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The BARD1 Cys557Ser variant and risk of familial breast cancer in a South-American population

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Abstract Since the discovery of the *BRCA1* and *BRCA2* genes, much work has been carried out to identify further breast cancer (BC) susceptibility genes. *BARD1* (BRCA1-associated ring domain) was originally identified as a BRCA1-interacting protein but has also been described in tumor-suppressive functions independent of BRCA1. Some association studies have suggested that the *BARD1* Cys557Ser variant might be associated with increased risk of BC, but others have failed to confirm this finding. To date, this variant has not been analyzed in Spanish or South-American populations. In this study, using a case–control design, we analyzed the C-terminal Cys557Ser change in 322 Chilean BC cases with no mutations in *BRCA1* or *BRCA2* and in 570 controls in order to evaluate its possible association with BC susceptibility. *BARD1* Cys557Ser was associated with an increased BC risk ($P = 0.04$, OR = 3.4 [95 % CI 1.2–10.2]) among cases belonging to families with a strong family history of BC. No difference between single cases affected with age <50 years at diagnosis ($n = 117$) and controls was observed for carriers of Cys/Ser genotype. It is likely that this variant is not involved in BC risk in this group of women. We also analyzed a possible interaction between *BARD1* 557Ser/*XRCC3* 241Met variants considering the role of both genes in the maintenance of genome integrity. The combined genotype Cys/Ser-carrier with the *XRCC3* 241Met allele was associated with an increased BC risk ($P = 0.02$, OR = 5.01 [95 % CI 1.36–18.5]) among women belonging to families with at least three BC and/or ovarian cancer cases. Our results suggest that BARD1 557Ser and *XRCC3* 241Met may play roles in BC risk in women with a strong family history of BC. Nevertheless there is no evidence of an interaction between the two SNPs. These findings should be confirmed by other studies and in other populations.

Keywords Familial breast cancer · *BARD1*-Cys557Ser · *XRCC3*-Thr241Met · Polymorphism

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Introduction

Susceptibility to breast cancer (BC) is likely the result of susceptibility alleles in many different genes. Since the discovery of the *BRCA1* and *BRCA2* genes, much attention has been focused on characterizing the remaining genetic risk for BC [1, 2]. Worldwide studies estimates that 70–85 % of BC cases are negative for mutations in *BRCA1* and *BRCA2* [3]. To explain family clustering of *BRCA1/2*-negative BC cases, a polygenic model in which a large number of low- to moderate-penetrance genes are collectively responsible for the disease, has been proposed [4, 5]. The *BARD1* gene is reportedly to be targeted by germline and somatic mutations in a subset of breast and ovarian cancers and has been considered a candidate for association with cancer susceptibility [6].

The gene *BARD1* (BRCA1-associated ring domain) is located on 2q34–35 and codes for a protein of 777 amino acids. BARD1 participates in various important cellular processes such as DNA repair, RNA processing, transcription, cell cycle regulation, and apoptosis [7]. Reduced expression of *BARD1* in vitro results in complex changes to mammary epithelial cells including altered cell shape, increased cell size and aberrant cell cycle progression, further suggesting that *BARD1* repression could give rise to a premalignant phenotype [8]. BARD1 interacts with BRCA1 in vivo. BRCA1 principally forms a heterodimer with BARD1. BRCA1 and BARD1 are related structurally and functionally. Both proteins have an amino-terminal RING domain and two BRCT carboxyl-terminal domains [9]. Because the germline mutations in these domains segregate with susceptibility to BC and breast-ovarian syndrome [10], *BARD1* has been considered a gene suppressor for tumors independent of *BRCA1*.

