*Null Results in Brief*

# Polymorphisms in *RAD51*, *XRCC2*, and *XRCC3* Are Not Related to Breast Cancer Risk

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## Introduction

Highly penetrant, but rare, mutations in genes involved in double-strand break repair (i.e., *BRCA1* and *BRCA2*) are associated with a risk for breast cancer of 40% to 65% by age 70 years (1, 2). Polymorphisms in other double-strand break repair genes are thought to contribute to the risk for the disease, either independently or through modifying the risk associated with rare mutations.

This study focuses on polymorphisms in three genes involved in the homologous recombination of double-strand breaks: *RAD51* 5′ untranslated region 135 G>C (rs1801320), X-ray repair cross-complementing group 2 (*XRCC2*) Arg$^{188}$His (rs3218536), and *XRCC3* Thr$^{241}$Met (rs861539) in relation to breast cancer risk in the New York University Women's Health Study cohort.

## Materials and Methods

The New York University Women's Health Study cohort collected questionnaires and blood samples from 14,274 healthy women ages 35 to 65 years in 1985 to 1991 (3). The current nested case-control study is matched for age and date at blood donation and includes incident cases of invasive breast cancer diagnosed before March 1998, with further methodologic details described by Shore et al. (4).

DNA was isolated using Qiagen QIAamp Blood Mini Kits (Qiagen, Inc.; ref. 4). Genotyping was done using PCR-RFLP methods described previously (ref. 4; see Appendix 1 for gene-specific PCR conditions and primer sequences). Blood clots and/or cell aggregates were available for 48% of the women. For the remaining women, serum specimens were used. Genotype results from clots/red cells and serum showed excellent concordance between repeated samples ($n = 73$) in pilot studies (97% for *RAD51* 135 G>C, 99% for *XRCC2* Arg$^{188}$His, and 98% for *XRCC3* Thr$^{241}$Met). Quality control duplicates showed 100% concordance for all three polymorphisms.

**Statistical Methods.** Deviation from Hardy-Weinberg equilibrium was assessed in controls using the $\chi^2$ goodness-of-fit test. The relationship between genotype and breast cancer risk was evaluated using conditional logistic regression and the additive coding model. The dominant model was also assessed for *RAD51* and *XRCC2* because of the small number of individuals with the homozygous variant genotype. Tests for interaction between genotype and ethnicity, family history, body mass index, and smoking were planned a priori.

Given our sample size (612 cases and 612 controls) and the allelic frequencies in our population, we had sufficient power (99% for *RAD51* 135 G>C, 99% for *XRCC2* Arg$^{188}$His, and 88% for *XRCC3* Thr$^{241}$Met) to detect associations of the magnitude observed by Kadouri et al. (5) for *RAD51* 135 G>C and Kuschel et al. (6) for *XRCC2* Arg$^{188}$His and *XRCC3* Thr$^{241}$Met.

## Results

Genotype frequencies did not deviate from Hardy-Weinberg equilibrium ($P > 0.5$). Variant allele frequencies were comparable with those previously reported for populations of Caucasians of European descent for *XRCC2* Arg$^{188}$His (8%; refs. 6-9) and *XRCC3* Thr$^{241}$Met (36%; refs. 8-14), but the variant allele frequency for *RAD51* 135 G>C of 9% was somewhat lower than previous reports (5, 6, 9).

Table 1 describes study subject characteristics. As expected, significant differences in body mass index and parity/age at first full-term pregnancy were observed between cases and controls. However, these variables were not associated with genotype. Ethnicity was significantly associated with breast cancer risk and genotype. Asian and Hispanic women had a lower

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risk for breast cancer than non-Jewish White women (odds ratio, 0.49; 95% confidence interval, 0.29-0.81); this association is as expected (15). Ethnicity was significantly related to genotype for *RAD51* GC/CC ($P < 0.0001$) and *XRCC3* CT/TT ($P < 0.0001$) genotypes. Among Black women, 37.4% had at least one copy of the *RAD51* 135 G>C variant allele (non-Jewish White, 15.9%; Jewish White, 9.6%; others, 17.3%). The *XRCC3* $Thr^{241}Met$ variant was most common (67.3%) among Jewish White women (non-Jewish White, 60.3%; Black, 38.4%; others, 40.8%). *XRCC2* $Arg^{188}His$ variant was not significantly related to ethnicity.

Unadjusted and ethnicity-adjusted odds ratios and 95% confidence intervals are presented in Table 2. Although ethnicity was found to be related to genotype and risk, adjusting for ethnicity altered the odds ratios only slightly. In this population, none of the polymorphisms was found to influence breast cancer risk. The sum of variant alleles was also not related to risk (data not shown). Similar results were obtained when the analysis was restricted to Caucasians (data not shown). No significant interaction was found between genotype and ethnicity, body mass index, smoking, parity, or family history.

