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## Synergistic interaction of variants in *CHEK2* and *BRCA2* on breast cancer risk

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**Abstract** We studied the effects of *BRCA2* and *CHEK2* variants on breast cancer risk in two case-control series from Poland and Belarus. The missense *BRCA2* variant T1915M was associated with a significant reduction in breast cancer risk (OR = 0.62; 95% CI 0.49–0.79;  $P = 0.0007$ ). Modest increases of breast cancer risk were observed for the four analysed *CHEK2* variants (I157T, 1100delC, IVS2 + 1G > A and del5395) (OR = 2.2; 95% CI 1.7–2.8;  $P = 0.0001$ ). The highest risk was observed among women who carried both a *BRCA2* and a *CHEK2* variant (OR = 5.7; 95% CI 1.7–19;  $P = 0.006$ ). We observed a statistically significant interaction effect

between *CHEK2* mutations and the *BRCA2* substitution ( $P = 0.03$ ). These data suggest that the *BRCA2* T1915M polymorphism alone might be associated with a reduced risk of breast cancer, but among *CHEK2* mutation carriers, it may lead to an unexpectedly high risk.

**Keywords** Breast cancer · *CHEK2* · *BRCA2* · Breast cancer · Gene interaction

### Introduction

Major genes for susceptibility to breast cancer include *BRCA1* and *BRCA2*; these genes are characterized by high lifetime penetrance of cancer, with corresponding odds ratios (OR) in excess of ten [1]. Mutations in the *CHEK2* gene confer a more modestly elevated risk of breast cancer (OR two to three) [2–4]. The examination of families with mutations suggests that *CHEK2* may have an important role in modifying the effects of other cancer susceptibility genes, or vice versa [5, 6]. For example, we reported that a *CHEK2* mutation increases the risk of prostate cancer only among men with a specific genotype of *CDKN1B* (encoding the tumour suppressor p27<sup>KIP1</sup>) [7]. The risk of breast cancer in *CHEK2* mutation carriers is not significantly modified by the *CDKN1B* genotype, but other genes which are related to cell cycle control or to DNA damage response might interact with *CHEK2* in breast carcinogenesis. *BRCA2* is such a candidate gene. There are no highly penetrant *BRCA2* founder mutations in Poland, but there is a low-penetrance missense variant (C5972T) which predisposes to early-onset breast cancer (age at diagnosis below 40 years) (OR = 1.4) and to ductal cancer in situ (DCIS) with micro-invasion (OR = 2.8) [8]. DCIS with micro-invasion is also over-represented among women

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with a *CHEK2* mutation.<sup>1</sup> We have developed a breast cancer registry that contains clinical information, pathology details and DNA specimens from unselected patients with breast cancer for the purpose of identifying the principal genes involved in susceptibility to breast cancer and their interactions. We hypothesized that *CHEK2* and *BRCA2* mutations might have an interactive effect on breast cancer risk (and possibly be synergistic).

## Methods

### Cases

The study was approved by the ethics committee of the Pomeranian University. Cases consisted of 1,022 consecutive women with invasive breast cancer diagnosed in the city of Szczecin, Poland. The patient was invited to participate in person during her hospital stay or through a mailed invitation. During the interview the goals of the study were explained, informed consent was obtained, genetic counseling was given and a blood sample was taken for DNA analysis. A detailed family history of cancer was taken (first, and second-degree relatives included) and a risk factor questionnaire was completed. The medical record and pathology report were reviewed. The median age at diagnosis was 56 years.

A second, independent series of cases was 1,756 women with breast cancer who had been diagnosed during the years 1998–2007 at the Belorussian Institute for Oncology and Medical Radiology Aleksandrov N.N. in Minsk or at one of the regional oncology centers in Gomel, Mogilev, Grodno, Brest or Vitebsk. The series mainly consisted of consecutive patients unselected for family history, with the exception of additional 28 cases with familial breast cancer ascertained at the center in Minsk. Median age at diagnosis in the Belarus cohort was 48 years, and a total of 305 patients (16%) reported a first-degree relative with breast cancer.

### Controls

The Polish control subjects consisted of 1,022 healthy women who had no past history of cancer and a negative cancer family history in first- and second-degree relatives. The controls were selected from a database derived from a population-based study of the 1.5 million residents of west Pomerania. During the interview the goals of the study were explained, informed consent was obtained, genetic

counseling was given and a blood sample was taken for DNA analysis. A detailed family history of cancer was taken (first and second-degree relatives included) and a risk factor questionnaire was completed. Women affected with any malignancy or with one or more cancers diagnosed among first- or second degree relatives were excluded from the controls. For each case, a single control was selected. Cases and controls were matched for sex, year of birth (within 2 years) and geographical origin.

