Short Communication

Breast Cancer Risk Reduction Associated with the RAD51 Polymorphism among Carriers of the BRCA1 5382insC Mutation in Poland

Anna Jakubowska, Steven A. Narod, David E. Goldgar, Marek Mierzejewski, Bartłomiej Masojć, Katarzyna Nej, Jowita Huzarska, Tomasz Byrski, Bohdan Gorski, and Jan Lubinski

Hereditary Cancer Centre [J. H., T. B., J. J.], Department of Genetics and Pathology [A. J., M. M., B. M., B. G., J. L.], Pomeranian Academy of Medicine, Szczecin 70-115, Poland, Centre for Research on Women’s Health, University of Toronto, Ontario, Canada [S. A. N.]; Unit of Genetic Epidemiology, International Agency for Research on Cancer, Lyon, France [D. E. G.]; and Department of Molecular Biology, Inter-University Unit, University of Szczecin and Pomeranian Academy of Medicine, Szczecin, Poland [K. N.]

Abstract

The observed heterogeneity of breast cancer risk among women who carry the same BRCA1 mutation suggests the existence of modifying environmental and genetic factors. The product of the RAD51 gene functions with BRCA1 and BRCA2 in the repair of double-stranded DNA breaks. To establish whether polymorphic variation of RAD51 modifies risk for hereditary breast cancer, we conducted a matched case-control study on 83 pairs of female carriers of the BRCA1 5382insC mutation. Cases consisted of women with breast cancer, and controls were women with the same mutation but who were unaffected. The frequency of the RAD51 135C variant allele was established in cases and controls using RFLP-PCR. The RAD51 135C allele was detected in 37% of unaffected and in 17% of affected BRCA1 carriers. Among 27 discordant matched pairs, the RAD51 135C allele was found in the healthy carrier on 22 occasions and in the affected carrier on only five occasions (odds ratio = 0.23; 95% confidence interval, 0.07–0.62; P = 0.0015). This finding suggests that RAD51 is a genetic modifier of breast cancer risk in BRCA1 carriers in the Polish population. It will be of interest to confirm this in other populations as well.

Introduction

The observed heterogeneity of breast cancer risk among women who carry the same BRCA1 mutation suggests the existence of modifying environmental and genetic factors. The identification of these modifiers may be important for providing accurate risk assessments for carriers who face difficult choices regarding prophylactic mastectomy and oophorectomy. Some potential genetic and nongenetic modifiers have been identified (1). RAD51, a homologue of RecA of *Escherichia coli*, functions in mitotic recombination and in DNA repair in association with BRCA1 and BRCA2 (2). Overexpression of RAD51 may contribute to the development of sporadic breast cancer (3). Two previous studies suggest that a single nucleotide polymorphism (135 G to C) in the 5′ untranslated region of RAD51 may influence breast cancer risk in BRCA mutation carriers (4, 5). Wang et al. (4) reported the presence of the RAD51 135C allele in 13% of 224 BRCA1 carriers affected with breast cancer and in 11% of 179 healthy BRCA1 carriers (OR = 1.14; P = 0.68). For carriers of BRCA2 mutations the OR was 2.8 (P = 0.04). In a second study of Ashkenazi Jewish women (5), the RAD51 135C allele was detected in 9.9% of 121 affected BRCA1 mutation carriers and in 6.1% of 49 healthy BRCA1 carriers (OR = 1.7; P = 0.56). The RAD51 135C allele was identified in 17.4% of 46 affected BRCA2 carriers and in 4.9% of 41 healthy BRCA2 carriers (OR = 4.2; P = 0.09). These earlier studies did not control for potential differences in risk between different BRCA1 and BRCA2 mutations, for differences in ethnicity, or for other known risk factors for breast cancer.

The 5382insC mutation accounts for ~55% of all Polish families with BRCA1 mutations (6). Because of this strong founder effect and because Poland contains a relatively stable and ethnically homogeneous population, there is an opportunity to perform association studies of modifying genes that are not influenced to a great extent by allelic or ethnic variation. We attempt to confirm the previously reported positive association between the RAD51 135C allele and breast cancer risk in the Polish population with 5382insC BRCA1 mutations.

