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Association of genetic variants at *TOX3*, 2q35 and 8q24 with the risk of familial and early-onset breast cancer in a South-American population

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Abstract Recent Genome-Wide Association Studies have identified several single nucleotide polymorphisms (SNPs) associated with breast cancer (BC) among women of Asian, European, and African-American ancestry. Nevertheless, the contribution of these variants in the South American population is unknown. Furthermore, there is little information about the effect of these risk alleles in women with early BC diagnosis. In the present study, we evaluated the association between rs3803662 (*TOX3,* also known as *TNRC9*), rs13387042 (2q35), and rs13281615 (8q24) with BC risk in 344 Chilean *BRCA1/2*-negative BC cases and in 801 controls. Two SNPs, rs3803662 and rs13387042, were significantly associated with increased BC risk in familial BC and in non-familial early-onset BC. The risk of BC increased in a dose-dependent manner with the number of risk alleles (P-trend < 0.0001 and 0.0091, respectively). The odds ratios for BC in familial BC and in early-onset non-familial BC were 3.76 (95 %CI 1.02–13.84, $P = 0.046$) and 8.0 (95 %CI 2.20–29.04, $P = 0.002$), respectively, for the maximum versus minimum number of risk alleles. These results indicate an additive effect of the *TOX3* rs3803662 and 2q35 rs13387042 alleles for BC risk. We also evaluated the interaction between rs3803662 and rs13387042 SNPs. We observed an additive interaction only in non-familial early-onset BC cases (AP = 0.72 (0.28–1.16), $P = 0.001$). No significant association was observed for rs13281615 (8q24) with BC risk in women from the Chilean population. The strongly increased risk associated with the combination of low-penetrance risk alleles supports the polygenic inheritance model of BC.

Keywords Breast cancer · Polymorphisms · *TOX3* · *TNRC9* · 2q35 · 8q24

Introduction

Breast Cancer (BC) is one of the most common malignancies affecting women worldwide. In Chile, BC has the first-

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highest mortality rate among cancers (15.02/100,000 women), and its incidence is on the rise among all age groups [1]. Genetic factors play an important role in BC development. Several genes are known to be associated with increased susceptibility to BC, including *BRCA1, BRCA2, ATM* and others [2], but only about 5 % of BC incidence can be explained by these high-penetrance mutations [3]. Moreover, these genes are responsible only for about 16–20 % of the risk for familial BC. Therefore, the genetic basis of 80 % of familial cases remains unexplained [4]. Genome-Wide Association Studies (GWAS) have identified genetic susceptibility loci that are associated with BC risk. All of the loci identified to date have been so-called low-penetrance polymorphisms, with weak associations with BC risk as compared to the high-penetrance mutations [5]. Although each low-penetrance variant confers only a small increase in the risk of BC, a combination of single variants may act cumulatively to increase risk significantly [6].

The *TOX3*/LOC643714 (also known as *TNRC9*) locus on chromosome 16q12 was one of the first BC regions to be identified through GWAS in populations of European and East Asian origin [7]. Several single nucleotide polymorphisms (SNPs) associated with risk of BC have been identified in this locus. Of these, rs3803662 is the most strongly correlated with disease. Each copy of the allele T of the rs3803662 SNP is associated with a 20 % increase in the risk of BC [8]. Two other SNPs, rs13387042 (2q35) and rs13281615 (8q24), located in non-coding regions, were also found to be associated with BC risk [7, 9]. The common susceptibility loci (*FGFR2*, *TOX3*, *MAP3K1*, *LSP1*, 2q35, and 8q24) reported in 2007 by Easton et al. [7], Hunter et al. [10] and Stacey et al. [9] have been verified in other studies [11–13] in European, Asian and African-American populations. Nevertheless, the contribution of these variants in the South American population is unknown. Furthermore, there is little information about the effect of these risk alleles in women diagnosed with BC at $\leq$ 50 years of age. The Chilean population is the result of the admixture between Asian and Spanish population; therefore, whether these genetic variants are applicable marker SNPs in Chilean women is unknown. In a previous study, only 18 % of Chilean BC patients with a family history of breast and ovarian cancer carry *BRCA1/2* point mutations [14], and none of the women with non-familial early-onset BC studied were carriers of *BRCA1/2* point mutations. Our group has also studied mutations in other susceptibility genes, but these are not frequent enough to explain the all of the *BRCA*-negative BC cases [15–20]. Working under the assumption that the trait is polygenic, we evaluated the association of *TOX3* rs3803662, 2q35 rs13387042, and 8q24 rs13281615 with familial BC and early-onset non-familial BC in non-carriers of *BRCA1/2* mutations from a South American population.

