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EPIDEMIOLOGY

Variants in DNA double-strand break repair genes and risk of familial breast cancer in a South American population

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Abstract The double-strand break (DSB) DNA repair pathway has been implicated in breast cancer (BC). RAD51 and its paralogs XRCC3 and RAD51D play an important role in the repair of DSB through homologous recombination (HR). Some polymorphisms including *XRCC3*-Thr241Met, *RAD51*-135G>C, and *RAD51D*-E233G have been found to confer increased BC susceptibility. In order to detect novel mutations that may contribute to BC susceptibility, 150 patients belonging to 150 Chilean *BRCA1/2*-negative families were screened for mutations in *XRCC3*. No mutations were detected in the *XRCC3* gene. In addition, using a case–control design we studied the *XRCC3*-Thr241Met, and *RAD51D*-E233G polymorphisms in 267 BC cases and 500 controls to evaluate their possible association with BC susceptibility. The *XRCC3* Met/Met genotype was associated with an increased BC risk ($P = 0.003$, OR = 2.44 [95%CI 1.34–4.43]). We did not find an association between E233G polymorphism and BC risk. We also analyzed the effect of combined genotypes among *RAD51*-135G>C, Thr241Met, and E233G polymorphisms on BC risk. No interaction was observed between Thr241Met and 135G>C. The combined genotype Thr/Met–E/G was associated with an increased BC risk among women who (a) have a family history of BC, (b) are *BRCA1/2*-negative, and (c) were <50 years at onset ($n = 195$) ($P = 0.037$, OR = 10.5 [95%CI 1.16–94.5]). Our results suggested that the variability of the DNA HR repair genes *XRCC3* and *RAD51D* may play a role in BC risk, but this role may be underlined by a mutual interaction between these genes. These findings should be confirmed in other populations.

Keywords Familial BC · *XRCC3*-Thr241Met · *RAD51D*-E233G · *RAD51*-135G>C · Polymorphism

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Introduction

Breast Cancer (BC) is the most common cancer in women in the world. It has been estimated that one out of every nine women will develop BC during their lives [1]. In Chile, mortality in women due to BC has risen, from 8.5 deaths per 100,000 women in 1985 to 12.8 per 100,000 women in 1995 [2]. Currently, it has the second-highest mortality rate among

cancers (13.8/100,000 women) [3]. Incidence increased from 16.2/100,000 women in 1998 to 26.2/100,000 women in 2002 [4]. Hereditary BC accounts for around 5–15% of all BC cases [5]. Together, germline mutations in the two major susceptibility genes *BRCA1* and *BRCA2* (*BRCA1/2*) account for approximately 20% of the familial BC cases [6]. This indicates the potential involvement of additional susceptibility genes that would account for low to moderate BC risk. This discovery would represent an important step in defining individual risks for hereditary BC.

Several studies have reported that mutations in genes involved in DNA repair and maintenance of genome integrity may be responsible for increase of cancer risk. Double-strand break (DSB) damage is the most injurious lesion observed because it causes cell death or loss of genetic material. Genes involved in DSB DNA repair are good candidates for low-penetrance genes that contribute to the development of BC. The human *RAD51* gene, a homolog of *Escherichia coli* RecA, has a key role in recombination and DNA repair of DSBs through homologous recombination (HR). This gene interacts with BRCA1 and BRCA2 [7, 8]. Several Rad51-related proteins, *XRCC2*, *XRCC3*, *RAD51C*, and *RAD51D,* also interacts with Rad51 as part of a multiprotein complex that forms a heterodimer with each of the genes involved in DSB repair through HR [7]. Some of these genes (*RAD51*, *XRCC2*, and *XRCC3*) have already been analyzed for genetic variants in BC cases, with some polymorphisms found to confer increased susceptibility [9]. Polymorphisms in DSB genes that could potentially alter function have been evaluated in relation to BC risk in a number of epidemiological studies. Some of the studied polymorphisms include *RAD51*-135G>C [10–13], *XRCC3*-Thr241Met [9, 13–20], and *RAD51D*-E233G [21, 22].