The Belorussian controls were 1,019 female volunteers who had been ascertained during the same time period at the Institute for Inherited Diseases in Minsk, Belarus. Subjects were excluded as controls if they reported a personal history of breast cancer, but further details about family history were not obtained. The controls had the same geographical origin as the patients, but were not matched for year of birth. The median age was 51 years.

### Laboratory methods

We evaluated the presence of four *CHEK2* variants in the cases and controls, including one missense variant (I157T) and three truncating variants (I100delC, IVS2 + 1G > A and del5395). The techniques for detection of these mutations have been described previously [9, 10]. We evaluated the presence of the common *BRCA2* variant (T1915M) in the Polish study using a RFLP PCR technique described previously [8]. Duplicate genotyping for quality control was performed for 92 randomly selected samples for *BRCA2* and 462 for *CHEK2*, but no discrepancies with the initial results were found. As a further check, all mutation-positive cases (positive for the *BRCA2* and/or any *CHEK2* mutation) were confirmed by sequencing (ABI DNA Sequencer 377, Perkin Elmer), but again with no discrepancies.

In the Belarus study, a 5'-exonuclease allelic discrimination assay was newly established using the primer pair 5'-GGATGATTCAGAGGATATTCTTCATAACTC-3' and 5'-GTTGTAAAATTTCTTCACTCTGAATGTCAG-3', with the allele-specific probes 5'-FAM-AATGTAGCACGCATT CACATAAG-BHQ-3' and 5'-YakimaYellow-AATGTAGC ATGCATTCACATAAG-BHQ-3' (variant nucleotide underlined). Primers and probes were obtained from Eurogentec. Allelic discrimination assays were run on an SDS 7000 Sequence Detection System (Applied Biosystems). The call rate was 100% for both the case and the control series. The presence of a mutation was independently confirmed by direct sequencing using BigDye chemistry and an Avant 3100 Genetic Analyser (Applied Biosystems).

### Statistical analysis

The prevalences of each of the five alleles were compared in cases and controls. Both controls and cases were in

<sup>1</sup> Cybulski C, Huzarski T, Byrski T, Lubiński J. Estrogen Receptor Status in CHEK2-positive Breast Cancers: Implications for Chemoprevention (submitted for publication).

Hardy–Weinberg equilibrium for the *CHEK2* variants (when all four variants considered as whole, and when considered one by one) and for the *BRCA2* variant, both in the Polish sample and in the Belorussian sample. Odds ratios were generated from two-by-two tables and statistical significance was assessed using the Chi-square Test (with Yates' correction). To evaluate the statistical significance of the observed gene to gene interaction, a multivariate logistic regression was conducted using a model which contained terms for the main effects of the *CHEK2* and *BRCA2* variants separately, as well as an interaction term for the combined presence of the *CHEK2* and *BRCA2* variants. For the analysis of the combined data set the model was adjusted for the study group (Poland and Belarus).

## Results

Initially, the main effects of the alleles in the two genes were estimated in the Polish subjects. At least one of the four *CHEK2* variants (I157T, 1100delC, IVS2 + 1G > A or del5395) was detected in 11.5% of the breast cancer cases and in 6% of the controls (OR = 2.1 95% CI 1.5–2.9;  $P = 0.0001$ ). The odds ratio associated with the missense variant I157T was 1.8 (95% CI 1.3–2.6;  $P = 0.0015$ ) and the odds ratio associated with a truncating mutation was 3.1 (95% 1.5–6.3;  $P = 0.0024$ ). The T1915M variant of *BRCA2* was detected in 5% of the cases and 6% of the controls (OR = 0.9; 95% CI 0.6–1.3).

The distribution of *CHEK2* and *BRCA2* genotypes in the Polish cases and controls are presented in Table 1. There was a modest increase in the risk of breast cancer associated with a *CHEK2* mutation alone (OR = 1.9; 95% CI

1.3–2.6;  $P = 0.0003$ ). Carriers of the *BRCA2* allele alone were more abundant among controls, but no protective effect could be demonstrated (OR = 0.7; 95% CI 0.5–1.1;  $P = 0.14$ ). However, compared to women who carried neither mutation, women who carried a mutation in both genes were at elevated risk (OR = 6.8; 95% CI 1.5–30;  $P = 0.008$ ). To test whether this result reflected a significant synergistic interaction, a logistic regression analysis was performed, which included an interaction term. The results of the logistic model indicate that the effect of the joint genotype was five times greater than expected under a linear model, and the interaction was statistically significant ( $P = 0.04$ ).