Methods

Subjects

A total of 347 BC patients belonging to 347 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 index cases were tested for *BRCA1* and *BRCA2* mutations as described [14]. 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, 13.3 % (46/347) had cases of bilateral BC; 8.4 % (29/347) had cases of both BC and OC; and 2.6 % (9/347) had male BC. In the BC group, the mean age of diagnosis was 42.2 years, and 81.3 % had age of onset $<$ 50 years. BC was verified by the original pathology report for all probands.

The sample of healthy Chilean controls ($n = 801$) was recruited from CONAC files. DNA samples were taken from unrelated individuals with no personal or familial history of cancer and who had given consent for anonymous testing. These individuals were interviewed and informed as to the aims of the study. DNA samples were obtained according to all ethical and legal requirements. The control sample was matched to the cases for age and socioeconomic strata.

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 participants.

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	88 (25.4 %)
Two family members with breast and/or ovarian cancer	127 (36.6 %)
Single affected individual with breast cancer $\leq$ age 35	67 (19.3 %)
Single affected individual with breast cancer between age 36 and 50	65 (18.7 %)
TOTAL	347

Genotyping analysis

Genomic DNA was extracted from peripheral blood lymphocytes of 347 cases belonging to the high-risk selected families and 801 controls. Samples were obtained according to the method described by Chomczynski and Sacchi [21].

Genotyping of rs3803662 (*TOX3*), rs13387042 (2q35), and rs13281615 (8q24) was carried out using pre-designed TaqMan SNP Genotyping Assays (Applied Biosystems) (assay ID C__25968567_10, C__32048042_10, and C___1332250_10, respectively). The reaction was performed in a 10 uL final volume containing 5 ng of genomic DNA, 1X TaqMan Genotyping MasterMix and 1X TaqMan SNP Genotyping Assay. Polymerase chain reaction was carried out in a StepOne Plus 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 DNA controls confirmed by sequencing. The alleles were assigned using the software SDS 2.2 (Applied Biosystems). As a quality control, we repeated the genotyping on $\sim$ 10 % of the samples, and all genotype scoring was performed and checked separately by two reviewers unaware of the case–control status.

Statistical analyses

The Hardy–Weinberg equilibrium assumption was assessed in the control sample using a goodness-of-fit Chi square test (HWChisq function included in "HardyWeinberg" package v 1.4.1). Fisher's exact test was used to test the association of *TOX3*, 2q35, and 8q24 genotypes and/or alleles in cases and controls. The odds ratio (OR) and its 95 % confidence interval (CI) were calculated to estimate the strength of the association in cases and controls (oddsratio.fisher function included on "epitools" package v 0.5–6). A two-sided P value < 0.05 was used as the criterion for significance. The Cochran-Armitage trend test was performed to test additive genetic effect model (CATT function included on "Rassoc" package v 1.03). A Chi-squared test for trend was performed to examine additive combined effects of risk alleles (Stata/SE 10.0 for Unix - StataCorp, TX, USA- using "ptrend" package). The interaction on the additive scale was assessed by measuring the attributable proportion due to interaction (AP) [22]. The confidence interval (CI) and P-value were calculated according to Hossmer et al. [23] (expected value under the null hypothesis = 0). The interaction on the multiplicative scale was assessed by logistic regression analysis (Stata/SE 10.0 for Unix -StataCorp, TX, USA) 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 of < 0.05 was used as the criterion for statistical significance. All statistical analyses were performed using the R statistical environment (available at http://www.r-project.org/), unless indicated otherwise.

