Double-Strand Break Repair Gene Polymorphisms and Risk of Breast or Ovarian Cancer

Penelope M. Webb, 1 John L. Hopper, 4 Beth Newman, 3 Xiaqing Chen, 2 Livia Kelemen, 2 Graham G. Giles, 5 Melissa C. Southey, 4 Georgia Chenexvix-Trench, 2 and Amanda B. Spurdle 2

1Population and Clinical Sciences Division and 1Cancer and Cell Biology Division, Queensland Institute of Medical Research and University of Queensland; 2School of Public Health, Queensland University of Technology, Brisbane, Queensland, Australia; Centre for Genetic Epidemiology, University of Melbourne; and 2Cancer Epidemiology Centre, Council of Victoria, Carlton, Victoria, Australia

Abstract

Deficiencies in DNA repair have been hypothesized to increase cancer risk and excess cancer incidence is a feature of inherited diseases caused by defects in DNA damage recognition and repair. We investigated, using a case-control design, whether the double-strand break repair gene polymorphisms RAD51 5′ untranslated region –135 G > C, XRCC2 R188H G > A, and XRCC3 T241M C > T were associated with risk of breast or ovarian cancer in Australian women. Sample sets included 1,456 breast cancer cases and 793 age-matched controls ages under 60 years of age, 549 incident ovarian cancer cases, and 335 controls of similar age distribution. For the total sample and the subsample of Caucasian women, there were no significant differences in genotype distribution between breast cancer cases and controls or between ovarian cancer cases and combined control groups. The crude odds ratios (OR) and 95% confidence intervals (95% CI) associated with the RAD51 GC/CC genotype frequency was OR, 1.10; 95% CI, 0.80-1.41 for breast cancer and OR, 1.22; 95% CI, 0.92-1.62 for ovarian cancer. Similarly, there were no increased risks associated with the XRCC2 GA/AA genotype (OR, 0.98; 95% CI, 0.76-1.26 for breast cancer and OR, 0.93; 95% CI, 0.69-1.25 for ovarian cancer) or the XRCC3 CT/TT genotype (OR, 0.92; 95% CI, 0.77-1.10 for breast cancer and OR, 0.87; 95% CI, 0.71-1.08 for ovarian cancer). Results were little changed after adjustment for age and other measured risk factors. Although there was little statistical power to detect modest increases in risk for the homozygote variant genotypes, particularly for the rare RAD51 and XRCC2 variants, the data suggest that none of these variants play a major role in the etiology of breast or ovarian cancer. (Cancer Epidemiol Biomarkers Prev 2005;14(2):319–23)

Introduction

Deficiencies in the DNA repair process have been hypothesized to increase cancer risk. Excess cancer incidence is a feature of inherited disease caused by defects in DNA damage recognition and repair, including mutations of the BRCA1, BRCA2, ATM, and Chek2 genes (1-5). Epidemiologic evidence suggests that double-strand DNA damage may contribute to the etiology of breast cancer in that ionizing radiation, which primarily causes double-strand DNA breaks, increases risk of breast cancer (6-8). Furthermore, in vitro studies have shown reduced double-strand break repair (DSBR) proficiency after radiation-induced damage in breast cancer patients both with and without a reported family history of breast cancer (9). It has been proposed that common variants in genes of the DSBR pathways that potentially result in altered protein expression or function may confer a modest increase in breast cancer susceptibility. Ionizing radiation has also been associated with increased incidence of ovarian cancer among survivors of atomic bomb explosions (10) but the association with therapeutic radiation is inconsistent (11-13).

To date, the largest population-based case-control study assessing the role of DSBR gene variants in breast cancer susceptibility investigated 15 variants in seven DSBR genes, in up to 2,200 cases and controls (14). This study reported a significant increased risk associated with the rare homozygote genotype for two amino acid substitution polymorphisms, the XRC2 R188H G > A AA genotype [odds ratio (OR), 2.6; 95% confidence interval (95% CI), 1.0-6.7] and the XRCC3 T241M C > T TT genotype (OR, 1.3; 95% CI, 1.1-1.6). Although there was no statistically significant increase in risk associated with the RAD51 5′ untranslated region –135 G > C polymorphism, the point estimate was increased for the rare homozygote (OR, 2.5; 95% CI, 0.6-10.9).