Materials and Methods

To determine whether the RAD51 135C allele is a modifier of BRCA1 mutation penetrance, we conducted a matched case-control study. Women with breast cancer and a BRCA1 5382insC mutation (cases) were selected from the Hereditary Cancer Registry in the Pomeranian Academy of Medicine of Szczecin, Poland. Pedigrees were taken, which included the sites and ages of cancer diagnoses in all first-degree relatives of probands. For each case, an attempt was made to identify a matched unaffected female control who also carried the BRCA1 5382insC mutation. Controls were required to be older than the age at which breast cancer was diagnosed in the corresponding matched case. Cases and controls were matched on hormone replacement therapy (ever/never), oral contraceptives (ever/never), replacement therapy (ever/never), oral contraceptives (ever/never), and ethnically homogeneous population, there is an opportunity to perform association studies of modifying genes that are not influenced to a great extent by allelic or ethnic variation. We attempt to confirm the previously reported positive association between the RAD51 135C allele and breast cancer risk in the Polish population with 5382insC BRCA1 mutations.

Received 8/1/02; revised 2/14/03; accepted 2/14/03.

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.

1 This work was supported by EC Project Grant No. QLRT-1999-00063.

2 To whom requests for reprints should be addressed, at Department of Genetics and Pathology, Pomeranian Academy of Medicine, Ul. Połabska 4, Szczecin 70-115, Poland. Phone/Fax: 48-91-466-15-33; E-mail: aniaj@sci.pam.szczecin.pl.

3 The abbreviations used are: OR, odds ratio; CI, confidence interval.
never), parity, age at first pregnancy (±2 years), and breastfeeding (±3 months). It was possible to identify 83 matched pairs; 11 pairs were sisters, 3 pairs were more distant relatives, and 69 pairs were unrelated. In addition, we genotyped 82 consecutive breast cancer cases and 189 healthy individuals selected at family doctors from the city of Szczecin.

Primers and PCR conditions have been reported previously (5). The PCR-RFLP technique was applied to establish the RAD51 135C polymorphism status. An example of the images produced by the variant 135C and the wild-type alleles is shown in Fig. 1. The accuracy of the PCR-based technique was verified by direct DNA sequencing of 12 cases with variant bands and of 10 cases with wild-type bands. In all cases, the results of the two techniques were concordant. The unadjusted OR for the association of breast cancer risk and the RAD51 135C allele were estimated from the ratio of discordant pairs, and the significance of this association was tested using McNemar’s test. We also analyzed the data as an unmatched case-control study, adjusting for year of birth and age at first pregnancy and parity. The estimated OR for the RAD51 variant was 0.35 (95% CI, 0.17–0.74; P = 0.005). Among the 83 affected carriers, women with the RAD51 135C variant were diagnosed on average at an age 3.2 years older than women without the variant. This association is consistent with the hypothesis that the RAD51 135C allele is protective against breast cancer, but this age difference was not statistically significant (P = 0.12).

**Results**

We measured the frequency of the RAD51 135C variant allele in 83 cases and 83 controls (who carried the BRCA1 5382insC mutation), in 82 consecutive breast cancer patients and in 189 healthy controls. All study subjects were of Polish ethnicity. The cases and controls are compared in Table 1. None had used hormone replacement therapy for >5 years, and only 2% had used oral contraceptives for >5 years. Among the 166 parents of the cases, there were 50 malignant tumors reported (including 20 breast cancers), and among the 166 parents of the controls, there were 53 malignant tumors reported (including 22 breast cancers).

