism, but Heikkinen et al. (51) reported that amino acid residues 136, 137, and 176 are critical for phosphorylation of *NBS1* after irradiation and forming complexes with meiotic recombination 11 homologue. Rare amino acid substitutions in other adjacent regions of *NBS1* (e.g., codon 150 Leu/Phe) contribute to increased risk of breast and ovarian cancer (51). Healey et al. (41) observed a deficiency

Table 4. ORs for radiation exposure variables and breast cancer stratified by the number of variant genotypes in DSB repair genes

Exposure category	0-1 variant*		2-4 variants*	
	Cases/controls	OR (95% CI) †	Cases/controls	OR (95% CI) †
History of radiation to chest				
No	943/834	Reference	882/840	Reference
Yes	78/69	1.1 (0.8-1.6)	80/56	1.4 (1.0-2.1)
LRT ‡ (P)	0.24			
No. chest X-rays §				
0	202/142	Reference	190/142	Reference
1-5	267/263	0.7 (0.6-1.0)	231/257	0.7 (0.5-0.9)
6-10	94/79	0.9 (0.6-1.3)	95/86	0.8 (0.6-1.3)
≥11	66/42	1.1 (0.7-1.8)	82/46	1.5 (0.9-2.3)
P _{trend}		0.89		0.27
LRT ‡ (P)	0.51			
No. mammograms §				
0	137/93	Reference	87/80	Reference
1-2	82/68	1.0 (0.6-1.5)	59/117	0.5 (0.3-0.8)
3-5	138/138	0.9 (0.6-1.3)	128/119	1.4 (0.9-2.2)
6-10	127/123	0.9 (0.6-1.3)	133/104	1.7 (1.1-2.7)
≥11	152/106	1.1 (0.7-1.7)	196/116	2.2 (1.4-3.5)
P _{trend}		0.86		<0.0001
LRT ‡ (P)	<0.001			
No. mammograms (2-y lag) §				
0	172/113	Reference	114/99	Reference
1-2	144/112	1.0 (0.7-1.5)	113/151	0.8 (0.5-1.1)
3-5	107/117	0.7 (0.5-1.0)	116/100	1.3 (0.8-1.9)
6-10	100/102	0.7 (0.4-1.0)	116/97	1.3 (0.9-2.1)
≥11	114/85	0.9 (0.6-1.4)	145/91	1.8 (1.2-2.8)
P _{trend}		0.16		0.0003
LRT ‡ (P)	<0.001			
No. mammograms at age <40 y §				
0	471/396	Reference	412/401	Reference
1	53/57	0.8 (0.5-1.2)	52/55	1.2 (0.8-1.8)
≥2	106/74	1.1 (0.8-1.6)	131/77	2.1 (1.5-3.0)
P _{trend}		0.80		<0.0001
LRT ‡ (P)	0.12			
No. mammograms at age ≥40 y §				
0	166/116	Reference	120/112	Reference
1-2	91/73	1.2 (0.8-1.8)	70/108	0.8 (0.5-1.2)
3-5	133/130	0.9 (0.7-1.4)	112/16	1.4 (0.9-2.1)
6-10	119/114	0.9 (0.6-1.4)	135/103	1.9 (1.3-3.0)
≥11	128/96	1.1 (0.7-1.7)	168/99	2.4 (1.5-3.8)
P _{trend}		0.97		<0.0001
LRT ‡ (P)	<0.001			
No. mammograms at age ≥40 y (2-y lag) §				
0	225/150	Reference	178/140	Reference
1-2	124/106	1.0 (0.7-1.4)	93/131	0.7 (0.5-1.0)
3-5	105/107	0.8 (0.5-1.1)	106/99	1.1 (0.7-1.6)
6-10	86/94	0.7 (0.4-1.0)	103/97	1.0 (0.7-1.6)
≥11	97/72	0.9 (0.6-1.4)	125/71	1.7 (1.1-2.7)
P _{trend}		0.13		0.02
LRT ‡ (P)	0.004			

*Variant genotypes: *XRCC3* codon 241 any Met, *NBS1* codon 185 any Gln, *XRCC2* codon 188 any His, and *BRCA2* codon 372 any His.

† Adjusted for offsets, age, and race.

‡ LRT for interaction between exposure and number of variant genotypes.

§ Phase 2 (invasive and *in situ*) CBCS only.

of *BRCA2* codon 372 His/His homozygotes in women unaffected with cancer in North Europe and concluded that the His allele may decrease fetal survival. However, in the present study, we did not observe departures from Hardy-Weinberg equilibrium for *BRCA2* codon 372 genotypes in African-American controls ($P = 0.86$) or White controls ($P = 0.56$).

