The *RAD51* 135 G>C Polymorphism Modifies Breast Cancer and Ovarian Cancer Risk in Polish *BRCA1* Mutation Carriers

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Abstract

Breast and ovarian cancer penetrance in *BRCA1* mutation carriers is estimated to be between 15% and 80% by age 70 years. At present, it is not possible to predict with any certainty who is most likely to develop disease or which age it will develop. Previous studies have tried to correlate the sites of *BRCA1* mutations with disease risk; however, the results have not yielded any definitive association. An alternative explanation that could account for differences in the penetrance of *BRCA1* mutations is the action of modifier genes. In this study, we have investigated the role of the *RAD51*_135_G>C polymorphism in breast and ovarian cancer case-control populations of Polish women who have been matched for *BRCA1* mutation and year of birth. The results reveal that women who harbor the C allele have almost twice the reduction in breast and ovarian cancer risk compared with women who harbor only the G allele. These findings suggest that the effect of the *RAD51* C allele is an important risk modifier for malignancies occurring on a background of *BRCA1* mutations. In addition, we were able to show that the site of the *BRCA1* mutation does not influence the effect of the *RAD51* C allele, indicating that this polymorphism contributes to prevention of disease in *BRCA1* carriers. In conclusion, the *RAD51* C allele seems to protect against both breast and ovarian cancer in women harboring *BRCA1* mutations. (Cancer Epidemiol Biomarkers Prev 2007;16(2):270–5)

Introduction

Women harboring a *BRCA1* germ line mutation have a high lifetime risk of developing breast and ovarian cancer; for breast cancer in the range of 46% to 87% (1-3) and for ovarian cancer between 15% and 68% (3-6), the difference is due to the average age of disease diagnosis assessed from high-risk family studies compared with population-based studies. Such differences suggest that the penetrance of *BRCA1* mutations is influenced by other genetic or environmental factors. Identification of such risk-modifying factors in addition to *BRCA1* mutation status is important for an effective application of risk prediction or cancer prevention strategies in women carrying *BRCA1* mutations.

Three common founder mutations in *BRCA1*, 5382insC, 4153delA, and 300 T>G, account for ~90% of all detected *BRCA1* mutations in Polish families with breast and ovarian cancers (7, 8). Because of this strong founder effect and the Polish population being relatively stable and ethnically homogeneous, it is ideal for association studies of risk-modifying genes not influenced by *BRCA1* allelic or ethnic variation.

A candidate modifier of hereditary breast and ovarian cancer risk is the *RAD51* gene, a homologue of the *RecA* gene of *Escherichia coli*. The *RAD51* gene product acts together with BRCA1 and BRCA2 proteins in homologous recombination and repair of double-strand DNA breaks (9). A small study conducted among breast cancer patients from Japan suggested the existence of a disease-causing *RAD51* germ line mutation, $Arg^{150}Gln$, in two patients with bilateral breast cancer (10). Based on its cellular function and on rare *RAD51* germ line mutations giving rise to genetic disease (11), common *RAD51* variants that alter RAD51 protein expression levels may operate to influence disease risk and can therefore be considered as a genetic factor in cancer susceptibility (12). A functional single nucleotide polymorphism in the *RAD51* gene, changing a guanine to cytosine at position 135 in the 5′ untranslated region, has been identified, which is associated with enhanced promoter activity that may alter disease risk (13).