This overall prevalence of the RAD51 135C allele was 26% among 89 healthy population controls. However, among the BRCA1 carriers, the RAD51 135C allele was detected in 37% of the unaffected BRCA1 carriers (31 of 83) and in 17% of the affected carriers (14 of 83). There were 27 discordant matched pairs (i.e., one of two carried the RAD51 135C allele). Among these, the RAD51 135C allele was found in the healthy carrier on 22 occasions and in the affected carrier on only five occasions (Table 2). This corresponds to an OR of 0.23 (95% CI, 0.07–0.62; P = 0.0015; McNemar’s test). We also analyzed the data as an unmatched case-control study, adjusting for year of birth and age at first pregnancy and parity. The estimated OR for the RAD51 variant was 0.35 (95% CI, 0.17–0.74; P = 0.005). Among the 83 affected carriers, women with the RAD51 135C variant were diagnosed on average at an age 3.2 years older than women without the variant. This association is consistent with the hypothesis that the RAD51 135C allele is protective against breast cancer, but this age difference was not statistically significant (P = 0.12).

**Discussion**

The results of this matched case-control study suggest that the RAD51 135C allele is associated with a reduced risk of breast cancer among BRCA1 5382insC carriers in Poland, but not among the Polish population in general. In contrast to our study, two previous studies found the RAD51 135C allele to be associated with a higher risk of breast cancer (4, 5). Our study has several strengths. We included only BRCA1 carriers, whereas previous studies combined both BRCA1 and BRCA2 carriers, and the association was largely confined to BRCA1 carriers. We also confined our study population to carriers of a single BRCA1 mutation. It is possible that the action of Rad51 is mutation specific, and previous studies would have had only a few instances of this mutation in their data sets.

The mechanisms of cancer risk modification by RAD51 is unknown. RAD51 binds to BRCA1, and with BRCA2, forms a complex involved in repair of double-strand DNA breaks (2). In contrast to the products of other BRCA1 mutations, the truncated 5382insC protein is believed to be stable (7) and contains an intact RAD51 binding site (8). It is possible that this allele of RAD51 enhances the mutant or wild-type BRCA1 activity and diminishes the tumorigenicity of breast precursor cells. The 83

---

Table 1: Comparison of cases and matched controls

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current age (yr)</td>
<td>47.6</td>
<td>46.9</td>
<td>0.50</td>
</tr>
<tr>
<td>Age at breast cancer diagnosis (yr)</td>
<td>39.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity (mean)</td>
<td>2.1</td>
<td>2.2</td>
<td>0.32</td>
</tr>
<tr>
<td>Age first pregnancy (yr)</td>
<td>23.8</td>
<td>23.6</td>
<td>0.52</td>
</tr>
<tr>
<td>Breastfeeding (months)</td>
<td>7.5</td>
<td>8.8</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* Paired t test.

Table 2: Distribution of RAD51-135C polymorphism in the 83 matched pairs—carriers of BRCA1 5382insC

<table>
<thead>
<tr>
<th>Affected</th>
<th>Unaffected</th>
<th>No. of pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>–</td>
<td>47</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>9</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>22</td>
</tr>
</tbody>
</table>

* (+) RAD51-135C variant, (–) wild type RAD51.
cases studied were recruited on average 8 years after their diagnosis of breast cancer; thus, if the RAD51 135 C allele were associated with a poor prognosis, this allele would be underrepresented among long-term breast cancer survivors. Other possible differences between ours and previous studies include sample sizes, the different ethnic groups studied, and study design. We used a matched case-control design, and we controlled for many reproductive and lifestyle factors. Although ours was a relatively small study, the matched design provided 80% power to detect an OR of 0.28. The frequency of the variant allele was higher in Poland than in the other populations. We found the allele present in 26% of healthy Polish controls, whereas Levy-Lahad et al. reported a frequency of 10% in 257 Ashkenazi Jewish women (5), and Wang et al. (4) found a frequency of 12% in 186 individuals from Australia and the United States. Our findings suggest that RAD51 may be the strongest genetic modifier of breast cancer risk in BRCA1 carriers identified to date, but this should be confirmed in additional studies.

References
Breast Cancer Risk Reduction Associated with the RAD51 Polymorphism among Carriers of the BRCA1 5382insC Mutation in Poland

Anna Jakubowska, Steven A. Narod, David E. Goldgar, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/12/5/457

Cited articles
This article cites 8 articles, 2 of which you can access for free at:
http://cebp.aacrjournals.org/content/12/5/457.full#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/12/5/457.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, contact the AACR Publications Department at permissions@aacr.org.