We have previously shown that the *RAD51*-135G>C polymorphism presents an increased risk of familial BC in women with age < 50 years at diagnosis, and we proposed that this polymorphism may be a BC risk variant [23]. The X-ray repair cross-complementing group 3 (*XRCC3*) gene codes for a protein participating in DSB repair through HR. *XRCC3* has been of considerable interest as a candidate susceptibility gene for cancer. The Thr241Met substitution has been the most thoroughly investigated polymorphism in *XRCC3* [24]. *RAD51D*, a paralog of the mammalian *RAD51* gene, is an important component for DNA repair and telomere maintenance. A *RAD51D* variant, E233G, was identified as a potential susceptibility allele in high-risk, site-specific, familial BC [21].

To detect potential novel gene defects that may contribute to heredity breast or ovarian cancer susceptibility, 150 *BRCA1/2*-negative BC cases belonging to Chilean high-risk families ($n = 150$) were screened for mutations in *XRCC3* using conformation-sensitive gel electrophoresis and DNA sequencing. In addition, using a case–control design, we studied the *XRCC3*-Thr241Met, *RAD51*-135G>C, and *RAD51D*-E233G polymorphisms in order to investigate a potential influence of these variants on familial BC susceptibility.

Methods

Families

A total of 267 BC patients belonging to 267 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 [25]. 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

Table 1 Selection criteria for the families included in this study

Selection criteria	Families
2 Family members with breast cancer	53
2 Family members with breast cancer, onset before age 40 in one	36
≥2 Family members with breast cancer, and ovarian cancer in one	8
≥3 Family members with breast cancer	35
≥3 Family members with breast cancer, at least one with onset before age 40	21
3 Family members with breast cancer, one male cancer	3
Single affected individual with breast cancer < age 51	31
Single affected individual with breast cancer < age 31	74
Breast cancer in a male	6
Total	267

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, 11.2% (30/267) presented cases of bilateral BC; 8.2% (22/267) presented cases of both breast and ovarian cancer, and 2.8% (7/267) presented male BC. In the BC group, the mean age of diagnosis was 43.9 years and 72.3% 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 population

The samples of healthy Chilean controls ($n = 500$) 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

DNA extraction

Genomic DNA was extracted from peripheral blood lymphocytes of 267 cases belonging to the high-risk selected families. Samples were obtained according to the method described by Chomczynski and Sacchi [26].

XRCC3 complete sequence analysis

A subset of 150 high-risk BC index cases negative for *BRCA1* and *BRCA2* point mutations were screened for *XRCC3* sequence variants. The whole coding sequence and exon–intron boundaries of the *XRCC3* gene were amplified by polymerase chain reaction (PCR). Primers were designed using PRIMER3 software (http://frodo.wi.mit.edu/primer3/) (Table 2). Each 35 ul of the reaction contained 40 ng genomic DNA, 10 pmol of each primer, 200uM each of dATP, dCTP, dGTP, dTTP (Bioron, Germany), 2 mM $MgCl_2$, 1X reaction buffer, and 1U Taq (Biotools B and M Labs, Spain). The thermal cycles were initiated for 5 min at 94°C, followed by 30 cycles of 30 s at 95°C, 30 s at 48°C, 30 s at 72°C, and 10 min final extension at 72°C. The fragments obtained were analyzed for sequence variants using conformation-sensitive gel electrophoresis (CSGE) [27]. Amplified samples were held at 95°C for 5 min and at 65°C for 1 h to generate a heteroduplex. The products were diluted 1:2 in sucrose buffer and loaded in a partially denaturing MDE® gel (Cambrex, UK) at 7 W constant power for different periods depending on the size of the fragment. Gels were silver-stained and dried in a vacuum gel dryer. All sequence variants detected by CSGE were identified by reamplification of the original DNA sample. Direct sequencing was performed in an ABI Prism 310 automated fluorescence-based cycle sequencer and a Rhodamine dye terminator system (Perkin Elmer Applied Biosystems, Foster City, CA). The CSGE assay is technically simple and has a high sensitivity for the detection of mutations. The main factors influencing sensitivity are the gel matrix and the identity of base mismatch [28, 29]. MDE matrix is a polyacrylamide-like matrix that is highly sensitive to DNA conformation differences. Separation on a MDE gel provides superior results when compared with standard polyacrylamide gels [30, 31].