Using a case-control design, we assessed whether these three polymorphisms (RAD51 5′ untranslated region –135 G > C, XRCC2 R188H G > A, XRCC3 T241M C > T) were associated with risk of breast or ovarian cancer in Australian women.

Subjects and Methods

Approval for this study was obtained from the ethics committees of The University of Melbourne, the New South Wales Cancer Council, The Cancer Council Victoria, and the Queensland Institute of Medical Research. Written informed consent was obtained from all participants.

Details of the methods of enrollment, study conduct, collection of peripheral blood, and DNA extraction have been published previously (15-19) and a brief description is given below.

Breast cancer cases (n = 1,456) and controls (n = 793) were from a population-based case-control-family study, the Australian Breast Cancer Family Study, conducted in 1992 to 2000 (15). Cases comprised women aged ≥60 years living in Sydney or Melbourne who were diagnosed with a first primary invasive breast cancer; controls were a randomly selected population-based sample of unaffected women recruited using the electoral rolls, frequency matched to the cases by age. Interviews...
were conducted for 1,579 (69%) of 2,304 eligible cases and 1,021 (67%) of 1,531 eligible controls, and DNA was available for 1,456 (92%) of participating cases and 1,205 (96%) of controls. Genotyping success rates methodology as previously described (16). Details of primers Detection System (Applied Biosystems, Foster City, CA) frequencies were compared across groups using the 

95% CI were calculated using unconditional logistical regress-

sion, with and without adjustment for measured risk factors. OR and

were no variation in genotype frequencies by reported ancestry and/

among the subset of individuals reporting Caucasian ancestry

and Australian country of birth (P = 0.2). Given apparent differences in genotype frequencies by reported ancestry and/or country of birth and nonhomogeneity of the relatively small samples of individuals randomly grouped as reporting birth outside Australia or non-Caucasian ethnicity, a term for

country of birth was included in all adjusted models and analyses were carried out including all women and also women reporting Caucasian ethnicity.

**Breast Cancer Risk.** There were no differences in genotype frequencies between breast cancer cases and controls for RAD51 (P = 0.5), XRCC2 (P = 0.8), or XRCC3 (P = 0.6). There were no significant associations between genotypes and breast cancer risk (Table 1). Estimates were little changed (<3%) by the exclusion of BRCA1 or BRCA2 mutation carriers, and there were no significant differences in genotype distribution between cases and controls for subgroups defined by age at onset (<40 or ≥40 years), or reported family history (all

P > 0.5). Analysis of the effects of combined chest X-ray/radiation exposure (ever/never) or smoking exposure (ever/never or pack-years) provided no consistent evidence that these environmental exposures modified the effect of the RAD51, XRCC2, or XRCC3 genotypes (data not shown). In addition, the combined effect of the three genetic variants was assessed, and based on the individual estimates RAD51 GC/CC, XRCC2 GA/AA, and XRCC3 TT were considered to be the likely at-risk genotypes. In the Caucasian group, a single control (0.2%) and three cases (0.3%) carried all three risk genotypes; moreover, there was no difference between cases and controls in the distribution of the different combinations of the three risk genotypes (P = 0.8) or in the total number of high-risk genotypes (0, 1, or 2-3) carried (P\text{trend} = 0.5).