Results

The selected characteristics of the 347 *BRCA1/2*-negative cases are summarized in Table 1. For the analysis, the whole sample was subdivided into two groups: cases belonging to families with two or more family members with BC and/or OC ($n = 215$) (subgroup A) and non-familial early-onset BC ($\leq$ 50 years) ($n = 132$) (subgroup B). The genotype distributions and allele frequencies of *TOX3* rs3803662 C > T, 2q35 rs13387042 G > A, and 8q24 rs13281615 G > A polymorphisms in the whole data set and in subgroups A and B with respect to the controls are shown in Table 2. The observed genotype frequencies for the three polymorphisms were all in Hardy–Weinberg equilibrium in the controls ($P = 0.79$ for rs3803662 C > T, $P = 0.98$ for rs13387042 G > A, and $P = 0.99$ for rs13281615 G > A, respectively).

In the single locus analyses, the genotype and allele distributions for rs3803662 C > T and rs13387042 G > A were significantly different in the whole sample *BRCA1/2*-negative cases and in subgroup A, with respect to the controls ($P < 0.05$). Furthermore, in the whole sample, the homozygous minor allele genotypes were associated with increased risk of BC [rs3803662 (TT genotype OR = 2.04, $P = 0.0002$), rs13387042 (AA genotype OR = 1.79, $P = 0.0018$)]. We also observed increased risk of BC in the whole sample for the carriers of the minor allele frequency for rs3803662 and rs13387042 (Table 2). In subgroup A, which included cases with family history of BC, significant associations were observed between the risk of BC and the homozygous minor allele genotype for *TOX3* rs3803662 (TT genotype OR = 2.38, $P = 0.0003$) and for 2q35 rs13387042 (AA genotype OR = 1.99, $P = 0.0015$). Furthermore, the P-trend test for the genotypes between cases and controls shows that the associations for allele variants were dose-dependent for *TOX3* rs3803662 and 2q35 rs13387042 in the whole sample and in subgroup A (Table 2). Nevertheless, no differences were observed in the genotype and allele distribution of rs3803662 and rs13387042 between non-familial early-onset BC (subgroup B) and controls ($P > 0.005$). In addition, no significant differences were observed in the genotype and allele distribution for 8q24 rs13281615 G > A, either in the whole data set or in subgroup A or B ($P > 0.005$).

Considering that it has been suggested that distinct BC-predisposing SNPs may act in an additive manner, and

Table 2 Genotype and allelic frequencies of rs3803662 (*TOX3*), rs13387042 (2q35), and rs13281615 (8q24) in *BRCA1/2*-negative breast cancer cases and controls