Conflicting data on the association of the *RAD51*_135_C allele with hereditary breast cancer risk exist. An association of the *RAD51*_135_C allele with an increased breast cancer risk among *BRCA1/2* mutation carriers has previously been reported but remained controversial, as initial data was not supported by more extensive analysis (14). Subgroup analyses by *BRCA* mutation revealed a significant association only in the subgroup of *BRCA2* mutation carriers (14). Similar findings were obtained in two other studies among female Ashkenazi Jewish carriers of the common *BRCA1* (185delAG, 5382insC) or *BRCA2* (6174delT) mutations (15, 16). Both studies reported an association of the C allele with an increased breast cancer risk among *BRCA2* mutation carriers, whereas no association was found in *BRCA1* mutation carriers. Recently, the opposite effect of reduced breast cancer risk was reported in a matched case-control study conducted on 83 pairs of female *BRCA1* 5382insC mutation carriers from Poland (17). In a study examining postmenopausal breast cancer cases, no association was observed with the *RAD51*_135_G>C allele, suggesting that this polymorphism is not associated with disease risk outside of the context of *BRCA1* and *BRCA2* (18). With respect to hereditary ovarian cancer risk, little and inconsistent data on a

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modifying effect of the C allele exist. Whereas one study reported an association of the C allele with a decreased ovarian cancer risk in *BRCA1/2* mutation carriers (14), no association was found in another study (15).

To elucidate the role of the *RAD51*_135_G>C polymorphism in breast and ovarian cancer, we did a case-control study on Polish *BRCA1* mutation carriers, including 258 breast cancer case-control pairs and 127 ovarian cancer case-control pairs.

Materials and Methods

Study Participants. The Hereditary Cancer Registry at the Pomeranian Medical University in Szczecin, Poland, contains clinical and epidemiologic data collected from 1997 to 2002 from 1,940 individuals carrying one of the three common Polish *BRCA1* founder mutations: 5382insC, 300 T>G, and 4153delA. Mutation carriers were selected from families with at least one breast cancer diagnosed before 50 years of age or ovarian cancer at any age or with a strong history of breast and/or ovarian cancer. A self-administered questionnaire was used to collect information on potential risk factors.

All study participants were of Polish origin and resided in Poland. They were considered as cases if they were diagnosed with invasive primary breast cancer or invasive primary ovarian cancer (excluding borderline ovarian carcinoma) and had not undergone prophylactic mastectomy or adnexectomy before the age of breast cancer diagnosis. They were considered as controls if they were unaffected by *in situ* carcinoma or any other type of cancer and had not undergone prophylactic mastectomy or adnexectomy before the age at which breast or ovarian cancer was diagnosed in the corresponding case. Controls were matched to cases by year of birth (within 2 years) and *BRCA1* mutation (5382insC, 300 T>G, and 4153delA).

A set of 385 case-control pairs, including 258 breast cancer case-control pairs and 127 ovarian cancer case control pairs, was selected. The number of pairs in the various subgroups and the median ages of breast and ovarian cancer diagnosis in the cases and in controls at the time when matching was done are shown in Table 1.

DNA samples were available from all study subjects. The research was approved by the Ethics Committee of Pomeranian Medical University in Szczecin, Poland, and all participants gave informed consent before enrolling in the study. All women received genetic counseling before and during the provision of their test results.

Genotyping Analysis. Genotyping of the 135_G>C polymorphism (rs1801320) in the *RAD51* gene (Genbank accession no. NT_010194) in 646 *BRCA1* mutation carriers was done by PCR and RFLP analysis because the single nucleotide polymorphism destroys an *Mva*I restriction site. Genomic DNA was isolated from peripheral blood leukocytes according to Lahiri and Schnabel (19). Primers and PCR as well as RFLP condition have been reported previously (15). Genotyping was done by two independent personnel who were blinded to the disease status of the samples. Several samples were sequenced on an ABI prism 377 DNA Sequencer (Perkin-Elmer, Foster City, CA) to confirm the genotypes. The reproducibility of the genotyping data was assessed by repeated analysis of 96 of 646 (15%) randomly selected DNA samples. Genotypes were obtained for all 646 breast and ovarian cancer cases and controls constituting PCR concordance rate of 100%.