The *BARD1* germline Cys557Ser variant (rs2897576) is the most studied mutation in this gene, and is located in a region of the gene necessary for induction of apoptosis and possibly for transcription regulation [11, 12]. It was originally classified as a harmless polymorphism [13], but has subsequently been considered to be a disease-associated variant [10, 14]. This variant was identified in an Italian family with five breast and one ovarian cancer (OC) case negative for *BRCA1/2* mutations, and marginal evidence suggested linkage between this variant and BC in that family (LOD score 2.89, $P = 0.06$) [14]. Two large Caucasian population-based studies have provided some support for the hypothesis that *BARD1* Cys557Ser variant is associated with BC risk [7, 15], in particular for a subset of cases with family history, early onset, or multiple primary breast cancers (OR = 2.41, 95 % CI 1.22–4.75, $P = 0.015$) [15]. Some studies have failed to confirm these findings [16–18]. To date the variant Cys557Ser has not been analyzed in Spanish, Asiatic or Hispano-American populations. This point is important considering that the Chilean population is the result of the admixture between Asian and Spanish populations.

In non carriers of *BRCA1/2* mutations, disease susceptibility also may be explained in terms of gene–gene interactions between alleles involved on the same pathways. Specifically, we are interested in interactions between genes involved in DNA repair and maintenance of genome integrity. We have previously shown that the Met/Met genotype was associated with an increased BC risk in *BRCA1/2*-negative cases with familial BC [19]. In this study, using a case–control design we study the *BARD1* 557Ser variant in order to investigate a potential influence of this variant on familial BC susceptibility. We also investigated a possible interaction between *BARD1* 557Ser/*XRCC3* 241Met variants considering the role of both genes in the maintenance of genome integrity.

Methods

Families

A total of 322 BC patients belonging to 322 high-risk *BRCA1/2*-negative Chilean families were selected from the files of the Servicio de Salud del Area Metropolitana de Santiago, Corporación Nacional del Cáncer (CONAC) and other private services of the Metropolitan Area of Santiago. All the index cases were tested for *BRCA1* and *BRCA2* mutations as described [20]. Pedigrees were constructed on the basis of an index case considered to have the highest probability of being a deleterious mutation carrier. None of the families met the strict criteria for other known syndromes involving BC, such as Li-Fraumeni, ataxia-telangiectasia, or Cowden disease.

Table 1 shows the specific characteristics of the families selected according to the inclusion criteria. All families participating in the study self reported Chilean ancestry dating from several generations, after extensive interviews with several members of each family from different generations. In the selected families, 14.0 % (45/322)

Table 1 Inclusion criteria for the families included in this study

Inclusion criteria	Families *n* (%)
Three or more family members with breast and/or ovarian cancer	87 (27.0)
Two family members with breast and/or ovarian cancer	118 (36.6)
Single affected individual with breast cancer ≤ age 35	57 (17.7)
Single affected individual with breast cancer ≤ age 50	60 (18.6)
Total	322 (100)

presented cases of bilateral BC; 9.3 % (30/322) presented cases of both BC and OC, and 2.8 % (9/322) presented male BC. In the BC group, the mean age of diagnosis was 42.5 years and 79.5 % had age of onset <50 years. BC was verified by the original pathology report for all probands.

This study was approved by the Institutional Review Board of the School of Medicine of the University of Chile. Informed consent was obtained from all the participants.

Control sample

The sample of healthy Chilean controls ($n = 570$) were recruited from the files of CONAC. DNA samples were taken from unrelated individuals with no personal or familial history of cancer, who gave their consent for anonymous testing. These individuals were interviewed and informed as to the aims of the study. DNA samples were obtained under considerations of all ethical and legal requirements. The control sample was matched by age and socioeconomic strata with respect to the cases.

Mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes of 322 cases belonging to the high-risk selected families and 570 controls. Samples were obtained according to the method described by Chomczynski and Sacchi [21].

TaqMan assay (Applied Biosystems, Foster City, USA) was used to genotype *BARD1*-Cys557Ser (c.1670G>C, rs28997576). The primers and probes were mixed with polymerase chain reaction reagents in 10 uL final volume containing 5 ng of genomic DNA, 1× TaqMan Genotyping MasterMix and 1× TaqMan SNP Genotyping Assay. Primers and allele-specific TaqMan probes were designed by Custom TaqMan® SNP Genotyping Assays (Applied Biosystems). Polymerase chain reaction was carried out in a StepOne RealTime PCR System (Applied Biosystems). The thermal cycles were initiated for 10 min at 95 °C, followed by 40 cycles each of 92 °C for 15 s and 60 °C for 1 min. Each genotyping run contained a heterozygous DNA control confirmed by sequencing. The alleles were assigned using the software SDS 2.1 (Applied Biosystems). As a quality control, we repeated the genotyping on ~10 % of the samples and all genotype scoring was performed and checked separately by two reviewers unaware of the case–control status.

