

Null Results in Brief

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

Jennifer Brooks,¹ Roy E. Shore,^{1,2} Anne Zeleniuch-Jacquotte,^{1,2} Diane Currie,³
Yelena Afanasyeva,¹ Karen L. Koenig,^{1,2} Alan A. Arslan,^{2,4}
Paolo Toniolo,^{2,4} and Isaac Wirgin^{2,3}

¹Division of Epidemiology, Department of Environmental Medicine; ²New York University Cancer Institute; ³Molecular Toxicology and Carcinogenesis Program, Department of Environmental Medicine; and ⁴Division of Epidemiology, Department of Obstetrics and Gynecology, New York University School of Medicine, New York, New York

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*) Arg¹⁸⁸His (rs3218536), and *XRCC3* Thr²⁴¹Met (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* Arg¹⁸⁸His, and 98% for *XRCC3* Thr²⁴¹Met). Quality control duplicates showed 100% concordance for all three polymorphisms.

Statistical Methods. Deviation from Hardy-Weinberg equilibrium was assessed in controls using the χ^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* Arg¹⁸⁸His, and 88% for *XRCC3* Thr²⁴¹Met) to detect associations of the magnitude observed by Kadouri et al. (5) for *RAD51* 135 G>C and Kuschel et al. (6) for *XRCC2* Arg¹⁸⁸His and *XRCC3* Thr²⁴¹Met.

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* Arg¹⁸⁸His (8%; refs. 6-9) and *XRCC3* Thr²⁴¹Met (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

Cancer Epidemiol Biomarkers Prev 2008;17(4):1016-9

Received 1/22/08; accepted 2/6/08.

Grant support: National Cancer Institute Center grant CA 16087 and National Cancer Institute grant CA091892, Department of Defense grant DAMD17-01-1-0578, and Komen Foundation grant BCTR 2000 685.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Anne Zeleniuch-Jacquotte, New York University School of Medicine, 650 First Avenue, 5th Floor, New York, NY 10016-3240. Phone: 212-263-6512; Fax: 212-263-8570. E-mail: anne.jacquotte@med.nyu.edu

Copyright © 2008 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-08-0065

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* Thr²⁴¹Met variant was most common (67.3%) among Jewish White women (non-Jewish White, 60.3%; Black, 38.4%; others, 40.8%). *XRCC2* Arg¹⁸⁸His 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) | | | Matched | |
| Median (25th, 75th percentile) | 60.3 (51.8, 66.6) | 60.3 (51.8, 66.6) | | |
| 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 | | | 1.00 | 0.02 |
| Non-Jewish | 222 (39.7) | 202 (36.9) | | |
| 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 n (%) | Controls n (%) | Unadjusted | | Ethnicity adjusted | |
|--------------------------------|----------------|-------------------|--------------------------------------|-------------------|--------------------------------------|-------------------|
| | | | 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 χ^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 Arg¹⁸⁸His 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 Thr²⁴¹Met 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 Thr²⁴¹Met 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.

References

- Begg CB, Haile RW, Borg A, et al. Variation of breast cancer risk among *BRCA1/2* carriers. *JAMA* 2008;299:194-201.
- Antoniou A, Pharoah P, Narod S, et al. Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2* mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet* 2003;72:1117-30.
- Zeleniuch-Jacquotte A, Shore R, Koenig K, et al. Postmenopausal levels of oestrogen, androgen, and SHBG and breast cancer: long-term results of a prospective study. *Br J Cancer* 2004;90:153-9.
- Shore R, Zeleniuch-Jacquotte A, Currie D, et al. Polymorphisms in *XPC* and *ERCC2* genes, smoking and breast cancer risk. *Int J Cancer* 2008;122:2101-5.
- Kadoury L, Kote-Jarai Z, Hubert A, et al. A single-nucleotide polymorphism in the *RAD51* gene modifies breast cancer risk in