Statistical Analysis. Risk estimates were calculated as crude odds ratios (OR_{crude}) with 95% confidence intervals (95% CI) using univariate conditional logistic regression for the 258 breast cancer and 127 ovarian cancer case-control pairs. For 177 breast cancer and 83 ovarian cancer case-control pairs, we adjusted for potential breast/ovarian cancer risk factors, called adjusted ORs (OR_{adj}), by including age at first live birth (0, ≤24, >24 years), lifetime cumulative months of breast-feeding (≤12 and >12 months), and smoking (<4 and >4 pack-years) in the multivariate logistic regression model. To account for a potential bias due to the presence of relatives, both in the case and in the control groups, we also did unadjusted analysis on 161 breast cancer case-control pairs and 100 ovarian cancer case-control pairs, and an adjusted analysis on 109 and 61 pairs, respectively, after the exclusion of the relative pairs.

Two-sided P values of ≤0.05 were considered as statistically significant. GC and CC genotypes were combined for regression analysis due to the low proportion of women with the CC genotype (<1% in patients and controls). Cases and controls were compared for their characteristics using the Mann-Whitney U test for continuous variables. The statistical analyses were done using the SAS/STAT(r) software, version 9.1, with the procedures LOGISTIC and SURVEYLOGISTIC (20).

Results

A total of 385 case-control pairs, including 258 breast cancer case-control pairs and 127 ovarian cancer case-control pairs, were included in this study. Breast and ovarian cancer cases

Table 1. Study groups: median ages of breast and ovarian cancer diagnosis of cases and of controls at the time of matching

No. case-control pairs	Risk factor data available	Median age of cancer diagnosis (range)	Median age of controls at the time of matching (range)*
Breast cancer case-control pairs			
258 (total)		40 (22-74)	45 (24-77)
177	Yes	40 (24-74)	45 (28-77)
81	No	39 (22-62)	45 (24-75)
161 (unrelated only)		39 (25-74)	45 (26-77)
109	Yes	40 (25-74)	45 (28-77)
52	No	38 (26-62)	44 (26-69)
Ovarian cancer case-control pairs			
127 (total)		46 (25-71)	49 (30-75)
83	Yes	45 (25-71)	49 (31-73)
44	No	48 (27-71)	51 (38-75)
100 (unrelated only)		46 (27-71)	49 (30-73)
61	Yes	46 (34-71)	49 (36-73)
39	No	48 (27-69)	51 (30-70)

*The median age difference between cases and controls was a result of matching the pairs by year of birth such that the age of the controls is the current age and for cases is the age of diagnosis. One hundred and twenty-four of the controls were used as controls for both breast cancer cases and ovarian cancer cases.

Table 2. Comparison of breast cancer cases and matched controls

Characteristic	Cases (N = 177)	Controls (N = 177)
	n (%)	n (%)
Year of birth (median)	1956 (range, 1926-1974)	1956 (range, 1925-1974)
Age of first live birth (median)	24 (range, 17-37)	23 (range, 16-36)
0	12 (7)	13 (7)
≤24	102 (58)	107 (61)
>24	63 (35)	57 (32)
Age at menarche (median, y)	13 (range, 10-18)	14 (range, 9-18)
≤11	16 (9)	9 (5)
12-13	74 (42)	71 (40)
≥14	87 (49)	97 (55)
BMI (median)	22 (range, 17-40)	24 (range, 17-44)
Parity		
0	12 (7)	13 (7)
1	39 (22)	31 (18)
2	84 (47)	81 (46)
3	28 (16)	38 (21)
4	8 (5)	9 (5)
>4	6 (3)	5 (3)
Breast-feeding (mo)*		
≤12	56 (34)	50 (30)
>12	109 (66)	114 (70)
OC use (y)		
<5	168 (95)	162 (92)
≥5	9 (5)	15 (8)
HRT		
Never	177 (100)	163 (92)
Ever	0 (0)	14 (8)
Smoking (pack-years) †		
<4	111 (64)	114 (64)
≥4	63 (36)	63 (36)

Abbreviations: BMI, body mass index; OC, oral contraceptive; HRT, hormone replacement therapy.
*Nulliparous women were excluded.
†Missing information on smoking status from three cases.