## Discussion

Genetic instability acquired through inefficient double-strand break repair is believed to be a component of breast cancer susceptibility. RAD51 plays a central role in homologous recombination, through direct interaction with XRCC2, XRCC3, BRCA1, BRCA2, etc., to form a complex essential for the repair of double-strand breaks and DNA cross-links (especially XRCC2 and XRCC3) and for the maintenance of chromosome stability (16).

Studies have suggested that *RAD51* 135 G>C modifies the breast cancer risk of women with a family history of breast cancer (17, 18) or carriers of *BRCA2* mutations (5, 18-21). However, results have been inconsistent (22-24). Studies of non–*BRCA2* mutation carriers or women without a family history have found no association between *RAD51* 135 G>C and breast cancer risk (5, 6).

**Table 1. Characteristics of cases and controls**

| Variables | Cases ($n$ = 612) | Controls ($n$ = 612) | Odds ratio (95% confidence interval)* | $P$* |
|---|---|---|---|---|
| Age at diagnosis (y) | | | | |
| Median (25th, 75th percentile) | 60.3 (51.8, 66.6) | 60.3 (51.8, 66.6) | Matched | |
| Body mass index (kg/m$^2$)[†,‡] | | | | |
| Median (25th, 75th percentile) | | | | |
| Age ≤52 y | 22.8 (20.9, 25.4) | 23.1 (21.4, 25.0) | 0.56 (0.11-2.75) | 0.47 |
| Age >52 y | 25.2 (22.5, 28.4) | 24.2 (22.0, 27.6) | 2.20 (1.00-4.82) | 0.05 |
| Height (cm) | | | | |
| Median (25th, 75th percentile) | 163 (157, 168) | 163 (157, 168) | 1.00 (0.99-1.02) | 0.72 |
| Ethnicity, $n$ (%) | | | | |
| Caucasian | | | | |
| Non-Jewish | 222 (39.7) | 202 (36.9) | 1.00 | 0.02 |
| Jewish | 254 (45.4) | 232 (42.4) | 1.02 (0.77-1.35) | |
| Black | 50 (8.9) | 59 (10.8) | 0.77 (0.49-1.21) | |
| Others (including Hispanic and Asian) | 33 (5.9) | 54 (9.9) | 0.49 (0.29-0.81) | |
| Unknown | 53 | 65 | | |
| Family history, $n$ (%) | | | | |
| None | 468 (76.5) | 475 (77.6) | 1.00 | 0.31[§] |
| 1 affected relative, >45 y | 76 (12.4) | 85 (13.9) | 0.91 (0.66-1.27) | |
| 1 affected relative, age unknown | 15 (2.5) | 12 (2.0) | 1.24 (0.58-2.65) | |
| >1 affected relative or 1 age <45 y | 53 (8.7) | 40 (6.5) | 1.33 (0.87-2.04) | |
| Age at menarche (y), $n$ (%) | | | | |
| <13 | 309 (50.5) | 286 (46.7) | 1.00 | |
| ≥13 | 303 (49.5) | 326 (53.3) | 0.87 (0.70-1.08) | 0.20 |
| Number of pregnancies, $n$ (%) | | | | |
| Nulliparous | 201 (37.2) | 180 (32.1) | 1.00 | 0.19[§] |
| 1 | 62 (11.5) | 81 (14.5) | 0.73 (0.48-1.10) | |
| 2 | 153 (28.3) | 173 (30.9) | 0.77 (0.56-1.06) | |
| ≥3 | 125 (23.1) | 126 (22.5) | 0.82 (0.58-1.15) | |
| Unknown | 71 | 52 | | |
| Age at first term pregnancy (y), $n$ (%) | | | | |
| <25 | 142 (23.2) | 183 (29.9) | 1.00 | 0.0002[§] |
| 25-29 | 168 (27.5) | 180 (29.4) | 1.21 (0.88-1.66) | |
| Nulliparous | 201 (32.8) | 180 (29.4) | 1.47 (1.08-1.99) | |
| >30 | 101 (16.5) | 69 (11.3) | 1.96 (1.32-2.89) | |
| Smoking status, $n$ (%) | | | | |
| Never | 253 (47.7) | 253 (48.8) | 1.00 | 0.35 |
| Ever | 278 (52.4) | 265 (51.2) | 0.99 (0.76-1.29) | |
| Unknown | 81 | 94 | | |

*Odds ratios and $P$ values are for conditional univariate regression analysis.
† Using ln of body mass index (at baseline) as a continuous variable.
‡ A division at the age of 52 y was decided upon a priori as a surrogate for menopausal status.
§ $P$ for trend using ordered categories shown in this table.