Table 2 Primer for *XRCC3* mutation analysis

Exon	Primer sequence (5′–3′)	Fragment length (bp)	3′ Intron boundary (bp)	5′ Intron boundary (bp)
4	F: TGCATCAGTGTCTGGAATGG	446	102	91
	R: ATCAGGGTAGGCAAAGGAAG			
5	F: GCTCTCACAGTGCTCTTAAC	431	77	176
	R: ATCTTCTGACCCGATGCTGT			
6	F: CTGACTCTGCATCTCAGGT	529	184	93
	R: AATGGTAGGAACAGCGCAAG			
7	F: GCACTTGATTTGTGTGGCAG	366	52	119
	R: ATGACCCATGCTGTGTTCAG			
8	F: TTTCAGACGGTCGAGTGAC	450	73	123
	R: CTTCTCGATGGTTAGGCACA			
9 and 10	F: TGTGCCTAACCATCGAGAAG	596	41	131[a]
	R: CTTCGGATGAGAAAGTGGAG			

[a] 3′UTR from stop codon

XRCC3-Thr241Met, RAD51-135G>C, and RAD51D-E233G variants analyses

A polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) assay was used to genotype *XRCC3*-Thr241Met (c.722C>T, rs861539), *RAD51*-135G>C (c.-98G>C, rs1801320), and *RAD51D*-E233G (*RAD51L3* c.639A>G, rs28363284) variants in 267 *BRCA1/2*-negative cases and 500 controls. PCR products were digested overnight with restriction enzymes (New England Biolabs, MA, USA) according to the manufacturer protocols and analyzed in 3% agarose gel electrophoresis. Primers, restriction enzymes, and the length of digested fragments are shown in Table 3.

Statistical analyses

The Hardy–Weinberg equilibrium assumption was assessed in the control sample using a goodness-of-fit chi-square test. Fisher's exact test was used to test the association of *XRCC3*-Thr241Met, *RAD51*-135G>C, and *RAD51D*-E233G 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 significance. All statistical analyses were performed using Intercooled Stata 8.2 for Windows (StataCorp, TX, USA).

Results

In the 150 BC patients negative for *BRCA1/2* point mutations, no variation except the Thr241Met polymorphism was detected in the exon or splice boundary regions of the *XRCC3* gene. The Thr241Met polymorphism was present in the 31.3% of the analyzed cases.

The *RAD51*-135G>C and *XRCC3*-Thr241Met polymorphisms and the *RAD51D*-E233G variant were analyzed in 267 *BRCA1/2*-negative cases with familial BC and in 500 controls. None of the genotype distributions differ significantly in controls ($P > 0.05$) from those predicted by the Hardy–Weinberg equilibrium. Table 4 shows the distribution of the genotypes and allele frequencies of the 135G>C, E233G, and Thr241Met variants. The frequencies of the genotypes and alleles of 135G>C and E233G variants did not differ significantly between cases and controls. With respect to the Thr241Met polymorphism, the frequency of the allele Met was higher in cases than controls ($P = 0.03$). Furthermore a higher frequency of Met/Met homozygotes was observed in BC cases (10.1%) than controls (4.4%), and this difference was statistically

Table 3 Primers, restriction enzymes and fragment lengths for *XRCC3*-Thr241Met, *RAD51*-135G>C and *RAD51D*-E233G variants