and controls were similar with respect to potential breast/ovarian cancer risk factors, including age at first live birth, age at menarche, body mass index, parity, breast-feeding, and smoking. In contrast, hormone replacement therapy use was less frequently reported by breast cancer cases than by controls (0 cases and 14 controls; Table 2) and hormone replacement therapy and oral contraceptive use of >5 years were less frequently reported by ovarian cancer cases than by controls (2 cases and 11 controls; 0 cases and 8 controls, respectively; Table 3). These differences were not considered relevant because of the small number of individuals. The characteristics of the subgroups of breast and ovarian cancer cases and their corresponding controls for which risk factor information was available are given in Tables 2 and 3, respectively.

Among *BRCA1* carriers, the prevalence of the C allele was significantly lower in breast cancer cases (48 of 516, 9.3%; 95% CI, 7.0-12.2 versus 72 of 516, 14%; 95% CI, 11.1-17.3; Fisher's exact test, P = 0.025) and in ovarian cancer cases [23 of 254 (9%); 95% CI, 5.9-13.4 versus 39 of 254 (15.3%); 95% CI, 11.3-20.5; Fisher's exact test, P = 0.041] compared with controls. The allele and genotype distributions between breast and ovarian cancer patients and their corresponding controls are shown in Tables 4 and 5, respectively.

Comparison of Genotype Frequencies among Breast Cancer Patients and Controls. For the breast cancer case-control pairs, risk factor information was available for 177 pairs and was partially or completely lacking for 81 pairs. There was no difference in the *RAD51*_GC+CC associated breast cancer risk in unadjusted analysis conducted on 258 pairs (OR_{crude}, 0.58; 95% CI, 0.38-0.91) compared with the adjusted analysis conducted on 177 pairs (OR_{adj}, 0.56; 95% CI, 0.32-0.97; Table 4). After exclusion of relatives, the same statistically significantly decreased risks were observed both in unadjusted and adjusted analyses (OR_{crude}, 0.5; 95% CI, 0.28-0.88; OR_{adj}, 0.38; 95% CI, 0.18-0.82).

Breast cancer risk was also assessed with respect to the underlying *BRCA1* mutation for 181 case-control pairs carrying the 5382insC mutation, and the 62 pairs positive for 300 T>G (Table 6). The GC+CC genotypes were associated with reduced breast cancer risk in carriers of 5382insC mutation (OR_{crude}, 0.57; 95% CI, 0.33-0.99) and 300 T>G (OR_{crude}, 0.67; 95% CI, 0.30-1.48). The latter, however, was not statistically significant; however, a trend in the patients harboring the 300 T>G mutation was consistent with the results observed for the 5382insC patients.

Breast cancer cases with the GC+CC genotype were diagnosed at a median age 1.5 years older than women with the GG genotype [41 years (range, 26-70 years) versus 39.5 years (range, 22-74 years)], which was not statistically significant (Mann-Whitney U test, P = 0.58).

Comparison of Genotype Frequencies among Ovarian Cancer Patients and Controls. Risk factor information was available for a subgroup comprising 83 ovarian cancer case-control pairs and was partially or completely lacking for 44 pairs. Unadjusted analyses conducted on 127 pairs and adjusted analyses conducted on 83 pairs revealed similar results (Table 5). A comparison of the *RAD51*_135_G>C frequencies among cases and controls identified combined GC+CC (OR_{crude}, 0.48; 95% CI, 0.03-0.91; OR_{adj}, 0.43; 95% CI, 0.18-1.00) genotypes as a statistically significant modifier of ovarian cancer risk. After exclusion of pairs with relatives, similar results were obtained in unadjusted and adjusted analyses (OR_{crude}, 0.59; 95% CI, 0.3-1.17; OR_{adj}, 0.52; 95% CI, 0.2-1.33).