Appendix A. Gene-Specific PCR Conditions and Primer Sequences

| | | |
|--------------------------------------|---|--|
| Cycling conditions | | |
| Rad51 | 95°C for 5 min 95°C for 30 s 65°C for 30 s 72°C for 60 s 72°C for 7 min | 33 cycles for red cells/clots, 37 cycles for serum |
| XRCC2/XRCC3 | 95°C for 5 min 95°C for 30 s 65°C for 30 s 72°C for 60 s 72°C for 7 min | |
| Primer pairs and restriction enzymes | | |
| Rad51 | TGGGAAGTCAACTCATCTGG GCGCTCTCTCAGCAG BstNI (60°C for 2 h) | |
| XRCC2 | GATTTGGATAGCCTGTCA AGAATCATCTTGTGGAG SexA1 (37°C for 3 h) | |
| XRCC3 | ATGGCTCGCCTGGTGGTCA CATCCTGGCTAAAAATACG NlaIII (37°C for 2 h) | |

- BRCA2 carriers, but not in BRCA1 carriers or noncarriers. *Br J Cancer* 2004;90:2002–5.
6. Kuschel B, Auranen A, McBride S, et al. Variants in DNA double-strand break repair genes and breast cancer susceptibility. *Hum Mol Genet* 2002;11:1399–407.
 7. Rafii S, O'Regan P, Xinarianos G, et al. A potential role for the XRCC2 R188H polymorphic site in DNA-damage repair and breast cancer. *Hum Mol Genet* 2002;11:1433–8.
 8. Garcia-Closas M, Egan KM, Newcomb PA, et al. Polymorphisms in DNA double-strand break repair genes and risk of breast cancer: two population-based studies in USA and Poland, and meta-analyses. *Hum Genet* 2006;119:376–88.
 9. Packer B, Yeager M, Burdett L, et al. SNP500Cancer: a public resource for sequence validation, assay development, and frequency analysis for genetic variation in candidate genes. *Nucleic Acids Res* 2006;34:D617–21.
 10. Manuguerra M, Saletta F, Karagas MR, et al. XRCC3 and XPD/ERCC2 single nucleotide polymorphisms and the risk of cancer: a HuGE review. *Am J Epidemiol* 2006;164:297–302.
 11. Smith TR, Miller MS, Lohman K, et al. Polymorphisms of XRCC1 and XRCC3 genes and susceptibility to breast cancer. *Cancer Lett* 2003b;190:183–90.
 12. Jacobsen NR, Nexø BA, Olsen A, et al. No Association between the DNA repair gene XRCC3 T241M polymorphism and risk of skin cancer and breast cancer. *Cancer Epidemiol Biomarkers Prev* 2003;12:584–5.
 13. Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002;11:1513–30.
 14. Han S, Zhang H-T, Wang Z, et al. DNA repair gene XRCC3 polymorphisms and cancer risk: a meta-analysis of 48 case-control studies. *Eur J Hum Genet* 2006;14:1136–44.
 15. Ries L, Melbert D, Krapcho M, et al., editors. SEER cancer statistics review, 1975–2004. Bethesda, MD: National Cancer Institute; 2006.
 16. Thacker J. The RAD51 gene family, genetic instability and cancer. *Cancer Lett* 2005;219:125–35.
 17. Costa S, Pinto D, Pereira D, et al. DNA repair polymorphisms might contribute differentially on familial and sporadic breast cancer susceptibility: a study on a Portuguese population. *Breast Cancer Res Treat* 2007;103:209–17.
 18. Jara L, Acevedo ML, Blanco R, et al. RAD51 135G>C polymorphism and risk of familial breast cancer in a South American population. *Cancer Genet Cytogenet* 2007;178:65–9.
 19. Levy-Lahad E, Lahad A, Eisenberg S, et al. A single nucleotide polymorphism in the RAD51 gene modifies cancer risk in BRCA2 but not BRCA1 carriers. *Proc Natl Acad Sci U S A* 2001;98:3232–6.
 20. Wang WW, Spurdle AB, Kolachana P, et al. A single nucleotide polymorphism in the 5' untranslated region of RAD51 and risk of cancer among BRCA1/2 mutation carriers. *Cancer Epidemiol Biomarkers Prev* 2001;10:955–60.