**Ovarian Cancer Risk.** Overall, there were no significant differences between the distributions of RAD51, XRCC2, or XRCC3 genotypes between ovarian cancer cases and the combined group of breast cancer and ovarian cancer controls (P = 0.3, 0.9, and 0.3, respectively), and no significant association with risk (Table 2). Further adjustment for oral contraceptive use and parity among the subgroup of women with this information reduced the OR by 5% to 10% (results not shown). There was no statistically significant variation in genotype frequency across the different histologic subtypes of ovarian cancer or by tumor stage or grade (data not shown). There was also no variation in XRCC3 or RAD51 between invasive and borderline tumors; however, the XRCC2 GA/AA genotype was significantly more common among women with
invasive cancer (16 versus 4%, \(P = 0.002\)). As a result, there was no association between the XRCC2 GA/AA genotype and risk of invasive cancer (OR, 1.09; 95% CI, 0.81-1.48) but a statistically significant inverse association was seen for borderline tumors (OR, 0.26; 95% CI, 0.09-0.71). A similar pattern was seen for both serous (13% versus 6%) and borderline tumors (OR, 0.26; 95% CI, 0.09-0.71). A similar statistically significant inverse association was seen for ovarian cancer (OR, 1.09; 95% CI, 0.81-1.48) but a no association between the XRCC2 GA/AA genotypes.

### Discussion

Our data provide no evidence to support an increase in risk of either breast cancer or ovarian cancer associated with the Rad51 –135 G > C, XRCC2 R188H G > A or XRCC3 T241M C > T polymorphisms. Risk estimates for breast or ovarian cancer were either close to unity, below unity, or driven by the heterozygote genotype. While our analysis of risk of breast cancer might be considered translatable to only women ages <60, there was no evidence for differences in allele or genotype frequency for different age groups or between our control group and those from other studies, suggesting it is also applicable to older women.

The nonsignificant increased risk for breast cancer associated with the RAD51 C variant was driven by the heterozygote genotype, with ORs below unity for the rare homozygous variant. Despite the same allele frequency in controls, this pattern contrasts with the 0.8-fold and 2.5-fold breast cancer risks reported by Kuschel et al. (14) for the GC and CC genotypes, respectively. The RAD51 C variant has not been investigated in any other large case-control studies although it has been reported to modulate breast cancer risk among BRCA1/2 mutation carriers (20-22). The relative risk estimates in our study and that by Kuschel et al. (14) for the GC and CC genotypes, respectively. The RAD51 C variant has not been investigated in any other large case-control studies although it has been reported to modulate breast cancer risk among BRCA1/2 mutation carriers (20-22). The relative risk estimates in our study and that by Kuschel et al. (14) for the GC and CC genotypes, respectively. The RAD51 C variant has not been investigated in any other large case-control studies although it has been reported to modulate breast cancer risk among BRCA1/2 mutation carriers (20-22). The relative risk estimates in our study and that by Kuschel et al. (14) for the GC and CC genotypes, respectively. The RAD51 C variant has not been investigated in any other large case-control studies although it has been reported to modulate breast cancer risk among BRCA1/2 mutation carriers (20-22). The relative risk estimates in our study and that by Kuschel et al. (14) for the GC and CC genotypes, respectively. The RAD51 C variant has not been investigated in any other large case-control studies although it has been reported to modulate breast cancer risk among BRCA1/2 mutation carriers (20-22). The relative risk estimates in our study and that by Kuschel et al. (14) for the GC and CC genotypes, respectively. The RAD51 C variant has not been investigated in any other large case-control studies although it has been reported to modulate breast cancer risk among BRCA1/2 mutation carriers (20-22). The relative risk estimates in our study and that by Kuschel et al. (14) for the GC and CC genotypes, respectively. The RAD51 C variant has not been investigated in any other large case-control studies although it has been reported to modulate breast cancer risk among BRCA1/2 mutation carriers (20-22). The relative risk estimates in our study and that by Kuschel et al. (14) for the GC and CC genotypes, respectively.