Table 3. Comparison of ovarian cancer cases and matched controls

Characteristic	Cases (N = 83)	Controls (N = 83)
	n (%)	n (%)
Year of birth (median)	1953 (range 1928-1971)	1953 (range 1929-1971)
Age of first live birth (median)	22 (range 16-38)	23 (range 17-35)
0	7 (8)	7 (8)
≤24	59 (71)	51 (62)
>24	17 (21)	25 (30)
Age at menarche (median, y)	14 (range 10-17)	14 (range 9-18)
≤11	10 (12)	4 (5)
12-13	24 (29)	28 (34)
≥14	49 (59)	51 (61)
BMI (median)	25 (range 19-38)	25 (range 17-44)
Parity		
0	7 (8)	7 (8)
1	11 (13)	12 (14)
2	43 (52)	41 (50)
3	13 (16)	17 (20)
4	7 (8)	4 (5)
>4	2 (3)	2 (3)
Breast-feeding (mo)*		
≤12	20 (26)	23 (30)
>12	56 (74)	53 (70)
OC use (y)		
<5	83 (100)	75 (90)
≥5	0 (0)	8 (10)
HRT		
Never	81 (97)	72 (87)
Ever	2 (3)	11 (13)
Smoking (pack-years) †		
<4	56 (67)	52 (63)
≥4	24 (29)	31 (37)

*Nulliparous women were excluded.
† Missing information on smoking status from three cases.

Table 4. *RAD51*_135_G>C polymorphism modifies breast cancer risk in *BRCA1* carriers

Genotype/allele	All breast cancer cases and controls			
	Cases (N = 258)	Controls (N = 258)	OR_{crude} (95% CI)	P
	Pn (%)	*Pn* (%)		
*RAD51*_135_G>C				
GG	210 (81)	188 (73)	1.00 (reference)	
GC+CC	48 (19)	70 (27)	0.58 (0.38-0.91)	0.018
C*	48 (9)	72 (14)		
Genotype/allele	Cases and controls with risk factor information			
	Cases (N = 177)	Controls (N = 177)	OR_{adj} † (95% CI)	P
	n (%)	*n* (%)		
*RAD51*_135_G>C				
GG	144 (81)	128 (72)	1.00 (reference)	
GC+CC	33 (19)	49 (28)	0.56 (0.32-0.97)	0.038
C*	33 (9)	50 (14)		

*Allele frequency is in agreement with published data (17).
† Adjusted for age of first live birth, breast-feeding, and smoking.

The risk estimations were also assessed with respect to the underlying *BRCA1* mutation. Risks were estimated for 96 case-control pairs carrying the 5382insC mutation, and the 23 pairs positive for 300 T>G (Table 7). The GC+CC genotypes were associated with a reduced ovarian cancer risk in carriers of the 5382insC mutation (OR_{crude}, 0.57; 95% CI, 0.28-1.16) and the 300 T>G change (OR_{crude}, 0.20; 95% CI, 0.02-1.71), which was not significant because of the reduced power of the analysis.

Among all ovarian cancer cases, women with the GC+CC genotypes were diagnosed at a median age 3 years older than women with the GG genotype [49 years (range, 35-59 years) versus 46 years (range, 25-71 years)], but this age difference was not statistically significant (Mann-Whitney U test, P = 0.27).

Discussion

We investigated the effect of *RAD51*_135_G>C 5′ untranslated region polymorphism on the risk of breast and ovarian cancer in highly selected breast/ovarian cancer populations from Poland. Although several investigations of the *RAD51*_135_G>C polymorphism in hereditary breast cancer populations have been previously reported (14-17), little data on the influence of this polymorphism on hereditary ovarian cancer risk exist (14, 15). Due to conflicting results obtained in these previous studies, we report evidence of a modifying effect of the *RAD51* polymorphism on ovarian cancer risk among Polish *BRCA1* carriers and confirm our previous findings that this polymorphism is a modifier of *BRCA1*-associated breast cancer risk in a larger sample set.