We previously genotyped *XRCC3*-Thr241Met (c.722C>T, rs861539) in 267 *BRCA1/2*-negative cases and 500 controls according to protocols published previously [19]. In this study we genotyped an additional sample to achieve the sample size required for this study. Therefore, each of the 322 *BRCA1/2* negative cases and 570 controls have genotype for *XRCC3*-Thr241Met.

Statistical analyses

The Hardy–Weinberg equilibrium assumption was assessed in the control sample using a goodness-of-fit χ^2 test. Fisher's exact test was used to test the association of *BARD1*-Cys557Ser genotypes and/or alleles in cases and controls. The association of combined genotypes was assessed by logistic regression analysis. The odds ratio (OR) and its 95 % confidence interval (CI) were calculated to estimate the strength of the association in cases and controls. A P value <0.05 was used as the criterion for statistical significance.

The interaction on the additive scale was assessed by measuring the relative excess risk due to interaction (RERI) [22]. The confidence interval (CI) and P value were calculated according to Hosmer et al. [23] (expected value under the null hypothesis = 0). The interaction on the multiplicative scale was assessed by logistic regression analysis and by calculating the ratio of the combined OR divided by the independent ORs of the SNPs considered in this study (expected value under the null hypothesis = 1). A P value <0.05 was used as the criterion for statistical significance. All statistical analyses were performed using Intercooled Stata 8.2 for Windows (StataCorp, TX, USA).

Results

Table 2 shows the allele and genotype frequencies of *BARD1*-Cys557Ser in the whole sample of *BRCA1/2*-negative BC cases ($n = 322$) and in the subgroups of cases belonging to families with three or more family members with BC and/or OC ($n = 87$) (subgroup a), and single affected women with BC without family history of BC or OC and age of diagnosis before 50 years ($n = 117$) (subgroup b). We found a low frequency of *BARD1*-557Ser among the control population (0.01) and in the 322 *BRCA1/2*-negative BC cases (0.01). No homozygosity was detected in either the cases or controls. The frequency of *BARD1*-Cys557Ser genotypes and alleles did not differ significantly between all BC cases and controls ($P = 0.47$). Nevertheless, within subgroup a, which was comprised of families with at least three BC and/or OC cases, a higher frequency of *BARD1*-Cys557Ser carriers was observed in cases (5.7 %) than in controls (2.5 %), and the difference was statistically significant ($P = 0.04$, OR = 3.4 [95 % CI 1.2–10.2]). Therefore, carriers of *BARD1*-Cys557Ser have a 3.4-fold increase of BC risk only among women belonging to families with a strong family history of BC. No association between single cases affected with age <50 years at diagnosis ($n = 117$) (subgroup b) and controls was observed in the carriers of *BARD1* Cys/Ser genotype (Table 2). In addition we also compared the

Table 2 Genotype and allele frequencies of *BARD1*-Cys557Ser in *BRCA1/2*-negative breast cancer cases and controls