Variant	Primer sequence	Annealing temperature	Restriction enzyme	Fragment length	Reference
XRCC3 Thr241Met (c.722C>T, rs861539)	F: 5′-TTTCAGACGGTCGAGTGAC-3′ R: 5′ CTTCTCGATGGTTAGGCACA 3′	48°C	*Nla*III	Thr allele: 299 bp + 149 bp; Met allele: 194 bp + 149 bp + 105 bp	Smith et al. [16]
RAD51 135G>C (c.-98G>C, rs1801320)	F: 5′-TGGGAACTGCAACTCATCTGG-3′ R: 5′-GCGCTCCTCTCTCCAGCAG-3′	56°C	*Bst*NI	G allele: 86 bp + 71 bp; C allele: 157 bp	Levy-Lahad et al. [10]
RAD51D E233G (*RAD51L3* c.639A>G, rs28363284)	F: 5′-GGCCTTGATGATGCTGCTG-3′ R: 5′-GCTCGCAATAACTAGAAATCAA-3′	56°C	*Alu*I	E allele: 28 bp + 87 bp; G allele: 115 bp	[a]

[a] Primers and protocols were designed by the authors

Table 4 Genotype and allelic frequencies of *RAD51*-135G>C, *RAD51D*-E233G and *XRCC3*-Thr241Met in *BRCA1/2*-negative breast cancer cases and controls

Gene	Genotype or allele	BC cases (%) ($n = 267$)	Controls (%) ($n = 500$)	P value[a]	OR [95%CI]
RAD51	GG	232 (86.9%)	441 (88.2%)	–	1.00 (reference)
	GC	33 (12.4%)	58 (11.6%)	0.410	1.08 [0.68–1.71]
	CC	2 (0.7%)	1 (0.2%)	0.276	3.80 [0.34–42.17]
	G	497 (0.93)	940 (0.94)	-	1.00 (reference)
	C	37 (0.07)	60 (0.06)	0.272	1.17 [0.76–1.78]
RAD51D	E/E	262 (98.1%)	492 (98.4%)	–	1.00 (reference)
	E/G	5 (1.9%)	8 (1.6%)	0.494	1.17 [0.38–3.63]
	G/G	0 (0.0%)	0 (0.0%)	–	–
	E	529 (0.99)	992 (0.99)	–	1.00 (reference)
	G	5 (0.01)	8 (0.01)	0.494	1.17 [0.38–3.60]
XRCC3	Thr/Thr	149 (55.8%)	296 (59.2%)	–	1.00 (reference)
	Thr/Met	91 (34.1%)	182 (36.4%)	0.520	0.99 [0.72–1.37]
	Met/Met	27 (10.1%)	22 (4.4%)	0.003	2.44 [1.34–4.43]
	Thr	389 (0.73)	774 (0.77)	–	1.00 (reference)
	Met	145 (0.27)	226 (0.23)	0.030	1.28 [1.00–1.63]

[a] Fisher's exact test

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

significant ($P = 0.003$, OR = 2.44 [95%CI 1.34–4.43]). Therefore, Met/Met homozygotes have a 2.44-fold increase of BC risk. No effect between cases with age < 50 years at diagnosis ($n = 195$) and controls was observed in the carriers of Met/Met genotype (data not shown). In relation to the E233G variant, we found a low frequency among control population and in the 267 *BRCA1/2*-negative BC cases selected by family history. No homozygosity was found; the variant appeared heterozygous with a frequency of 1.6% among controls and 1.9% in the cases with a familial aggregation pattern ($P = 0.494$) (Table 4).

Table 5 shows the distribution of combined genotypes of the Thr241Met and 135G>C polymorphisms. There was a significant difference in the distribution of combined genotypes between BC patients and controls (chi-squared = 13.4, $P = 0.02$). None of the combined genotypes showed a protective role against BC. The only combined genotype that showed a significant difference was Met/Met–GG, but GG is the wild-type genotype of *RAD51*-135G>C. The distribution of the Met/Met–GG combined genotype is the same as that obtained for the Met/Met genotype (Table 4). These results do not show an interaction between Thr241Met and 135G>C and suggest that the 135G>C polymorphism does not modify the BC risk among Met/Met carriers. In a previous manuscript, our group reported that *RAD51*-135G>C genotypes (G/C and C/C) were associated only in cases: (a) with a family history of BC, (b) *BRCA1/2*-negative, and (c) with age at onset < 50 years [23]. Table 6 shows the frequencies of combined genotypes of the Thr241Met and 135G>C polymorphisms for *BRCA1/2*-negative cases with age at diagnosis < 50 years ($n = 195$) and controls ($n = 500$).