 21. Antoniou A, Similnikova O, Simard J, et al. RAD51 135G—C modifies breast cancer risk among BRCA2 mutation carriers: results from a combined analysis of 19 studies. *Am J Hum Genet* 2007;81:1186–200.
 22. Lee K-M, Choi J-Y, Kang C, et al. Genetic polymorphisms of selected DNA repair genes, estrogen and progesterone receptor status, and breast cancer risk. *Clin Cancer Res* 2005;11:4620–6.
 23. Webb PM, Hopper JL, Newman B, et al. Double-strand break repair gene polymorphisms and risk of breast or ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:319–23.
 24. Jakubowska A, Gronwald J, Menkiszak J, et al. The RAD51 135 G>C polymorphism modifies breast cancer and ovarian cancer risk in Polish BRCA1 mutation carriers. *Cancer Epidemiol Biomarkers Prev* 2007;16:270–5.
 25. Han J, Hankinson SE, Ranu H, et al. Polymorphisms in DNA double-strand break repair genes and breast cancer risk in the Nurses' Health Study. *Carcinogenesis* 2004;25:189–95.
 26. Han J, Hankinson SE, Zhang SM, et al. Interaction between genetic variations in DNA repair genes and plasma folate on breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2004;13:520–4.
 27. Matullo G, Palli D, Peluso M, et al. XRCC1, XRCC3, XPD gene polymorphisms, smoking and 32P-DNA adducts in a sample of healthy subjects. *Carcinogenesis* 2001;22:1437–45.
 28. Au W, Salama S, Sierra-Torres C. Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. *Environ Health Perspect* 2003;111:1843–50.
 29. Fuller L, Painter R. A Chinese hamster ovary cell line hypersensitive to ionizing radiation and deficient in repair replication. *Mutat Res* 1988;193:109–21.
 30. Caldecott K, Jeggo P. Cross-sensitivity of gamma-ray-sensitive hamster mutants to cross-linking agents. *Mutat Res* 1991;255:111–21.
 31. Sangrajarang S, Schmezer P, Burkholder I, et al. The XRCC3 Thr241Met polymorphism and breast cancer risk: a case control study in a Thai population. *Biomarkers* 2007;12:523–32.
 32. Smith TR, Levine EA, Perrier ND, et al. DNA-repair genetic polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2003a;12:1200–4.
 33. Thyagarajan B, Anderson KE, Folsom AR, et al. No association between XRCC1 and XRCC3 gene polymorphisms and breast cancer risk: Iowa Women's Health Study. *Cancer Detect Prev* 2006;30:313–21.
 34. The Breast Cancer Association Consortium. Commonly studied single-nucleotide polymorphisms and breast cancer: results from the Breast Cancer Association Consortium. *J Natl Cancer Inst* 2006;98:1382–96.
 35. Reliene R, Bishop AJR, Schiestl RH, et al. Involvement of homologous recombination in carcinogenesis. *Adv Genet* 2007;58:67–87.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association
for Cancer Research

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

Jennifer Brooks, Roy E. Shore, Anne Zeleniuch-Jacquotte, et al.

Cancer Epidemiol Biomarkers Prev 2008;17:1016-1019.

Updated version Access the most recent version of this article at:
<http://cebp.aacrjournals.org/content/17/4/1016>

Cited articles This article cites 33 articles, 9 of which you can access for free at:
<http://cebp.aacrjournals.org/content/17/4/1016.full#ref-list-1>

Citing articles This article has been cited by 1 HighWire-hosted articles. Access the articles at:
<http://cebp.aacrjournals.org/content/17/4/1016.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cebp.aacrjournals.org/content/17/4/1016>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.