		All BC cases ($n = 347$)			Families with ≥ 2 BC and/or OC cases ($n = 215$)			Single affected, diagnosis ≤ 50 years ($n = 132$)		
Genotype or allele	Controls (%) ($n = 801$)	BC cases (%)	*P* value (a)	OR [95 %CI]	BC cases (%)	*P* value (a)	OR [95 %CI]	BC cases (%)	*P* value (a)	OR [95 %CI]
rs3803662 (*TOX3*)										
C/C	330 (41.2 %)	100 (28.8 %)	0.0002 (b)	Ref.	54 (25.1 %)	**<0.0001 (b)**	Ref.	46 (34.9 %)	0.194 (b)	Ref.
C/T	371 (46.3 %)	185 (53.3 %)	**0.0004**	**1.64 (1.22–2.21)**	122 (56.7 %)	**0.0001**	**2.00 (1.39–2.91)**	63 (47.7 %)	0.1990	1.21 (0.79–1.87)
T/T	100 (12.5 %)	62 (17.9 %)	**0.0002**	**2.04 (1.35–3.06)**	39 (18.2 %)	**0.0003**	**2.38 (1.44–3.90)**	23 (17.4 %)	0.0519	1.65 (0.90–2.93)
P-trend (c)			**<0.0001**			**<0.0001**			0.0768	
C/T + T/T	471 (58.8 %)	247 (71.1 %)	**<0.0001**	**1.73 (1.30–2.29)**	161 (74.9 %)	**<0.0001**	**2.08 (1.47–2.98)**	86 (65.1 %)	0.0993	1.30 (0.87–1.96)
C	0.64	0.55		Ref.	0.53		Ref.	0.59		Ref.
T	0.36	0.45	**<0.0001**	**1.44 (1.20–1.74)**	0.47	**<0.0001**	**1.57 (1.25–1.95)**	0.41	0.0456	1.26 (0.96–1.66)
rs13387042 (2q35)										
G/G	329 (41.1 %)	112 (32.3 %)	0.0061 (b)	Ref.	72 (33.5 %)	0.0065 (b)	Ref.	40 (30.3 %)	0.056 (b)	Ref.
G/A	369 (46.1 %)	172 (49.6 %)	**0.0165**	**1.36 (1.02–1.83)**	98 (45.6 %)	0.1502	1.21 (0.85–1.72)	74 (56.1 %)	**0.0105**	**1.64 (1.07–2.55)**
A/A	103 (12.8 %)	63 (18.1 %)	**0.0018**	**1.79 (1.20–2.66)**	45 (20.9 %)	**0.0015**	**1.99 (1.25–3.14)**	18 (13.6 %)	0.1515	1.43 (0.74–2.69)
P-trend (c)			**0.0014**			**0.0032**			0.0682	
G/A + A/A	472 (58.9 %)	235 (67.7 %)	**0.0029**	**1.46 (1.11–1.92)**	143 (66.5 %)	**0.0254**	**1.38 (1.00–1.92)**	92 (69.7 %)	**0.0115**	**1.60 (1.06–2.44)**
G	0.64	0.57		Ref.	0.56		Ref.	0.58		Ref.
A	0.36	0.43	**0.0009**	**1.34 (1.11–1.61)**	0.44	**0.0018**	**1.38 (1.11–1.73)**	0.42	0.0421	1.27 (0.96–1.67)
rs13281615 (8q24)										
A/A	152 (19.0 %)	68 (19.6 %)	0.0925 (b)	Ref.	42 (19.5 %)	0.5348 (b)	Ref.	26 (19.7 %)	0.0505 (b)	Ref.
A/G	394 (49.2 %)	148 (42.6 %)	0.1808	0.83 (0.58–1.20)	97 (45.1 %)	0.3239	0.89 (0.58–1.37)	51 (38.6 %)	0.1724	0.75 (0.44–1.31)
G/G	255 (31.8 %)	131 (37.8 %)	0.2508	1.14 (0.79–1.66)	76 (35.4 %)	0.4070	1.07 (0.69–1.96)	55 (41.7 %)	0.2218	1.26 (0.74–2.18)
P-trend (c)			0.2417			0.5858			0.1715	
A/G + G/G	649 (81.0 %)	279 (80.4 %)	0.4325	0.96 (0.69–1.34)	173 (80.5 %)	0.4606	0.96 (0.65–1.44)	106 (80.3 %)	0.4628	0.95 (0.59–1.58)
A	0.44	0.41		Ref.	0.42		Ref.	0.39		Ref.
G	0.56	0.59	0.1288	1.11 (0.92–1.34)	0.58	0.3109	1.06 (0.85–1.32)	0.61	0.0934	1.20 (0.91–1.59)

(a) Fisher's exact test, (b) ×2 for independence, (c) Cochran-Armitage trend test

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

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

Table 3 Combined effects of rs3803662 (*TOX3*) and rs13387042 (2q35) on the risk of breast cancer