Table 5 Distribution of combined genotypes of *XRCC3*-Thr241Met and *RAD51*-135G>C in *BRCA1/2*-negative breast cancer cases and controls

Combine genotype (*XRCC3*-Thr241Met–*RAD51*-135G>C)	BC cases (%) ($n = 267$)	Controls (%) ($n = 500$)	P value[a]	OR [95%CI]
Thr/Thr–GG	124 (46.4%)	257 (51.4%)	–	1.00 (reference)
Thr/Thr–GC	23 (8.6%)	38 (7.6%)	0.49	1.25 [0.72–2.20]
Thr/Thr–CC	2 (0.7%)	1 (0.2%)	0.25	4.15 [0.37–46.2]
Thr/Met–GG	81 (30.3%)	164 (32.8%)	0.89	1.02 [0.73–1.44]
Thr/Met–GC	10 (3.7%)	18 (3.6%)	0.73	1.15 [0.51–2.57]
Thr/Met–CC	0 (0.0%)	0 (0.0%)	–	–
Met/Met–GG	27 (10.1%)	20 (4.0%)	0.001	2.80 [1.51–5.18]
Met/Met–GC	0 (0.0%)	2 (0.4%)	–	–
Met/Met–CC	0 (0.0%)	0 (0.0%)	–	–

[a] Fisher's exact test

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

Table 6 Genotype and allelic frequencies of *RAD51*-135G>C, and combined frequencies of *XRCC3*-Thr241Met–*RAD51*-135G>C in *BRCA1/2*-negative breast cancer cases with age < 50 years at diagnosis and controls

Genotype or allele (*RAD51*-135G>C)	BC cases (%) ($n = 195$)	Controls (%) ($n = 500$)	*P* value[a]	OR [95%CI]
GG	159 (81.5%)	441 (88.2%)	–	1.00 (reference)
GC	34 (17.4%)	58 (11.6%)	0.027	1.63 [1.03–2.58]
CC	2 (1.0%)	1 (0.2%)	0.175	5.55 [0.50–61.63]
GC + CC	36 (18.5%)	59 (11.8%)	0.016	1.69 [1.08–2.66]
G	352 (0.90)	940 (0.94)	–	1.00 (reference)
C	38 (0.10)	60 (0.06)	0.012	1.68 [1.10–2.57]
Combine genotype (*XRCC3*-Thr241Met–*RAD51*-135G>C)				
Thr/Thr–GG	86 (44.1%)	257 (51.4%)	–	1.00 (reference)
Thr/Thr–GC	24 (12.3%)	38 (7.6%)	0.028	1.89 [1.07–3.33]
Thr/Thr–CC	2 (1.0%)	1 (0.2%)	0.146	5.98 [0.54–66.73]
Thr/Met–GG	55 (28.2%)	164 (32.8%)	0.991	1.00 [0.68–1.48]
Thr/Met–GC	10 (5.1%)	18 (3.6%)	0.220	1.66 [0.73–3.73]
Thr/Met–CC	0 (0.0%)	0 (0.0%)	–	–
Met/Met–GG	18 (9.2%)	20 (4.0%)	0.004	2.68 [1.36–5.32]
Met/Met–GC	0 (0.0%)	2 (0.4%)	–	–
Met/Met–CC	0 (0.0%)	0 (0.0%)	–	–

[a] Fisher's exact test

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

The distributions of Thr/Thr–GC and Thr/Thr–CC combined genotypes are similar to the G/C + C/C *RAD51*-135G>C genotypes, and Thr/Thr is the wild-type genotype. Therefore, it is possible to conclude that the Thr241Met polymorphism does not modify BC risk among women who are *RAD51* 135C carriers diagnosed at <50 years.

