Short Communication

Breast Cancer Risk Reduction Associated with the *RAD51* Polymorphism among Carriers of the *BRCA1* 5382insC Mutation in Poland[1]

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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 *BRCA*1 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[3] 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 *BRCA*2 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/

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[3] The abbreviations used are: OR, odds ratio; CI, confidence interval.

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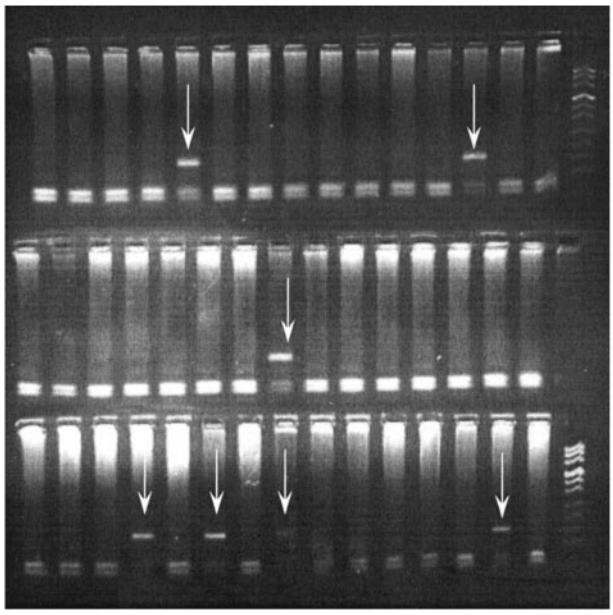

Fig. 1. An example of the gel-image with samples with and without the *RAD51* 135C allele variant. Wild-type allele is digested by *Mva*I, resulting in 86 and 71 bp products; *RAD51* 135C allele is not digested by *Mva*I, resulting in 157 bp product (*arrows*).

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. The adjusted OR was estimated using unconditional logistic regression.

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 27% among the 166 carriers of *BRCA1* 5382insC mutations, was 25% among 82 consecutive breast cancer patients, and was 26% among 189 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 ($P = 0.12$).

Table 1 Comparison of cases and matched controls

	Cases $n = 83$	Controls $n = 83$	P^a
Current age (yr)	47.6	46.9	0.50
Age at breast cancer diagnosis (yr)	39.7		
Parity (mean)	2.1	2.2	0.32
Age first pregnancy (yr)	23.8	23.6	0.52
Breastfeeding (months)	7.5	8.8	0.10

[a] Paired *t* test.

Table 2 Distribution of *RAD51*-135C polymorphism in the 83 matched pairs—carriers of *BRCA1* 5382insC[a]

Affected	Unaffected	No. of pairs
−	+	22
+	+	9
+	−	5
−	−	47

[a] (+) *RAD51*-135C variant, (−) wild type *RAD51*.

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 *BRCA2* 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

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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.

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