Number of risk alleles (a)	Controls (n = 801) (%)	All BC cases (n = 347)			Families with ≥ 2 BC and/or OC cases (n = 215)			Single affected, diagnosis ≤ 50 years (n = 132)		
		BC cases (%)	OR [95 % CI]	P value (b)	BC cases (%)	OR [95 % CI]	P value (b)	BC cases (%)	OR [95 % CI]	P value (b)
0 risk allele	128 (16.0 %)	27 (7.8 %)	Ref.		17 (7.9 %)	Ref.		10 (7.6 %)	Ref.	
1 risk allele	309 (38.6 %)	115 (33.1 %)	**1.76 (1.10–2.81)**	**0.017**	61 (28.4 %)	1.48 (0.83–2.64)	0.177	54 (40.9 %)	**2.23 (1.10–4.52)**	**0.025**
2 risk allele	263 (32.8 %)	132 (38.1 %)	**2.37 (1.49–3.78)**	**<0.001**	88 (40.9 %)	**2.51 (1.43–4.41)**	**0.001**	44 (33.3 %)	**2.14 (1.04–4.39)**	**0.038**
3 risk allele	93 (11.6 %)	64 (18.4 %)	**3.26 (1.93–5.50)**	**<0.001**	45 (20.9 %)	**3.64 (1.96–6.76)**	**<0.001**	19 (14.4 %)	**2.61 (1.16–5.88)**	**0.020**
4 risk allele	8 (1.0 %)	9 (2.6 %)	**5.33 (1.8–15.07)**	**0.002**	4 (1.9 %)	**3.76 (1.02–13.84)**	**0.046**	5 (3.8 %)	**8.0 (2.20–29.04)**	**0.002**
P-trend (c)				**<0.0001**			**<0.0001**			**0.0091**
Global P (d)				**<0.001**			**<0.001**			**0.013**

(a) 0 risk allele: C/C + G/G; 1 risk allele: C/C + G/A, C/T + G/G; 2 risk alleles: C/C + A/A, C/T + G/A, T/T + G/G; 3 risk alleles: C/T + A/A, T/T + G/A; 4 risk alleles: T/T + A/A

(b) Fisher's exact test, (c) Chi squared test for trend, (d) Chi squared test for independence

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

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

TOX3 rs3803662 T and 2q35 rs13387042 A alleles were associated with increased BC risk, we evaluated their combined effect. For the analysis, the subjects were divided into five groups based on number of risk alleles [subjects with 0 (group 1), 1 (group 2), 2 (group 3), 3 (group 4), and 4 (group 5)]. As shown in Table 3, the distribution of the combined genotypes in the whole sample, and in subgroups A and B, significantly differed from the controls ($p < 0.001$, < 0.001, and 0.013, respectively), and the risk of BC increased in a dose-dependent manner in the whole sample and in subgroups A and B as the number of risk alleles increase (P-trend < 0.0001, < 0.0001, and 0.0091, respectively). Using group 1 as the reference group, the OR of group 5 for BC was 5.33 (95 %CI, 1.8–15.1, $P = 0.002$) for the whole sample, 3.76 (95 %CI, 1.02–13.84, $P = 0.046$) for familial BC, and 8.0 (95 %CI, 2.20–29.0, $P = 0.002$) for early-onset non-familial BC. These results indicate an additive effect of the *TOX3* rs3803662 T and 2q35 rs13387042 A alleles on risk of breast cancer.

Considering the additive effect observed between *TOX3* rs3803662 T and 2q35 rs13387042 A alleles on risk of familial and early-onset non-familial BC, we next evaluated the interaction between *TOX3* rs3803662 and 2q35 rs13387042 SNPs. We observed an additive interaction only in subgroup B, which included non-familial early-onset BC cases (AP = 0.72 (0.28–1.16), $P = 0.001$) (Table 4).

Discussion

Breast cancer is a polygenic disease. In addition to the highly penetrant (*BRCA1*, *BRCA2*, and *TP53*) and moderately penetrant (*CHEK2, BRIP1, ATM* and *PALB2*) genes, BC has a component of inheritance due to low-penetrance and common genetic variants. The more recently published Genome-Wide Association Studie (GWAS) in BC have reported low-risk alleles in at least 41 different loci [24]. Although each low-penetrance variant confers only a small increase in the risk of BC, together they are thought to represent roughly 8 % of the familial BC cases [13]. If so, identifying such combinations might be a useful tool for targeted cancer prevention.