Table 7 shows the distribution of combined genotypes of *XRCC3*-Thr241Met and *RAD51D*-E233G in *BRCA1/2*-negative BC cases and controls. The frequency of the double heterozygous condition (Thr/Met–E/G) was higher in cases (1.5%) than controls (0.2%), but the difference was borderline significant ($P = 0.076$, OR = 7.84 [95%CI 0.87–70.75]). These results allow us to conclude that E233G does not modify the BC risk due to Thr241Met. Table 7 also gives the distribution of combined genotypes for cases with age < 50 years at diagnosis ($n = 195$) and controls ($n = 500$). The frequency of the double heterozygous condition (Thr/Met–E/G) was 1.3% in cases and 0.2% in controls ($P = 0.037$, OR = 10.5 [95%CI 1.16–94.5]). Therefore, the carriers of combined genotype Thr/Met–E/G have a 10.5-fold increase of BC risk in women age < 50 years at diagnosis.

Discussion

Mutations in *BRCA1* and *BRCA2* are associated with susceptibility to breast and ovarian cancer. However, at present, these mutations account for only a portion of familial cases, and consequently additional targets are being sought intensively. Genetic instability acquired through inefficient DSB repair is believed to be a component of BC susceptibility. The X-ray repair cross-complementing group 3 gene (*XRCC3*) is a highly suspected candidate gene for cancer susceptibility. XRCC3 plays a role in homologous recombination through direct interaction with *RAD51*, *RAD51D*, *XRCC2*, *BRCA1*, *BRCA2*, etc. to form a complex essential for the repair of DSBs and DNA cross-links and for the maintenance of chromosome instability [7]. XRCC3 may also play a critical role in the restoration of damaged or collapsed replication forks, which may be of great significance for proliferating cancer cell activity [32]. Therefore, it seems justified to seek genetic variations in *XRCC3* and an association between genetic variability of the *RAD51*, *RAD51D*, and *XRCC3* genes and BC.

Therefore, in this study, we first screened for germline mutations in *XRCC3* that could be responsible for increased risk of familial BC. However, no disease-related alterations were observed in the *XRCC3* coding sequence, with exception of the Thr241Met polymorphism. To date, no reports have been published that have investigated the coding regions of *XRCC3* for variations involved in BC. In the *XRCC3* genetic variation database (www.ensembl.org; accession number ENSG00000126215) the nucleotide diversity is lower in the coding region than the noncoding region. It is likely that the lower diversity in the coding region may be due to selective pressure that eliminates

Table 7 Distribution of combined genotypes of *XRCC3*-Thr241Met and *RAD51D*-E233G in *BRCA1/2*-negative BC cases in the subgroup with age < 50 years at diagnosis and controls

Combine genotype (*XRCC3*-Thr241Met–*RAD51D*-E233G)	BC cases (%)	Controls (%) ($n = 500$)	*P* value (a)	OR [95%CI]
All BC cases ($n = 267$)				
Thr/Thr–E/E	148 (55.4%)	290 (58.0%)	–	1.00 (reference)
Thr/Thr–E/G	1 (0.4%)	6 (1.2%)	0.302	0.33 [0.04–2.74]
Thr/Thr–G/G	0 (0.0%)	0 (0.0%)	–	–
Thr/Met–E/E	87 (32.6%)	181 (36.2%)	0.716	0.94 [0.68–1.30]
Thr/Met–E/G	4 (1.5%)	1 (0.2%)	0.067	7.84 [0.87–70.75]
Thr/Met–G/G	0 (0.0%)	0 (0.0%)	–	–
Met/Met–E/E	27 (10.1%)	21 (4.2%)	0.002	2.51 [1.38–4.61]
Met/Met–E/G	0 (0.0%)	1 (0.2%)	–	–
Met/Met–G/G	0 (0.0%)	0 (0.0%)	–	–
BC cases, age < 50 years at diagnosis ($n = 195$)				
Thr/Thr–E/E	111 (55.3%)	290 (58.0%)	–	1.00 (reference)
Thr/Thr–E/G	1 (0.4%)	6 (1.2%)	0.444	0.43 [0.05–3.66]
Thr/Thr–G/G	0 (0.0%)	0 (0.0%)	–	–
Thr/Met–E/E	61 (31.9%)	181 (36.2%)	0.492	0.88 [0.61–1.27]
Thr/Met–E/G	4 (1.3%)	1 (0.2%)	0.037	10.5 [1.16–94.5]
Thr/Met–G/G	0 (0.0%)	0 (0.0%)	–	–
Met/Met–E/E	18 (11.1%)	21 (4.2%)	0.018	2.23 [1.15–4.36]
Met/Met–E/G	0 (0.0%)	1 (0.2%)	–	–
Met/Met–G/G	0 (0.0%)	0 (0.0%)	–	–

(a) Fisher's exact test. BC, breast cancer; OR, odds Ratio; CI, Confidence Interval

variation linked to deleterious mutations, in order to maintain the protein sequence.