The *RAD51*_135_G>C polymorphism has previously been shown to be associated with hereditary ovarian cancer risk. One study on *BRCA1/2* mutation carriers, including 33 ovarian cancer cases and 179 controls that all carry a *BRCA1* mutation, suggested that the C allele is associated with about half the reduced ovarian cancer risk (14). However, in a study conducted on Ashkenazi Jewish *BRCA1/2* carriers comprising 30 ovarian cancer cases and 49 controls with *BRCA1* mutations, no such association was observed (15). An association of the C allele with hereditary breast cancer risk has been suggested by several reports (14-16). Wang et al. (14) reported an association of the C allele with a 3-fold increased breast cancer risk in the subgroup of 216 *BRCA2* mutation carriers, but not in the

Table 5. *RAD51*_135_G>C polymorphism modifies ovarian cancer risk in *BRCA1* carriers

Genotype/allele	All ovarian cancer cases and controls			
	Cases (N = 127)	Controls (n = 127)	OR_{crude} (95% CI)	P
	n (%)	*n* (%)		
*RAD51*_135_G>C				
GG	104 (82)	89 (70)	1.00 (reference)	
GC+CC	23 (18)	38 (30)	0.48 (0.03-0.91)	0.02
C*	23 (9)	39 (15)		
Genotype/allele	Cases and controls with risk factor information			
	Cases (N = 83)	Controls (n = 83)	OR_{adj} † (95% CI)	P
	n (%)	*n* (%)		
*RAD51*_135_G>C				
GG	68 (82)	57 (69)	1.00 (reference)	
GC+CC	15 (18)	26 (31)	0.43 (0.18-1.00)	0.05
C*	15 (9)	26 (16)		

*Allele frequency is in agreement with published data (17).
† Adjusted for age of first live birth, breast-feeding, and smoking.

Table 6. Breast cancer risks in women carrying different *BRCA1* mutations

Genotype	Carriers of the 5382insC mutation			
	Cases (N = 181)	Controls (N = 181)	OR_{crude} (95% CI)	P
	n (%)	n (%)		
*RAD51_135*_G>C				
GG	147 (81%)	132 (73%)	1.00 (reference)	
GC+CC	34 (19%)	49 (27%)	0.57 (0.33-0.99)	0.046
Genotype	Carriers of the 300 T>G mutation			
	Cases (N = 62)	Controls (N = 62)	OR_{crude} (95% CI)	P
	n (%)	n (%)		
*RAD51_135*_G>C				
GG	51 (82%)	46 (74%)	1.00 (reference)	
GC+CC	11 (18%)	16 (26%)	0.67 (0.30-1.48)	0.32

subgroup of *BRCA1* mutation carriers, including 199 breast cancer cases and 179 controls. No altered risk was found in two other studies in the subgroups of *BRCA1* mutation carriers, including 78 breast cancer cases and 49 controls (15) and 121 breast cancer cases and 50 controls (16). In our study on 385 breast/ovarian cancer case-control pairs, we have observed that the *RAD51*_GC+CC genotypes are associated with about half of the decrease in the risk of breast and ovarian cancer. The current study on 258 breast cancer case-control pairs and 127 ovarian cancer case-control pairs provided a 78% power to detect an OR of 0.58 for breast cancer and a 72% power to detect an OR of 0.48 for ovarian cancer.

The decrease in breast cancer risk in the breast-control pairs observed in our study confirms the previous results obtained in a smaller study of 83 Polish matched case-control pairs (17). Because the current study extends the number of breast and ovarian cancer cases and the data confirms our previous report, the numbers of patients used in this study is sufficient to reveal a true association of the C allele with decreased risks in breast and ovarian cancer.