Several previous epidemiologic studies examined the association between DSB repair gene polymorphisms and breast cancer risk. Few examined interactions with environmental exposures, and none included exposure to ionizing radiation (28). Healey et al. (41) in northern Europe and Spurdle et al. (42) in Australia reported weak positive associations for the *BRCA2* codon 372 His/His genotype and breast cancer: ORs (95% CIs) were 1.3 (1.1-1.6) and 1.4 (1.0-2.0), respectively. Freedman et al. (52) found no association for *BRCA2* codon 372 genotype and breast cancer among U.S. women. For the *XRCC2* codon 188 His/His genotype and breast cancer, Kuschel et al. (29) reported an OR (95% CI) of 2.6 (1.0-6.7) in northern Europe and Rafii et al. (43) observed an OR (95% CI) of 2.1 (0.7-7.1) in the United Kingdom. In the Nurses' Health Study (United States), Han et al. (45) observed no overall association for *XRCC2* genotype and breast cancer (OR, 1.1; 95% CI, 0.9-1.4) but reported a positive association for the His allele in the presence of low plasma folate. In the same study population, the protective effect for high plasma β -carotene levels was abolished among *XRCC2* codon 188 His carriers (44). The *XRCC3* codon 241 Met allele was associated with increased risk of bladder cancer and malignant melanoma but not lung cancer (for review, see ref. 28). *XRCC3* codon 241 Met/Met genotype showed a weak positive association with breast cancer among women in northern Europe (OR, 1.3; 95% CI, 1.1-1.6; ref. 29), Finland (OR, 1.7; 95% CI, 0.9-3.2; ref. 40), Canada (OR, 1.4; 95% CI, 0.9-2.2; ref. 53), and the United States (North Carolina; OR, 1.5; 95% CI, 0.9-2.5; ref. 25). No associations were observed among women in Denmark (54) or the Nurses' Health Study (44, 45). *NBS1* codon 185 Gln/Gln was not associated with breast cancer in northern Europe (29) or Finland (40).

In the present study, ORs for DSB repair genotypes were close to the null in African-American and White women (Table 2). 95% CIs for each locus overlapped estimates from most previous studies. Taken together, these results suggest that DSB repair genotypes do not have a strong independent association with breast cancer risk. ORs were also close to the null for combinations of DSB repair genotypes (Table 3). Combining genotypes across loci that cooperate in a damage repair process represents an attempt to construct a pathway genotype (27). The approach has been used successfully to analyze polymorphisms in estrogen metabolism genes (55, 56). Combinations of polymorphisms in the nonhomologous end-joining arm of DSB repair (57) and other DNA repair genes (25) have been investigated, but there are no published reports for combinations of HRR gene variants and breast cancer risk.

Mohrenweiser et al. (27) hypothesized that pathway genotypes consisting of multiple DNA repair gene polymorphisms would increase power to detect the carcinogenic effects of ionizing radiation and other environmental exposures. The rationale for this approach is based on the fact that the components in DSB repair and other DNA repair processes act as part of multiprotein pathways, with considerable interactions between proteins (58, 59). We observed evidence for modification of ORs for ionizing radiation exposure and breast cancer by combinations of DSB repair genotypes (Table 4). Our results are compatible with mouse models showing that amino acid substitutions in DSB repair genes increase susceptibility to ionizing radiation (60). For number of lifetime mammograms, women with two to four variants in DSB repair genes showed a positive association between increasing exposure and breast cancer risk. These results support the hypothesis of Swift et al. (18, 19), who initially

proposed that mammograms and other medical sources of exposure to radiation could increase risk of breast cancer among women who are genetically susceptible. The results also support previous researchers who proposed that the DSB repair pathway contains a set of candidate genes for susceptibility to ionizing radiation (2, 6, 24, 25).

A major limitation of our study is the fact that radiation exposure was based on participant recall of specific medical procedures. We did not verify exposures using medical records, and we did not ask participants to distinguish between screening and diagnostic mammograms. Thus, among cases and controls, we could not separate mammograms conducted as part of routine screening in the absence of symptoms and mammograms done as part of a work-up for benign breast disease or other nonmalignant conditions. It is likely that most of the mammograms reported at age <40 years were conducted as part of the work-up or clinical follow-up for benign breast conditions, but we did not collect detailed information on the histology of such diagnoses. Because interviews were conducted after diagnosis of breast cancer in cases, we asked women to exclude mammograms conducted as part of the clinical work-up for breast cancer. In Table 4, cases listed as having no previous mammograms are a product of this exclusion. However, because we did not verify exposures using medical records, we did not reliably exclude all mammograms conducted after diagnosis in cases. To address the issue of diagnostic and postdiagnostic exposure in cases, we conducted analyses in which we excluded mammograms conducted within 2 years of enrollment in the CBCS for both cases and controls using information specifically collected for this purpose. Even after incorporating a 2-year lag, ORs remained elevated for number of mammograms in women with two to four variant DSB repair genotypes. Response rates were lower among controls than cases, and we do not have information on radiation exposure for women who could not be located or refused participation in the study. However, for bias to account for the results observed in Table 4, enrollment in the study, misclassification of the number of mammograms, and/or the presence of underlying breast conditions leading to receipt of mammograms would have to be related to DSB repair genotypes, which is unlikely. Among CBCS controls, there was no difference between participants with zero to one and two to four DSB repair variants for total number of mammograms ($P = 0.83$), mammograms at age <40 years ($P = 0.54$), or mammograms at age ≥ 40 years ($P = 0.66$).