Genotype or allele	Controls (%) ($n = 570$)	All BC cases ($n = 322$)			Families with ≥3 BC and/or OC cases ($n = 87$)			Single affected, diagnosis ≤50 years ($n = 117$)		
		BC cases (%)	*P* value[a]	OR [95 % CI]	BC cases (%)	*P* value[a]	OR [95 % CI]	BC cases (%)	*P* value[a]	OR [95 % CI]
Cys/Cys	560 (98.2)	314 (97.5)	–	Ref.	82 (94.3)	–	Ref.	116 (99.1)	–	Ref.
Cys/Ser	10 (1.8)	8 (2.5)	0.47	1.4 [0.6–3.7]	5 (5.7)	**0.04**	**3.4 [1.2–10.2]**	1 (0.9)	0.70	0.5 [0.1–3.8]
Ser/Ser	0 (0.0)	0 (0.0)	–	–	0 (0.0)	–	–	0 (0.0)	–	–
Cys	1,130 (99.1)	636 (98.8)	–	Ref.	169 (97.1)	–	Ref.	233 (99.6)	–	Ref.
Ser	10 (0.9)	8 (1.2)	0.63	1.4 [0.6–3.6]	5 (2.9)	**0.04**	**3.3 [1.1–9.9]**	1 (0.4)	0.70	0.5 [0.1–3.8]

BC breast cancer, *OC* ovarian cancer, *OR* odds ratio, *CI* confidence interval

[a] Fisher's exact test

Bold values are statistically significant ($P < 0.05$)

allelic and genotype frequencies in subgroup a versus subgroup b. The *BARD1*-557Ser allele was higher in subgroup a being the difference significant ($\chi^2 = 4.12$, $P = 0.04$), and a higher frequency of *BARD1*-557Ser carriers was observed in subgroup a than in subgroup b (Table 2) ($\chi^2 = 4.18$, $P = 0.04$).

The familial BC cases and controls had been genotyped previously in 267 BC cases and in 500 controls for the *XRCC3*-Thr241Met SNP. Therefore, it was possible to analyze whether an association between *BARD1* and BC risk was present in women with a predisposing allele of this SNP. In the present study, the *XRCC3*-Thr241Met polymorphism was analyzed in 322 *BRCA1/2*-negative BC cases and 570 controls. The genotype distributions did not differ significantly in controls ($P = 0.17$) from those predicted by the Hardy–Weinberg equilibrium. Table 3 shows the distribution of the genotypes and allele frequencies of the Thr241Met variant. A higher frequency of Met/Met homozygotes was observed in all BC cases (9.9 %) than in controls (4.6 %), and this difference was statistically significant ($P = 0.002$, OR = 2.5 [95 % CI 1.4–4.4]). We also observed an association between Met/Met homozygote genotypes and increase in BC risk in the cases belonging to families with a strong family history of BC (subgroup b) ($P = 0.006$ OR = 3.2 [95 % CI 1.4–7.2]). The frequency of the Met/Met genotype was higher in the single cases of affected women with age <50 years at diagnosis (8.5 %) (subgroup b) than in controls (4.6 %) but the difference was borderline significant ($P = 0.06$, OR = 2.1 [95 % CI 1.0–4.7]). The analyses of *XRCC3*-Thr241Met in subgroups a and b were not done in our previous article [19].

Table 4 shows the distribution of combined genotypes of the *BARD1* Cys557Ser and *XRCC3*-Thr241Met polymorphisms. The frequency of combined genotypes did not differ significantly between all BC cases and controls ($\chi^2 = 8.81$, $P = 0.177$). Nevertheless there was a significant difference in the distribution of combined genotypes between BC cases of subgroup a and controls ($\chi^2 = 12.32$, $P = 0.031$). The frequency of the double heterozygous condition (Cys/Ser–Thr/Met) was higher in cases of subgroup a (3.4 %) than in controls (1.1 %), with the difference being borderline significant ($P = 0.07$, OR = 3.76 [95 % CI 0.90–15.6]). Nonetheless in subgroup a the frequency of carriers of combined genotype Cys/Ser-carriers of allele *XRCC3* 241Met (Thr/Met + Met/Met) was 4.5 % in cases as compared with 1.1 % in controls ($P = 0.02$, OR = 5.01 [95 % CI 1.36–18.5]) (Table 4). These results could indicate a possible interaction between *BARD1* 557Ser/*XRCC3* 241Met, in women belonging to families with at least three BC and/or OC. To estimate interaction on an additive scale, we calculated RERI. The RERI was 2.89 (CI 95 % −4.72 to 10.49; $P = 0.46$. The measure of interaction on a multiplicative scale, the ratio of OR, was