The decreased breast and ovarian cancer risks were found consistently in independent unrelated study subjects as well as in the entire study population. The reduction in statistical significance for the decreased ovarian cancer risk was expected given the decreased number of analyzed subjects. The discrepancies in our results compared with previous studies may be explained by differences in populations (Ashkenazi versus non-Ashkenazi), in the selection criteria (*BRCA1/2* carriers versus *BRCA1* carriers), matching criteria (not matched versus matched by mutation, year of birth), study size, and in risk evaluation with or without adjustment for known breast/ovarian cancer risk factors. It is unlikely, however, that any of these factors could adequately explain the differences between this investigation and the others. The most plausible explanation are type 1 or type 2 errors that are a result of insufficient study population sizes.

In the present study, risks were adjusted for known and putative breast/ovarian cancer risk factors, including age of first live birth, breast-feeding, and smoking (21-24). In the multivariate analysis, body mass index, age at menarche, parity, oral contraceptive use, and hormone replacement therapy were not included in the adjustment of analysis as the numbers of participants reporting either use or percentage variation was too low to influence breast or ovarian cancer risk.

Our findings indicate that the observed decrease in breast and ovarian cancer risk is attributed to the influence of the polymorphic *RAD51* gene. The potential role of the C allele as a risk modifier is consistent with the observation that women harboring this change tended to be diagnosed with disease at a later age compared with those harboring the G allele (41 versus 39.5 years and 49 versus 46 years, respectively). Given that the age difference is not great, larger confirmatory cohort studies need to be done to substantiate this finding.

The *RAD51*_135_G>C polymorphism located in the 5′ untranslated region seems to be of functional relevance. There is evidence to suggest that this change enhances the activity of the *RAD51* promoter, which may result in increased RAD51 expression (13). Altered protein levels may influence the activity of the multiprotein DNA-repair complex, including BRCA1, BRCA2, and RAD51. Thus, the functional consequence of this change on the expression of RAD51 suggests that the RAD51 polymorphism may modify disease risk itself and is

Table 7. Ovarian cancer risks in women carrying different *BRCA1* mutations

Genotype	Carriers of the 5382insC mutation			
	Cases (N = 96)	Controls (N = 96)	OR_{crude} (95% CI)	P
	n (%)	n (%)		
*RAD51_135*_G>C				
GG	76 (82%)	67 (70%)	1.00 (reference)	
GC+CC	20 (18%)	29 (30%)	0.57 (0.28-1.16)	0.12
Genotype	Carriers of the 300 T>G mutation			
	Cases (N = 23), n (%)	Controls (n = 23), n (%)	OR_{crude} (95% CI)	P
*RAD51_135*_G>C				
GG	21 (91%)	17 (74%)	1.00 (reference)	
GC+CC	2 (9%)	6 (26%)	0.20 (0.02-1.71)	0.14

not due to any other sequence change in a regulatory region of the gene or in a nearby gene that is in linkage disequilibrium with this polymorphism.

One of the confounding affects of studying modifier genes in populations of *BRCA1* mutation carriers is the potential differential contributions to disease by mutation site. In this study, we were able to assess whether two different mutations in *BRCA1* (300 T>G and 5382insC) contributed to disease risk in the presence or absence of the *RAD51*_135_G>C change. The results revealed that the difference in disease risk was solely attributable to the presence or absence of the *RAD51*_135_G>C allele and not the site of the *BRCA1* mutation. Due to the small numbers of 4153delA mutation carriers, a rigorous statistical analysis for this mutation was not possible.

Our study benefits from matching of cases and controls according to relevant risk factors as well as from a reasonable size for these kinds of studies. The study population consisted of women who harbored mutations in a gene known to confer a high risk of disease against a group of women who harbor the same germ line mutation but have not developed malignancy.

In summary, we have reported a modifying effect for the *RAD51*_135_G>C polymorphism on ovarian cancer risk in Polish *BRCA1* mutation carriers and confirmed the effect on breast cancer risk that had previously been reported on a smaller sample set. The knowledge of decreased breast and ovarian cancer risks in healthy *BRCA1* mutation carriers could lead to the modification of prevention and follow-up strategies for such women.

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