Several other association studies have reported attempts to replicate the XRCC2 findings of Kuschel et al. (14). A British case-control study (521 cases/895 controls) supported an
association with increased breast cancer risk (GA: OR, 1.5; 95% CI, 1.0-2.2; AA: OR, 2.4; 95% CI, 0.5-12.2; ref. 23) and provided suggestive evidence that the risk might be greatest among young women with a family history. Our Australian study, which oversampled women aged <40 years and had detailed family history information on cases and controls, found no evidence of this. A nested case-control study from the Harvard Nurses’ Health Study (1,004 cases/1,385 controls; ref. 24) like us found no evidence for an association with the XRCC2 polymorphism (GA: OR, 1.1; 95% CI, 0.8-1.4; AA: OR, 1.3; 95% CI, 0.4-4.2). The frequency of the rare allele differed little between control groups of the four studies (0.06-0.08), and a combined analysis of the crude results from all studies (4,494 cases, 4,104 controls) suggests that there is no increased risk associated with the GA genotype (OR, 1.02; 95% CI, 0.90-1.15) but that an association with the rare AA genotype cannot be excluded (OR, 1.65; 95% CI, 0.95-2.86). However, even assuming a causal relationship, the population-attributable risk for this rare variant would be minor, accounting for <0.4% of all breast cancers.

Our study also found no evidence of an increased cancer risk associated with the XRCC3 T241M C → T polymorphism, although the upper confidence limit of 1.4 for our estimate of breast cancer risk for the TT genotype was similar to the 1.3-fold risk reported by Kuschel et al. (14). Three of five subsequent reports support our findings, reporting OR (95% CI) of 0.9 (0.7-1.1) and 1.0 (0.8-1.3) for the Nurses Health study nested case-control study (24), 0.9 (0.5-1.4) and 1.1 (0.5-2.3) for a relatively small U.S. hospital-based case-control study (25), and 1.0 (0.8-1.4) and 0.9 (0.6-1.4) for a Danish nested case-control study (26). Two other studies have, however, reported an increased risk for TT homozygotes with OR (95% CI) of 0.9 (0.6-1.3) and 1.4 (0.8-2.4) for a hospital-based case-control study (27) and 0.9 (0.7-1.3) and 1.4 (0.9-2.1) for a Canadian population-based study (28). The frequency of the rare allele in control groups ranged from 0.36 to 0.41, and a combined analysis of the crude results from all seven studies (5,584 cases, 5,012 controls) gave OR (95% CI) of 1.00 (0.92-1.09) and 1.0 (0.8-1.4) and 0.9 (0.6-1.4) for a hospital-based case-control study (27) and 0.9 (0.7-1.3) and 1.4 (0.9-2.1) for a Canadian population-based study (28). The frequency of the rare allele in control groups ranged from 0.36 to 0.41, and a combined analysis of the crude results from all seven studies (5,584 cases, 5,012 controls) gave OR (95% CI) of 1.00 (0.92-1.09) and 1.14 (1.01-1.28) for the CT and TT genotypes, respectively. This suggests that the XRCC3 homozygote variant genotype may be associated with a very modest increased risk of breast cancer, which, if true, would account for ~1.9% of all breast cancers.

The results from the ovarian cancer case-control comparisons largely mirrored those seen for breast cancer. We observed at most a nonsignificant 1.2-fold increase in ovarian cancer risk associated with the RAD51 C variant under