Table 3 Genotype and allelic frequencies of *XRCC3*-Thr241Met in *BRCA1/2*-negatives breast cancer cases and controls

Genotype or allele	Controls (%) ($n = 570$)	All BC cases ($n = 322$)			Families with ≥3 BC and/or OC cases ($n = 87$)			Single affected diagnosis ≤50 years ($n = 117$)		
		BC cases (%)	*P* value[a]	OR [95 % CI]	BC cases (%)	*P* value[a]	OR [95 % CI]	BC cases (%)	*P* value[a]	OR [95 % CI]
Thr/Thr	335 (58.8)	187 (58.1)	–	Ref.	45 (51.7)	–	Ref.	68 (58.1)	–	Ref.
Thr/Met	209 (36.7)	103 (32.0)	0.45	0.9 [0.7–1.2]	32 (36.8)	0.62	1.1 [0.7–1.9]	39 (33.3)	0.74	0.9 [0.6–1.4]
Met/Met	23 (4.6)	32 (9.9)	**0.002**	**2.5 [1.4–4.4]**	10 (11.5)	**0.006**	**3.2 [1.4–7.2]**	10 (8.5)	0.06	2.1 [1.0–4.7]
Thr/Met + Met/Met	232 (41.3)	135 (41.9)	0.78	1.0 [0.8–1.4]	42 (48.3)	0.20	1.3 [0.9–2.1]	49 (41.8)	0.92	1.0 [0.7–1.6]
Thr	879 (77.1)	477 (74.1)	–	Ref.	122 (70.1)	–	Ref.	175 (74.8)	–	Ref.
Met	261 (22.9)	167 (25.9)	0.14	1.2 [0.9–1.5]	52 (29.0)	0.06	1.4 [1.0–2.0]	59 (25.2)	0.45	1.1 [0.8–1.6]

BC breast cancer, *OC* ovarian cancer, *OR* odds ratio, *CI* confidence interval

[a] Fisher's exact test Bold values are statistically significant ($P < 0.05$)

2.14 (95 % CI 0.16–27.99; $P = 0.56$). Therefore, considering the obtained *P* values, there is no evidence of an interaction between the two SNPs.

Discussion

Mutations in *BRCA1* and *BRCA2* are associated with susceptibility to BC and OC. At present, however, these mutations account for only a portion of familial cases, and consequently there is an intensive search for additional susceptibility targets. One of these is *BARD1*, which participates in various important cellular processes such as DNA repair, DNA processing, transcription, cell cycle regulation and apoptosis [7]. BARD1 interacts with BRCA1 in vivo, and it is required for BRCA1 stability and nuclear localization [22]. Both proteins interacts via their respective amino terminal RING finger domains. *BARD1* also interacts genetically with *BRCA2*, and a physical interaction of the BARD1 and BRCA2 pathway in mitosis was also described recently [24, 25].

Since 2004, there have been several publications analyzing BC and genetic variations in *BARD1*. The most studied mutation in *BARD1* in the missense variant Cys557Ser, which is located in a region of the gene necessary for induction of apoptosis and possibly for transcription regulation [11, 12]. The *BARD1* Cys557Ser variant, has been previously reported to contribute to BC risk. Karppinen et al. [10] screened for *BARD1* mutations in 126 cases of familial BC in Finnish population. The variant Cys557Ser had a frequency of 5.6 % in cases vs. 1.4 % in controls (OR = 4.2, [CI 1.7–10.7] $P = 0.001$). The authors concluded that the variant could be a common BC susceptibility allele. In a second study, Karppinen et al. [7] carried out a case–control study in 2,906 cases with BC/OC and 3,591 controls, in a Nordic population. The frequency of the variant was greater in *BRCA1/2*-negative cases (6.8 %) vs. controls (2.7 %) (OR = 2.6 [95 % CI 1.7–4.0], $P < 0.001$). The authors concluded that Cys557Ser increases risk for BC by 2.6 in *BRCA1/2*-negative families and may interact with other susceptibility genes in the context of a polygenic model. Stacey et al. [15] analyzed Cys557Ser in an Iceland population and showed that the frequency of the variant is 0.037 in cases with familial BC vs. 0.016 in controls (OR = 2.41 [CI 95 % 1.22–4.75] $P = 0.015$). Jakubowska et al. [17] found no correlation between the variant Cys557Ser and familial BC in a Polish population but reported an association with the subgroup of women diagnosed at a very early age (OR = 2.9 [CI 95 % 1.2–7.1], $P = 0.03$). Johnatty et al. [26], in an Australian population, reported a non-significant increased prevalence of the variant for cases with high BC predisposition, and concluded that the *BARD1*