**Table 2. DNA repair polymorphisms and breast cancer risk**

| Genotype* | Cases | Controls | Unadjusted | | Ethnicity adjusted | |
|---|---|---|---|---|---|---|
| | *n* (%) | *n* (%) | Odds ratio (95% confidence interval) | *P* | Odds ratio (95% confidence interval) | *P* |
| Rad51 (*n* = 1,222) † | | | | | | |
| GG | 516 (84.5) | 513 (84.0) | 1.00 | 0.67 ‡ | 1.00 | 0.91 ‡ |
| GC | 88 (14.4) | 88 (14.4) | 0.99 (0.72-1.36) | | 1.05 (0.76-1.45) | |
| CC | 7 (1.1) | 10 (1.6) | 0.67 (0.24-1.87) | | 0.68 (0.24-1.94) | |
| GG vs GC/CC | | | 1.04 (0.76-1.41) | 0.82 | 1.02 (0.74-1.39) | 0.92 |
| XRCC2 (*n* = 1,204) † | | | | | | |
| GG | 515 (85.5) | 519 (86.2) | 1.00 | 0.82 ‡ | 1.00 | 0.77 ‡ |
| GA | 83 (13.8) | 78 (13.0) | 1.07 (0.77-1.50) | | 1.08 (0.77-1.52) | |
| AA | 4 (0.7) | 5 (0.8) | 0.81 (0.22-3.01) | | 0.83 (0.22-3.12) | |
| GG vs GA/AA | | | 1.06 (0.76-1.47) | 0.74 | 1.07 (0.77-1.48) | 0.71 |
| XRCC3 (*n* = 1,222) † | | | | | | |
| CC | 254 (41.6) | 249 (40.8) | 1.00 | 0.47 ‡ | 1.00 | 0.77 ‡ |
| CT | 259 (42.4) | 286 (46.8) | 0.88 (0.68-1.13) | | 0.83 (0.64-1.08) | |
| TT | 98 (16.0) | 76 (12.4) | 1.28 (0.89-1.83) | | 1.20 (0.83-1.72) | |

*Using the $\chi^2$ test, no significant difference in genotype frequencies was observed between cases and controls.
† Matched pairs were excluded if either member of the pair could not be definitively genotyped.
‡ *P* for trend.

Results for *XRCC2* Arg$^{188}$His have been similarly mixed (6-8, 23). It is thought that this polymorphism has only a small effect on gene activity (7), although it may modify risk in those with low levels of plasma α-carotene (25) or plasma folate (26).

*XRCC3* Thr$^{241}$Met has been found to be associated with increased DNA adducts (27), chromosomal deletions (28), and sensitivity to ionizing radiation and cross-linking agents (29, 30). Some (6, 17, 31) but not all (10, 23, 25, 32, 33) studies have found *XRCC3* Thr$^{241}$Met to be related to an increased risk for breast cancer. Pooled analyses and meta-analyses show a small but significant increase in risk (8, 14, 22, 34).

Disruption of double-strand break repair is thought to contribute to carcinogenesis through the accumulation of genetic errors and genetic instability (35). However, in this study, the *RAD51*, *XRCC2*, and *XRCC3* variants were found not to be associated with breast cancer risk. Unlike other reports, no relationship was found between *RAD51* 135 G>C and family history of breast cancer, perhaps because the participants in the study were not selected for having a family history of disease or being *BRCA1/2* mutation carriers.

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**Appendix A. Gene-Specific PCR Conditions and Primer Sequences**

| | | |
|---|---|---|
| Cycling conditions | | |
| Rad51 | 95°C for 5 min | |
| | 95°C for 30 s<br>65°C for 30 s<br>72°C for 60 s | 33 cycles for red cells/clots, 37 cycles for serum |
| | 72°C for 7 min | |
| XRCC2/XRCC3 | 95°C for 5 min | |
| | 95°C for 30 s<br>65°C for 30 s<br>72°C for 60 s | 30 cycles for red cells<br>32 cycles for clots<br>37 cycles for serum |
| | 72°C for 7 min | |
| Primer pairs and restriction enzymes | | |
| Rad51 | TGGGAACTGCAACTCATCTGG<br>GCGCTCCTCTCTCCAGCAG<br>BstN1 (60°C for 2 h) | |
| XRCC2 | GATTTTGGATAGCCTGTCA<br>AGAATCATCTTGTTTGGAG<br>SexA1 (37°C for 3 h) | |
| XRCC3 | ATGGCTCGCCTGGTGGTCA<br>CATCCTGGCTAAAAATACG NlaIII<br>(37°C for 2 h) | |

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