To date, most GWAS were conducted in populations of European and Asian ancestry. However, the contribution of those variants as predictors in South American women is unknown. Some studies have suggested substantial differences in genetic architecture among populations, such as allele frequencies and extent of linkage-disequilibrium [25]. In the present study, using a case–control deisgn, we evaluated the impact of *TOX3* rs3803662 C > T, 2q35 rs13387042 G > A, and 8q24 rs13281615 G > A

Table 4 Measures of interaction on additive and multiplicative scale between rs3803662 (*TOX3*) and rs13387042 (2q35) on the risk of breast cancer

Combined genotype rs3803662 (*TOX3*) and rs13387042 (2q35)	Controls (%) ($n = 801$)	All BC cases ($n = 347$)			Families with ≥ 2 BC and/or OC cases ($n = 215$)			Single affected, diagnosis ≤ 50 years ($n = 132$)		
		BC cases (%)	*P* value (a)	OR [95 % CI]	BC cases (%)	*P* value (a)	OR [95 % CI]	BC cases (%)	*P* value (a)	OR [95 % CI]
(C/C + C/T)–(G/G + G/A)	606 (75.6 %)	231 (66.6 %)		Ref.	135 (62.8 %)		Ref.	96 (72.7 %)		Ref.
T/T–(G/G + G/A)	92 (11.5 %)	53 (15.3 %)	**0.029**	**1.51 (1.04–2.18)**	35 (16.3 %)	**0.015**	**1.70 (1.10–2.62)**	18 (13.6 %)	0.451	1.23 (0.71–2.13)
(C/C + C/T)–A/A	95 (11.9 %)	54 (15.5 %)	**0.033**	**1.49 (1.03–2.15)**	41 (19.1 %)	**0.002**	**1.93 (1.28–2.92)**	13 (9.9 %)	0.643	0.86 (0.46–1.60)
T/T–A/A	8 (1.0 %)	9 (2.6 %)	**0.028**	**2.95 (1.12–7.74)**	4 (1.8 %)	0.192	2.24 (0.66–7.56)	5 (3.8 %)	**0.018**	**3.94 (1.26–12.31)**
Attributable proportion due to interaction (AP)		0.32 (−0.50–1.14), $p = 0.445$			−0.18 (−1.95–1.59), $p = 0.843$			**0.72 (0.28–1.16), $p = 0.001$**		
Measure of interaction on multiplicative scale (ratio of ORs)		1.31 (0.44–3.83), $p = 0.623$			0.68 (0.17–2.55), $p = 0.567$			3.69 (0.93–14.60), $p = 0.062$		

(a) Fisher's exact test

BC breast cancer, *OC* ovarian cancer, *OR* Odds Ratio, *CI* confidence interval

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

polymorphisms in women with familial and non-familial early-onset BC negative for point mutations in *BRCA1/2*, from the Chilean population.

Two SNPs (*TOX3* rs3803662 C > T, 2q35 rs13387042 G > A) were significantly associated with increased risk of familial BC in the Chilean population. *TOX3* is a gene located at chromosome 16q12, of uncertain function, and a newly described risk factor for BC [9]. *TOX3* contains a putative, high-mobility group box motif, suggesting its potential role as a transcription factor. It has been implicated in BC metastasis to bone [26]. Several polymorphisms have been identified in the *TOX3* gene, including the SNP rs3803662, a synonymous coding SNP which lies 8 Kb upstream of *TOX3*. Easton et al. [7] reported a strong association of rs3803662 with BC. The meta-analysis of Chen et al. [3] suggest that the *TOX3* rs3803662 polymorphism is significantly correlated with BC, and the T allele of the *TOX3* rs3803662 variant is a low-penetrance risk factor for developing breast cancer. Our results showed that rs3803662 was significantly associated with increased risk of familial BC (OR = 1.57 95 %CI 1.25–1.95), with higher OR values for the homozygous TT (OR = 2.38) with respect to the heterozygous CT (OR = 2.0). This results are compatible with those published by Latif et al. [27], which reported an association of *TOX3* rs3803662 with increased risk of BC in individuals with a family history of BC, without *BRCA1/2* mutations (OR = 2.39, 95 %CI 1.39–4.09, $P = 0.002$). Regarding the mechanism by which rs3803662 increases BC risk, Udler et al. [28] showed an association of this SNP with an increased expression of the *RBL2* gene, which is located near this risk allele, and proposed that this locus might differentially regulate more than one distant gene in cis or in trans. Riaz et al. [29] suggested that *TOX3* might act as a tumour suppressor gene, and the risk allele rs3803662 is significantly associated with lower expression of *TOX3*.