The Thr241Met substitution is the most thoroughly investigated polymorphism in *XRCC3* due to (C>T) transition at exon 7 (rs861539) [24]. The Thr241Met polymorphism in *XRCC3* changes the aminoacid from a neutral hydrophilic residue with a hydroxyl group to a hydrophobic one with a methyl sulfur group. This may result in a substantial change in protein structure and function [9]. Functional data also suggest that the *XRCC3*-Thr241Met polymorphism may be associated with slightly but not significantly decreased DNA repair capacity [33].

Some but not all previous studies have also found evidence to suggest that this polymorphism is a risk factor for cancer, including BC. A modest association between the homozygous variant genotype for *XRCC3*-Thr241Met and BC risk was first reported in a large population-based case-control study in the UK [9]. The authors reported that homozygous carriers of the *XRCC3*-241Met allele showed a 1.3-fold increased risk (95%CI 1.1–1.6). However, subsequent studies in Caucasian populations were unable to confirm this association [14–16, 18–20]. Figueiredo et al. [17] reported a marginal positive association for the Met/Met versus the Thr/Thr genotype (OR = 1.44 [95%CI 0.94–2.19]), but the heterozygous Thr/Met genotype was not associated with an increased in risk (OR = 0.96 [95%CI 0.71–1.32]). The authors also suggested that some polymorphisms may influence BC risk by modifying the effect of risk factors such as family history. Analyses of combined data from USA and Polish studies and a meta-analysis including the USA and Polish studies and the data of eight previously published studies in Caucasian women were consistent with a very small increased risk for Met/Met homozygous individuals, with no evidence for between-study heterogeneity [34]. Costa et al. [13] found that carriers of *XRCC3* 241Met genotypes without family history of BC have an increased susceptibility for BC ($P < 0.001$, OR = 2.21 [95%CI 1.42–3.44]) and suggested that this polymorphism is an important biomarker for sporadic BC susceptibility. A meta-analysis of 48 case-control studies including European, African-American, Asian, and mixed populations reported a significantly increased risk of BC ($P = 0.0004$, OR = 1.14 [95%CI 1.06–1.23], $P = 0.037$ for heterogeneity). The authors suggested that *XRCC3* might represent a low-penetrance susceptibility gene specific to BC [24]. Nevertheless, The Breast Cancer Association Consortium [35] reported that *XRCC3*-Thr241Met was not associated with BC risk. Our

results showed an association of the Met/Met genotype with increased BC risk among *BRCA1/2*-negative women with a family history of BC, suggesting that this polymorphism contributes to familial BC in the Chilean population. In accordance with the findings of Figueiredo et al. [17] we observed association for the Met/Met genotype compared with the Thr/Thr genotype, but the heterozygous Thr/Met was not associated with an increase in risk ($P = 0.52$, OR = 0.99 [95%CI 0.72–1.37]). Actually, it should be not uncommon for the same polymorphism to play a different role in cancer susceptibility across different populations, since cancer is a complex disease. Most of the studies that have analyzed the *XRCC3*-Thr241Met polymorphism have been conducted in European, African-American, and Asian populations. There were significant differences in terms of the *XRCC3*-241Met allele frequency between the two major ethnicities (European 36.1%; 95%CI 34.5–37.5; Asian, 8.22%, 95%CI 3.00–13.40; $P < 0.0001$) [24]. The contemporary Chilean population stems from the admixture of Amerindian peoples with the Spanish settlers arriving in the sixteenth and seventeenth centuries [36]. The relationships among ethnicity in the Amerindian admixture, genetic markers, and socioeconomic strata have been extensively studied in Chile [37–39]. The frequency of the *XRCC3*-241Met in the admixed Chilean population is 23% therefore intermediate between Caucasian and Asian, and is very similar to the Mexican-American (22.2%) [40], a population that also stems from the admixture of Amerindian peoples with Spanish settlers. Therefore, the different genetic background may contribute to the differing results.