We replicated this analysis in a separate set of cases and controls from Belarus (Table 2). At least one of the four *CHEK2* variants (I157T, 1100delC, IVS2 + 1G > A or del5395) was detected in 7% of the breast cancer cases and in 2.5% of the controls (OR = 2.9 95% CI 1.9–4.5;  $P = 0.0001$ ). The odds ratio associated with the missense variant I157T was 2.1 (95% CI 1.3–3.4;  $P = 0.002$ ) and the odds ratio associated with a truncating mutation was 8.5 (95% 2.6–27;  $P = 0.0001$ ). The T1915M variant of *BRCA2* was detected in 6% of the cases and 10% of the controls (OR = 0.6; 95% CI 0.4–0.8;  $P = 0.0005$ ).

In the Belorussian set, the presence of the *BRCA2* variant on its own appeared to be protective (OR = 0.6;  $P = 0.0003$ ). Again, the combination of *CHEK2* and *BRCA2* alterations was found to be associated with the highest risk (OR = 2.9; 95% CI 0.6–13;  $P = 0.25$ ). A two-gene interaction was plausible (OR = 1.8), but the interaction term was not statistically significant ( $P = 0.6$ ) (Table 2).

Evidence for an interaction remained strong when the cases and controls from the two studies were combined (Table 3). In this combined analysis, the *BRCA2* variant on

**Table 1** Combined effect of *CHEK2* and *BRCA2* T1915M variants on breast cancer risk (cases from Poland only)

	Cases <i>n</i> = 1,022	Controls <i>n</i> = 1,022	OR (95% CI)	<i>P</i> -value
Neither mutation	865	904	Baseline	
<i>CHEK2</i> mutation alone	104	58	1.87 (1.34–2.62)	0.0003
<i>BRCA2</i> T1915M alone	40	58	0.72 (0.48–1.09)	0.14
<i>CHEK2</i> mutation and T1915M	13	2	6.79 (1.53–30.2)	0.008
Interaction <i>CHEK2</i> × <i>BRCA2</i>			5.04 (1.04–24.4)	0.04

**Table 2** Combined effect of *CHEK2* and *BRCA2* T1915M variants on breast cancer risk (cases from Belarus only)

	Cases <i>n</i> = 1,756	Controls <i>n</i> = 1,019	OR (95% CI)	<i>P</i> -value
Neither mutation	1,534	896	Baseline	
<i>CHEK2</i> mutation alone	116	24	2.82 (1.81–4.42)	0.0001
<i>BRCA2</i> T1915M alone	96	97	0.56 (0.43–0.78)	0.0003
<i>CHEK2</i> mutation and T1915M	10	2	2.92 (0.64–13.4)	0.25
Interaction <i>CHEK2</i> × <i>BRCA2</i>			1.79 (0.36–8.93)	0.6

**Table 3** Combined effect of *CHEK2* and *BRCA2* T1915M variants on breast cancer risk (cases from Poland and Belarus combined)

	Cases <i>n</i> = 2,778	Controls <i>n</i> = 2,041	OR (95% CI)	<i>P</i> -value
Neither mutation	2,399	1,800	Baseline	
<i>CHEK2</i> mutation alone	220	82	2.18 (1.68–2.84)	0.0001
<i>BRCA2</i> T1915M alone	136	155	0.62 (0.49–0.79)	0.0007
<i>CHEK2</i> mutation and T1915M	23	4	5.71 (1.71–19.0)	0.006
Interaction <i>CHEK2</i> × <i>BRCA2</i>			3.53 (1.15–10.85)	0.03

**Table 4** Combined effect of *CHEK2* missense variant (I157T) and *BRCA2* T1915M variant on breast cancer risk (cases from Belarus and Poland combined)

	Cases <i>n</i> = 2,778	Controls <i>n</i> = 2,041	OR (95% CI)	<i>P</i> -value
Neither mutation	2,472	1,813	Baseline	
<i>CHEK2</i> I157T mutation alone	153	70	1.60 (1.20–2.14)	0.002
<i>BRCA2</i> T1915M alone	136	155	0.64 (0.51–0.82)	0.0004
<i>CHEK2</i> I157T and <i>BRCA2</i> T1915M	17	3	4.16 (1.22–14.2)	0.025
Interaction <i>CHEK2</i> × <i>BRCA2</i>			4.03 (1.12–14.5)	0.03

its own appeared protective (OR = 0.6; 95% CI 0.5–0.8;  $P = 0.0007$ ), whereas in women with only a *CHEK2* mutation, it was associated with an increased risk of breast cancer (OR = 2.2). Compared to women with neither variant, those with both variants were sixfold more prevalent among breast cancer cases (OR = 5.7; 95% CI 1.7–19;  $P = 0.006$ ). The interaction was statistically significant ( $P = 0.03$ ).

