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

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

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 (BRCH2) 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_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 hypothesized. 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 (G2 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
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 456 controls (70 African Americans and 386 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 also asked 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 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 DSBR 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 ≥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 μL reaction volumes. Reactions contained 0.7× 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 thermal cycler after the temperature had reached 50°C. Amplification was performed using the following conditions: 50°C for 2 minutes (AmpErase UNG Activation), 95°C for 10 minutes (AmpliTaq Gold Activation), and 40 cycles of 92°C for 15 seconds (denaturation) 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 DSBR repair loci of interest (http://snp500cancer.ncbi.nlm.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 χ² tests. Differences between allele or genotype frequencies in cases and controls were estimated using χ² 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 DSBR 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 DSBR 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 DSBR repair genes**

<table>
<thead>
<tr>
<th>Locus</th>
<th>SNP</th>
<th>Database reference sequence no.</th>
<th>Forward/reverse primers</th>
<th>Probe sequences,*</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRCC3</td>
<td>18067</td>
<td>rs861539</td>
<td>CCAGCACATGCTGACGTCCC/ACGCACACGGGCTCTGGA</td>
<td>Thr²⁴¹</td>
<td>62.0</td>
</tr>
<tr>
<td></td>
<td>C—T</td>
<td></td>
<td></td>
<td>VIC-TACGCCAGGCTGCCCC/</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thr²⁴⁴Met</td>
<td></td>
<td></td>
<td>Met²⁴¹(1)</td>
<td></td>
</tr>
<tr>
<td>NBS1</td>
<td>11122</td>
<td>rs1805794</td>
<td>TTAATTTTGGAGGCTGCTT/GAGCCTCAATGGAAGGCCAGAATA</td>
<td>Glu¹⁶⁸(1)</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>G—C</td>
<td></td>
<td></td>
<td>VIC-AGCATGTCAAGTCCAA/</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glu¹⁶⁸Gln</td>
<td></td>
<td></td>
<td>Glu¹⁶⁸(5)</td>
<td></td>
</tr>
<tr>
<td>XRCC2</td>
<td>31279</td>
<td>rs3218536</td>
<td>TTGCTCTAGTGTAGGAAGCTGT/</td>
<td>Arg¹⁸⁸(1)</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>A—C</td>
<td></td>
<td></td>
<td>VIC-ATGATCTATGGCTTGTT/</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A—C</td>
<td></td>
<td></td>
<td>Arg¹⁸⁸(5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asn²³²His</td>
<td></td>
<td></td>
<td>HIS¹⁷²(1)</td>
<td></td>
</tr>
<tr>
<td>BRCA2</td>
<td>1343</td>
<td>rs144848</td>
<td>ACGAATATGATAGCCTAATCGTTAG/</td>
<td>Asn²³²(5)</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>A—C</td>
<td></td>
<td></td>
<td>VIC-AAATGAGCAATCAGAAG/</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A—C</td>
<td></td>
<td></td>
<td>Asn²³²(5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asn²³²His</td>
<td></td>
<td></td>
<td>HIS¹⁷²(1)</td>
<td></td>
</tr>
</tbody>
</table>

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

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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 ≥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 breastfeeding). 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 appreciably across subgroups.

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...
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 Ps for the \( \beta \) coefficient in logistic regression models with exposure coded as an ordinal variable. All Ps 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 radiation to the chest** yielded highly imprecise ORs (data not shown), and the LRT was not statistically significant (\( P = 0.17 \)). 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 \( \geq 50 \) 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 domains.

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

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

*Adjusted for offsets and age.

³ Variant genotypes: XRCC3 codon 241 any Met, NBS1 codon 185 any Gin, XRCC2 codon 188 any His, and BRCA2 codon 372 any His.

Imprecise estimate.
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

<table>
<thead>
<tr>
<th>Exposure category</th>
<th>0-1 variant*</th>
<th>2-4 variants*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases/controls</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>History of radiation to chest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>943/834</td>
<td>1.0</td>
</tr>
<tr>
<td>Yes</td>
<td>78/69</td>
<td>1.0</td>
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<tr>
<td>LRT (P)</td>
<td>0.24</td>
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</tr>
<tr>
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<td>1.1</td>
</tr>
<tr>
<td>P&lt;0.001</td>
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<tr>
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<tr>
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<td>No. mammograms (2-y lag)</td>
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<td>172/113</td>
<td>0.8</td>
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<tr>
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<td>166/116</td>
<td>1.2</td>
</tr>
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</tr>
<tr>
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<td>225/150</td>
<td>1.0</td>
</tr>
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<td>105/107</td>
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<td>86/94</td>
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<td>97/72</td>
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<td>No. mammograms at age ≥40 y (2-y lag)</td>
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</table>

*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 un-
affected 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 environ-
mental 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 (1.0-1.6) and 1.2 (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
repair process that is susceptible 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 mammo-
grams conducted as part of routine screening in the absence of
symptoms and mammograms done as part of the 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
tackle 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 specifi-
cally collected for this purpose. Even after incorporating a
2-year lag, ORs remained elevated for number of mammo-
grams 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 mammo-
grams 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 poly-
morphisms. 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 pre-
diction programs and available functional data do not address
interactions among DSB repair genes or interactions with
the damage repair pathway contains a set of candidate genes for
susceptibility to ionizing radiation (2, 6, 24, 25).
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 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, 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).

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References

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

Robert C. Millikan, Jon S. Player, Allan Rene deCotret, et al.


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