There are several additional limitations to our study. To address genetic susceptibility to radiation exposure, we limited our analysis to four DSB repair genes. Based on previously determined criteria, we chose four genetic polymorphisms that were the most likely candidates for study. Even in a large study, such as the CBCS, power is limited to examine interactions between exposures with multiple levels (e.g., number of mammograms) and more than four genetic polymorphisms. DSB repair genes not measured in our study could contribute to susceptibility to ionizing radiation. Functional alleles may lie in linkage disequilibrium with the variants measured in our study, accounting for some or all of the observed effects for these four DSB genes. Freedman et al. (52) conducted a haplotype-based analysis of *BRCA2* and observed a high degree of linkage disequilibrium and several common haplotype blocks within the gene. The authors reported a positive association for a marker in intron 24 and breast cancer but not for codon 372 genotype. Healey et al. (41) investigated six variants in *BRCA2* and the only locus showing a positive association with breast cancer was codon 372 Asn/His. We chose variants based on potential to alter protein function and/or protein-protein interactions, but computer-based prediction programs and available functional data do not address interactions among DSB repair genes or interactions with

environmental exposures. To preserve adequate precision, we collapsed the number of variant genotypes into only two groups, zero to one and two to four variants. This method of categorization was also necessary to compare results in African Americans and Whites and to adjust for race in combined analyses. As shown in Table 3, the zero variants group is relatively small among Whites, and the three and four variants groups are small among African Americans.

Given the limitations of exposure assessment in the CBCS, it is difficult to estimate the amount of radiation received by study participants. Women who reported chest fluoroscopy or other coronary procedures probably received a radiation dose of ~9 mSv (4, 61, 62). Highly fractionated doses of radiation for treatment of tuberculosis can deliver doses of 10 mSv (1). Thus, the majority of women classified as having "History of radiation to the chest" probably received radiation doses in the range of 10 mSv per treatment. Few women reported radiation treatments for cancer, such as Hodgkin's disease, where radiation doses can exceed 40 Gy (2). The amount of radiation exposure from mammograms in the CBCS is difficult to estimate, given the wide calendar period of exposure reported by women in the study (~1950-2001). Currently, a single mammogram delivers ~3 mSv of radiation, approximately equal to the average yearly background dose from natural radiation exposure (4). Mammograms before the 1980s delivered higher doses of radiation, and repeated mammograms would deliver larger cumulative doses. However, the total cumulative dose of radiation from mammograms would still be lower than from repeated chest fluoroscopy or radiation treatments (61, 62). A single chest X-ray delivers ~0.01 mSv (63), considerably less radiation than a mammogram. The fact that we observed modification of ORs for mammograms by DSB repair genotypes, but not radiation treatments or chest X-rays, has at least one potential explanation. Misclassification of "History of radiation to the chest" due to lack of complete medical records, combined with the small number of women treated with high doses of radiation, could have reduced power to detect modification of ORs for this variable by DSB genotypes. The dose of radiation to the breast from chest X-rays may be too low to increase breast cancer risk even among individuals who are genetically susceptible. However, mammograms might represent a low-level radiation exposure that lies within the range where genetic susceptibility could contribute to increased risk of disease (5).

Based on a linear, no dose-threshold model, most experts assume that the excess breast cancer risk from yearly mammograms is minimal at the doses delivered by current X-ray machines (1, 2, 4, 62-64). However, a recombination mutagenesis mouse model (65) and studies of human cultured fibroblasts (66) suggest that linear extrapolation from high to low dose underestimates the extent of DNA damage resulting from exposure to low-dose ionizing radiation. The latter authors (66) provided evidence that cellular responses to radiation are not equally efficient at high and low doses. In particular, DSB repair may not be fully activated at X-ray doses of ≤ 1 mSv. Further research is needed on the role of DSB repair in response to low-dose radiation, particularly the range experienced by women from mammograms and other common medical procedures.

In conclusion, our results provide tentative support for a long-standing hypothesis that genetic factors modify the risk of breast cancer associated with exposure to low doses of ionizing radiation. Although we specifically designed our questionnaire to obtain information about exposures that preceded diagnosis in cases, we could not reliably exclude chest X-rays and mammograms conducted as part of a diagnostic work-up for breast cancer. In addition, we did not distinguish between screening mammograms and mammograms conducted as part of a work-up for other clinical breast conditions. Thus, our results are not directly relevant to ongoing discussions about

the risks and benefits of screening mammography (67-69). Given the preliminary nature of our findings, the DSB repair gene polymorphisms measured in the present study represent candidate genes for further study but are not appropriate for genetic testing. Future prospective epidemiologic studies incorporating polymorphisms in DSB repair genes, biomarkers of low-dose radiation exposure (65, 66), and radiation exposure validated through medical records could contribute to greater understanding of the mechanisms of action and dose-response relationships for ionizing radiation and human cancer.

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