We have previously shown that the odds ratios associated with truncating *CHEK2* mutations exceed that associated with the missense *CHEK2* variant I157T. We repeated the analysis of the combined cases of Poland and Belarus and their corresponding controls, but restricted the analysis to only the common missense *CHEK2* variant (Table 4). In combination, the missense variants of the two genes were associated with a fourfold increase in breast cancer risk and, although the sample size of women with both variants was small (17 cases and 3 controls), the interaction was statistically significant ( $P = 0.03$ ).

## Discussion

In Poland, about one in 500 women carries both a *CHEK2* mutation and the T1915M missense variant in *BRCA2*. According to the results from this study, these women may face a risk of breast cancer that is almost seven-fold greater than average. Assuming a background risk of cancer of approximately 5% to age 70, we estimate that Polish women with this combination may have a lifetime breast cancer risk of 30–35%. In comparison, about one in 300 Polish women carries a deleterious founder mutation in *BRCA1*, which is associated with an odds ratio of 15 and a lifetime risk of approximately 70% [11].

In isolation, neither the *CHEK2* I157T nor the *BRCA2* T1915M missense variant appear to lead to a strong influence in cancer risk, but when combined, the cancer risk may be increased to a range that becomes clinically important. However, there were relatively few individuals with both variants among our controls and the confidence interval surrounding the odds ratio is wide. It is important that these observations get extended to other populations where these or other founder mutations are present.

To our knowledge, *BRCA2* T1915M is at present the only low-penetrance missense variant reported for *BRCA2* that seems to be associated with a significant risk modification. As a caveat, our findings warrant confirmation in further replication studies. Another missense substitution, N372H, had initially been proposed as a low-penetrance allele in *BRCA2* [12] but was not confirmed in a subsequent large Consortium study [13]. However, it remains possible that other variants in *CHEK2* or *BRCA2* also participate in an epistatic relationship.

This is the second reported example of a gene-gene interaction involving *CHEK2*, and it remains to be seen whether it could be of relevance also for other malignancies. In 2006, we reported joint effects of *CDKN1B* (encoding the tumour suppressor p27<sup>KIP1</sup>) and *CHEK2* variants on prostate and colon cancer risk [7]. Modest effects on prostate cancer risk were observed for *CHEK2* variants (both missense and truncating). The excess cancer risk was restricted to men who were homozygous for the VV genotype in codon 109 of the *CDKN1B* gene. Among men with the VV genotype, the odds ratios associated with truncating and missense *CHEK2* mutations were 3.1 ( $P < 0.0001$ ) and 1.9 ( $P < 0.0001$ ), respectively. Among men with other *CDKN1B* genotypes (GG and VG), the odds ratios were 1.5 and 1.2 for truncating and missense

*CHEK2* mutations, respectively, and were not statistically significant. The interaction between *CHEK2* and *CDKN1B* was also present in patients with colon cancer, but not in breast cancer patients.

The evidence for a genetic interaction between *CHEK2* and *BRCA2* may reflect the functional interaction of their gene products, in DNA damage recognition and repair. For example, a loss of *CHEK2* would activate its substrate Cdc25 phosphatase which results in *BRCA2* phosphorylation by a cyclin-dependent kinase and subsequent block of homology-directed repair [14]. *CHEK2* may also mediate *BRCA2* expression at the transcript level via *FOXM1* phosphorylation in some cell types [15]. *CHEK2* also regulates the function of *BRCA1* and the loss of *CHEK2*-mediated *BRCA1* phosphorylation seems to be accompanied by a switch from the *BRCA2*-mediated homology-directed repair to the more error prone non homologous end-joining pathway [16]. The *BRCA2* T1915M variant is located in exon 11, coding for the domain that interacts with *RAD51* and this interaction is necessary for the activation of the *BRCA2*-mediated homology-directed repair [17].

Our unexpected and surprising observation is that the *BRCA2* missense allele appears to protect against breast cancer in most women, but in women who carry a *CHEK2* mutation, the cancer risk appears to be augmented. We hypothesize that the *BRCA2* T1915M variant alone may be optimal for the efficiency of homology-directed repair, but when combined with a *CHEK2* deficiency, this potentially protective effect could be lost due to a failure to switch the appropriate repair pathway. Functional studies will be required to support this speculation.

Our findings raise the possibility that polygenic risk models may be complicated by gene-gene interactions. It has recently been proposed that individual breast cancer risk can be measured by counting the number of deleterious variants present from among panel of seven [18]. This method is practical, but may be simplistic if situations similar to ours with *CHEK2* and *BRCA2* were more general, rather than exceptional. It appears likely that the *CHEK2* gene is an important modifier for the risk of cancer imparted by other genes. It may be possible to identify other genes which interact with *CHEK2* in risk modification, and it will be interesting to test additional genes involved in homology-directed repair in this regard.

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