Table 4 Distribution of combined genotypes of *BARD1*-Cys557Ser and *XRCC3*-Thr241Met in *BRCA1/2*-negative breast cancer cases and controls

Combine genotype (*BARD1*-Cys557Ser–*XRCC3*-Thr241Met)	Controls (%) ($n = 570$)	All BC cases ($n = 322$)			Families with ≥3 BC and/or OC cases ($n = 87$)		
		BC cases (%)	*P* value[a]	OR [95 % CI]	BC cases (%)	*P* value[a]	OR [95 % CI]
Cys/Cys–Thr/Thr	331 (58.1)	183 (56.8)	–	Ref.	44 (50.6)	–	Ref.
Cys/Cys–Thr/Met	203 (35.6)	100 (31.1)	0.45	0.89 [0.66–1.20]	29 (33.3)	0.78	1.07 [0.65–1.77]
Cys/Cys–Met/Met	26 (4.6)	31 (9.6)	**0.006**	**2.16 [1.24–3.74]**	9 (10.3)	**0.02**	**2.60 [1.14–5.92]**
Cys/Cys–Thr/Met + Met/Met	229 (40.2)	131 (40.7)	0.81	1.03 [0.45–7.31]	38 (43.6)	0.35	1.24 [0.78–1.99]
Cys/Ser–Thr/Thr	4 (0.7)	4 (1.2)	0.41	1.81 [0.45–7.31]	1 (1.1)	0.58	1.88 [0.21–17.2]
Cys/Ser–Thr/Met	6 (1.1)	3 (0.9)	0.89	0.90 [0.22–3.66]	3 (3.4)	0.07	3.76 [0.90–15.6]
Cys/Ser–Met/Met	0 (0.0)	1 (0.3)	–	–	1 (1.1)	–	–
Cys/Ser–Thr/Met + Met/Met	6 (1.1)	4 (1.2)	0.77	1.21 [0.34–4.33]	4 (4.5)	**0.02**	**5.01 [1.36–18.5]**
Ser/Ser–Thr/Thr	0 (0.0)	0 (0.0)	–	–	0 (0.0)	–	–
Ser/Ser–Thr/Met	0 (0.0)	0 (0.0)	–	–	0 (0.0)	–	–
Ser/Ser–Met/Met	0 (0.0)	0 (0.0)	–	–	0 (0.0)	–	–

Measure of interaction on additive scale: RERI = 2.89 (95 % CI −4.72 to 10.49); $P = 0.46$

Measure of interaction on multiplicative scale: ratio of ORs = 2.14 (95 % CI 0.16–27.99); $P = 0.56$

BC breast cancer, *OC* ovarian cancer, *OR* odds ratio, *CI* confidence interval

[a] Fisher's exact test Bold values are statistically significant ($P < 0.05$)

Cys557Ser variant is not associated with BC risk. In summary, to date this variant has been reported in samples from Iceland, Finland, Italy, and in Americans of European descendent, suggesting that a single ancient mutation has become geographically widespread in European-descendent populations. Stacey et al. [15] and others did not find *BARD1* Cys557Ser variant in samples from Yoruban, Han Chinese, Japanese and African-American individuals [13, 27]. Therefore, these authors suggested that the variant may be restricted to individuals with European ancestry and could contribute to the higher load of BC seen in this ethnic group. Nevertheless, to date this variant has not been investigated in Spanish, or Hispano-American populations.