Family history is the other difference between previous studies and our study. Most of the studies that have analyzed the *XRCC3*-Thr241Met polymorphism are population-based BC case-control designs that included cases not selected by family history. Only Figueiredo et al. [17] and Costa et al. [13] included a percentage of familial cases (25 and 11.5%, respectively). In our study the BC cases with high-risk family history were included based on the Proceedings of the International Consensus Conference on Breast Cancer Risk, Genetics, and Risk Management [41] (Table 1). Moreover, in our study controls were interviewed, age matched with cases, and included only individuals with no personal or family history of breast or other cancer. Cases and controls were from the same ethnic group and geographical area.

We also analyzed the effect of the combined genotypes of the *XRCC3*-Thr241Met and *RAD51*-135G>C polymorphisms in BC risk. In accordance with the findings of Krupa et al. [42], the distributions of the combined genotypes were different for BC cases and controls. Nevertheless we did not observe a protective role for the Thr/Met–CG combined genotype against BC as reported by Krupa et al. [42], probably due to the larger size of our control sample. The only combined genotype that showed a significant difference was Met/Met–GG, but GG is the wild-type genotype of *RAD51*-135G>C, thus the significance obtained is probably due to the *XRCC3* Met/Met genotype. We also observed that the Thr241Met polymorphism does not modify the BC risk conferred by the *RAD51*-135G>C genotype in women aged < 50 years at diagnosis. The XRCC3 protein participates in DNA double-strand break/recombinational repair and is a member of a family of Rad51-related protein that participates in HR to maintain chromosome stability and repair DNA damage. The *XRCC3*-241 Met variation is a nonconservative change, but it does not reside in the adenosine triphosphate-binding domain, the only functional domain identified in the protein [43]. Therefore it is likely that either the *XRCC3*-241Met mutation does not alter the direct interaction of XRCC3 and RAD51, or XRCC3 acts within other cellular pathways.

With respect to the *RAD51D*-E233G variant, only two studies have analyzed the role of the E233G variant in the BC susceptibility. Rodriguez-Lopez et al. [21] reported that the E233G variant could be overrepresented among site-specific, high-risk BC families (OR = 2.60 [95%CI 1.12–6.03]) and suggested a role for E233G as a low-penetrance susceptibility gene in this group of cases. On the contrary, Dowty et al. [22] found no evidence of association between *RAD51D*-E233G variant and BC risk in a group of Australian women. Our case-control study found no evidence of association between *RAD51D*-E233G and BC risk in Chilean *BRCA1/2*-negative women with BC who have a positive family history. As we previously discussed, the Chilean population is a mixed Spanish-Amerindians population, and the frequency of the E233G variant is lower than in Spanish population. It is probable that this variant was not present in the aboriginal peoples of the region. Considering that *XRCC3* and *RAD51D* are involved in the same pathway, we analyzed the distribution of *XRCC3*-Thr241Met–*RAD51D*-E233G combined genotypes. The frequency of the Thr/Met–E/G combined genotype was higher in cases than in controls, but the difference was borderline significant. Nadkarni et al. [44] suggested that the E233G variant affects RAD51D functions and protein interactions. A larger case-control study would likely be required to determine whether this composite genotype is associated with increased familial BC risk. Notwithstanding, our results showed an association of the Thr/Met–E/G combined genotype with *BRCA1/2*-negative family history ($n = 195$) and with age < 50 years at onset, suggesting that this composite genotype contributes to familial BC in the Chilean population.

Analysis of populations in which *XRCC3*-Thr241Met and *RAD51D*-E233G variants are prevalent would be of

extreme interest in determining their possible interaction in a BC susceptibility polygenic model.

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