### Table 2. Odds ratios for ovarian cancer by genotype according to different models of inheritance

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Genotype</th>
<th>Cases, n (%)</th>
<th>Controls*, n (%)</th>
<th>Crude OR</th>
<th>Adjusted OR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAD51</td>
<td>Codominant</td>
<td>GG 457 (83.7%)</td>
<td>971 (86.2%)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GC 85 (15.6%)</td>
<td>145 (12.9%)</td>
<td>1.25 (0.93-1.66)</td>
<td>1.19 (0.85-1.66)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC 4 (0.7%)</td>
<td>10 (0.9%)</td>
<td>0.85 (0.27-2.72)</td>
<td>0.80 (0.23-2.82)</td>
<td></td>
</tr>
<tr>
<td>XRCC2</td>
<td>Codominant</td>
<td>GG 451 (86.1%)</td>
<td>952 (85.2%)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA 68 (13.0%)</td>
<td>156 (14.0%)</td>
<td>0.92 (0.68-1.25)</td>
<td>0.84 (0.60-1.19)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA 5 (1.0%)</td>
<td>10 (0.9%)</td>
<td>1.06 (0.36-3.11)</td>
<td>1.05 (0.33-3.38)</td>
<td></td>
</tr>
<tr>
<td>XRCC3</td>
<td>Codominant</td>
<td>CC 229 (42.2%)</td>
<td>438 (38.9%)</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT 238 (43.8%)</td>
<td>538 (47.8%)</td>
<td>0.85 (0.68-1.06)</td>
<td>0.80 (0.62-1.03)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT 76 (14.0%)</td>
<td>149 (13.2%)</td>
<td>0.98 (0.71-1.34)</td>
<td>0.96 (0.67-1.38)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Totals vary because of missing genotype information. Recessive model is not shown for RAD51 and XRCC2 genotypes due to rarity of homozygote variant genotype.

*The combined group of breast cancer and ovarian cancer controls. Genotypes for unstratified ovarian cancer controls were as follows: -Rad51: 291, 40, 2; XRCC2: 274, 54, 3; XRCC3: 128, 161, 43.

† Adjusted for age group (<40, 40-49, 50-59, 60+ years) and country of birth (Australia, overseas, missing).
a dominant model of inheritance. However, as for the breast cancer case-control comparison, this was driven by the heterozygote genotype. All other genotypes examined had an overall risk close to or below unity. Although there was some variation in risk for XRCC2 when we stratified by invasive-ness, these comparisons were based on small numbers and any variation is likely to be due to chance. We conclude that these variants play no substantial role in determining risk of ovarian cancer. To our knowledge, the single other published study assessing ovarian cancer risk in relation to these variants only considered the RAD51 polymorphism among BRCA1/2 mutation carriers and found a reduction in risk of ovarian cancer associated with the C allele (20). It is relevant and of interest to consider possible functional consequences of the variants under investigation. The only insight we have pertaining to the XRCC2 A allele I88H variant is that it has been shown by cellular complementation assays to slightly increase sensitivity to damage (23). Numerious studies have assessed function of the XRCC3 T241M variant. These reports include no detrimental affect on homoygous recombination (29, 30), no association with glyopherin-A somatic mutation frequency (31), no increased levels of chromosomal aberrations and single-strand breaks (32), a suggestion of slightly increased resistance to Camptothecin (29), and a capability of rescuing the endoreduplication phenotype in XRCC3 −/− cells (33). It is likely that these insubstantial differences in function have at most minimal effects on cancer risk. Our study sample sizes were sufficiently large to detect dominantly inherited risks in the order of 1.4- to 1.5-fold for the rare RAD51 and XRCC2 variants, and 1.3- to 1.4-fold for the XRCC3 variant. Whereas we had 80% power to detect a 1.4- to 1.5-fold risk associated with the homozygote XRCC3 variant genotype, there was little statistical power to detect modest increases in cancer risk for the RAD51 and XRCC2 homozygote variant genotypes. We found no evidence that any of these DBSR variants were associated with a significant substantially increased risk of breast or ovarian cancer even after considering the effects of ethnicity on variant frequency and the effects of certain exposures where data were available. Similarly, combined analysis with other published studies indicated that there was at best evidence for a 1.14-fold associated risk of breast cancer, of borderline significance, associated with the XRCC3 T241M TT genotype. We conclude that the DBSR variants investigated in this study are unlikely to play a major role in the etiology of breast or ovarian cancer.

Acknowledgments

We thank the physicians, surgeons, and oncologists who endorsed this project; the interviewing staff; and the many women and their relatives who participated in this research; Margaret McCredie for her role in the establishment of Australian Breast Cancer Family Study; Adele Green for ovarian cancer case DNA and risk factor information; Nicholas Martin for ovarian cancer control DNA and risk factor information; Gillian Dite for database management; and Sarah Steinborner and Deon Venter for preparation of breast cancer case and control DNA.

References