In this study, we first analyzed whether the *BARD1* Cys557Ser variant could be responsible for increased risk of BC in an South-American population. We found a low frequency of *BARD1*-557Ser among controls (0.01) and in *BRCA1/2*-negative BC cases (0.01). The frequency in cases concur with those obtained by Karppinen et al. [7] in familial BC cases from an European Nordic population. Nevertheless, the frequency of *BARD1*-Cys557Ser in controls was slightly lower with respect the frequency reported for the Nordic population controls (1.8 vs 2.7 %, respectively). This difference could be consequence of the ethnic composition of the Chilean population or of the characteristics of the control sample. The contemporary Chilean population stems from the admixture of Amerindian peoples with the Spanish settlers arriving in the 16th and 17th centuries [28]. The relationships among ethnicity in the Amerindian admixture, genetic markers, and socioeconomic strata have been extensively studied in Chile [29–31]. Therefore, it is likely that the frequency of this variants was minor in the Amerindian peoples or in the Spanish population. On the other hand, our controls were healthy individuals without a family history of BC. In contrast, Nordic controls were anonymous voluntary and cancer-free blood donors.

The results of our case–control study showed an association of the *BARD1* 557Ser variant with increased BC risk only among *BRCA1/2*-negative woman belonging to families that had at least three BC and/or OC cases, suggesting that this mutation contributes to familial BC in the Chilean population. We also found that *BARD1* 557Ser variant is not involved in BC in the subgroup of single affected with BC without family history of BC or OC and age of diagnosis <50 years. Probably the BC in this group of women is associated with other gene variants such as low penetrance alleles [32]. Nevertheless, considering the reduced sample size of the subgroups, these findings should be confirmed by studies using a larger sample in order to substantially decrease the probability of false positive results.

We also analyzed the effect of the combined genotypes of the *BARD1*-Cys557Ser and *XRCC3*-Thr241Met polymorphism in BC risk considering the role of both genes in the maintenance of genome integrity. Our results showed a higher frequency of the double heterozygous condition (Cys/Ser–Thr/Met) in cases (3.4 %) belonging to families with a strong family history of BC (≥3 cases) than in controls (1.1 %) being the difference borderline significant (OR = 3.76 [95 % CI 0.90–15.6), $P = 0.07$). Nonetheless,

the combined genotype Cys/Ser-carrier with the *XRCC3* 241Met allele was associated with an increased BC risk ($P = 0.02$, OR = 5.01 [95 % CI 1.36–18.5]) among women belonging to families with at least three BC and/or ovarian cancer cases. The estimated measures of interaction both on the additive and multiplicative scales were greater than the sum of the estimated effects of each SNP alone, although without achieving statistical significance. Therefore there is no evidence of an interaction between the two SNPs. The reduced sample size of our study did not allow to reach statistical significance of interaction for the OR in either additive or multiplicative scales. Nevertheless, it has been proposed that other BC susceptibility alleles act together with Cys557Ser [15]. Inherited predisposition to BC is linked to mutations in genes that are involved in DNA repair pathways and functionally related with *BRCA1* and *BRCA2*. *BARD1* has been implicated in multiple crucial cellular processes including DNA repair [33–35]. Soon after the onset of DNA damage, BRCA1/BARD1 attract certain constitutive interacting partner proteins to sites of acute DNA damage. Forget et al. [36] provided the first demonstration that *XRCC3* forms a nuclear foci that localize to the sites of double strand breaks. Probably the XRCC3 protein could be one of the protein that interacts with the BRCA1/BARD1 complex. Furthermore, XRCC3 interacts with RAD51 as part of a multiprotein complex involved in double-strand breaks repair through homologous recombination [37] in which BRCA1 protein is involved.

In conclusion, our results suggest that *BARD1* 557Ser and *XRCC3* 241Met may play roles in BC risk in women with a strong family history of BC and no provides evidence of gene–gene interaction between *BARD1* 557Ser and *XRCC3* 241Met.

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Conflict of interest None.

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