Stacey et al. [9], in a GWAS, were the first to describe the association of SNP 2q35 rs13387042 with BC risk in individuals of European descent. Later, Milne et al. [30] confirmed the association between rs13387042 and BC in white women of European origin, and the odds ratio was higher when the analysis was restricted to case patients who were selected for a strong family history. The 2q35 rs13387042 has also been associated with BC in Chinese, African-American, and Taiwanese populations [25, 31, 32]. In this study, we found that the SNP rs13387042 was significantly associated with increased risk of familial BC in a mixed (Caucasian-Amerindian) South American population. SNP 2q35 rs13387042 lies in a 90 Kb region of high linkage disequilibrium that contains neither known genes nor noncoding RNAs [9]. Large collaborative studies will be necessary to evaluate the precise role of the 2q35 rs13387042 susceptibility variant.

The rs13281615 (8q24) was one of SNPs described by Easton et al. [7]. These authors reported evidence of associations between the SNP rs13281615 with family history of BC (OR = 1.06, 95 %CI 1.00–1.12, $P = 0.05$). Later, Latif et al. [27] confirmed an allelic association between increased cancer risk and the chromosome 8q variant ($P = 0.004$). The results of our case–control study showed no evidence of association between rs13281615 and BC risk, either in individuals with familial BC or non-familial early-onset BC. This result is in consistent with those obtained by Gorodnova et al. [11], who reported no association between 8q24 rs13281615 and BC risk in genetically enriched BC patients from the Russian population. Additionally, no association between the 8q locus and BC risk was observed by Zheng et al. [25] or Huo et al. [33] in African-American women. In Chinese women, Long et al. [34] and later Dai et al. [31] found no association of this locus with BC risk. The controversial results may be explained by the marked differences in rs13281615 and other SNP allele frequencies among populations [9]. In the European population, the rs13281615 G allele is the minor allele (0.46), while in the Asian population it is the major allele (0.60) and is associated with increased risk of BC. In the Chilean population, the rs13281615 G is the major allele (0.56), but we did not observe an association with BC risk. This finding is likely the consequence of the genetic structure of this population. The contemporary Chilean population stems from the admixture of Amerindian peoples with the Spanish settlers in the sixteenth and seventeenth centuries. Later migrations (Nineteenth century) of Germans, Italians, Arabs, and Croatians have had only a minor impact on the overall population (not more than 4 % of the total population) and are restricted to the specific locations of the country where they settled [35]. The relationship between ethnicity, Amerindian admixture, genetic markers, and socioeconomic strata has been extensively studied in Chile [36–38].

To evaluate the combined effects of the *TOX3* rs3803662 T and 2q35 rs13387042 A alleles, a genetic score was constructed based on the number of risk alleles. A dose–response association was observed between the number of risk alleles and BC risk, both in the subgroups with familial BC and non-familial early-onset BC (P-trend < 0.0001 and 0.0091, respectively). The presence of four risk alleles was associated with a 3.76-fold increased risk of familial BC and with an 8.0-fold increased risk in non-familial early-onset BC, compared with the presence of zero risk alleles. These results indicate that the *TOX3* rs3803662 and 2q35 rs13387042 SNPs have an additive effect on risk in both subgroups A and B. This result is particularly important in subgroup B (non-familial early-onset BC), in which *TOX3* rs3803662 and 2q35 rs13387042 alone did not increase BC risk, but the combined presence showed an additive effect. We also observed an additive interaction only in subgroup B, but considering the sample size, additional studies are needed to confirm this finding.

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Conflict of interest Corporación Nacional del Cáncer.The authors declare that they have no competing interests.

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