Spectrum of point mutations and genomic rearrangements in high-risk breast/ovarian cancer Chilean families

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TITLE PAGE

SPECTRUM OF *BRCA1/2* POINT MUTATIONS AND GENOMIC REARRANGEMENTS IN HIGH-RISK BREAST/OVARIAN CANCER CHILEAN FAMILIES

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ABSTRACT

The distribution of *BRCA1/2* germline mutations in breast/ovarian cancer (BC/OC) families varies among different populations. In the Chilean population there are only two reports of mutation analysis of *BRCA1/2* and these included a low number of BC and/or OC patients. Moreover, the prevalence of *BRCA1/2* genomic rearrangements in Chilean and in other South American populations is unknown. Here we present the mutation-detection data corresponding to a set of 326 high-risk families analyzed by conformation-sensitive gel electrophoresis and heteroduplex analysis. To determine the contribution of *BRCA1/2* LGRs in Chilean BC patients, we analyzed 56 high-risk subjects with no pathogenic *BRCA1/2* point mutations. Germline *BRCA1/2* point mutations were found in 23 (7.1%) of the 326 Chilean families. Families which had at least 3 BC and/or OC cases showed the highest frequency of mutations (15.9%). We identified 14 point pathogenic mutations. Three recurrent mutations in *BRCA1* (c.187_188delAG, c.2605_2606delTT, and c.3450_3453delCAAG) and three in *BRCA2* (c.4969_4970insTG, c.5374_5377delTATG, and c.6503_6504delTT) contributed to 63.6% and 66.7% of all deleterious mutations of each gene, that may reflect the presence of region-specific founder effects. Taken together *BRCA1/2* recurrent point mutations account for 65.2% (15/23) of the *BRCA1/2*(+) families. No large deletions or duplications involving *BRCA1/2* were identified in a subgroup of 56 index cases negative for *BRCA1/2* point mutations. Our study, which is the largest conducted to date in a South American population, provides a comprehensive analysis on the type and distribution of *BRCA1/2* mutations and allelic variants.

Keywords: Breast cancer. *BRCA1/2*. Chilean population. LGRs. recurrent mutations

INTRODUCTION

Breast cancer (BC) is the most common cancer among women worldwide. It has been established that one out of eight 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 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 familial BC cases [6]. Carriers of mutations in any of these genes have a 40-85% cumulative lifetime risk of BC by the age of 70 . Studies of *BRCA1/2* mutation frequencies have revealed significant differences in populations of different geographic regions and ethnicities. Disease-causing mutations are distributed throughout the entire coding regions of both genes. Since the identification of *BRCA1* and *BRCA2* as the principal genes responsible for inherited BC [7, 8], more than 3,400 distinct DNA sequence variants have been described in the BIC database (http://research.nhgri.nih.gov/bic/), of which 1,625 can be classified as pathogenic, including 1,454 truncating mutations (1,075 frameshift and 379 nonsense) and 139 splicing alterations. The remaining mutations, including 1,351 distinct missense changes, are unclassified variants of uncertain clinical consequences. In addition to point mutations that can be detected within a PCR fragment, *BRCA1* and *BRCA2* are both known to have germline mutations resulting from Large Genomic Rearrangements (LGRs) that result in duplications or deletions of one or more exons, usually producing premature stop codons.

Little is known about the contribution of *BRCA1/2* to hereditary BC in South American populations. In the Chilean population there are only two reports of mutation analysis of *BRCA1/2*, and these included a low number of BC and/or ovarian cancer (OC) patients [9, 10]. Moreover, the prevalence of *BRCA1/2* genomic rearrangements in Chilean and in other South American populations is unknown. Here we present the mutation-detection data corresponding to a set of 326 high-risk families analyzed by conformation-sensitive gel electrophoresis and heteroduplex analysis. Results of 64 out of these 326 high risk families were previously reported [9] showing that the *BRCA1/2* gene mutation spectrum was heterogeneous and broad. Our current study, which is the largest conducted to date in a South American population, provides a comprehensive analysis on the type and distribution of *BRCA1/2* mutations and allelic variants. To determine the contribution of *BRCA1/2* LGRs in Chilean BC patients, we analyzed 56 high-risk subjects with no pathogenic *BRCA1/2* point mutations.

METHODS

Families

In this study, 326 high-risk BC/OC 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 in the Metropolitan Area of Santiago. 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. All families participating in the study self reported Chilean ancestry dating several generations, after extensive interviews with several members of each family from different generations.

Table 1 shows the specific characteristic of the families selected according to the inclusion criteria. In the sample of selected families, 15.3% (50/326) had bilateral BC, 9.0% (29/326) had BC and OC, and 2.8% (9/326) presented male BC. From the 326 families which participated in this study, 348 women had only BC, two had both BC and OC, 5 had OC, and 7 had male BC. BC and OC for all cases included in the study were verified using the original pathology report.

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.

BRCA1 and *BRCA2* mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes of 362 index cases belonging to the 326 high-risk selected families. Samples were obtained according to the method described by Chomczynski and Sacchi [11].

The whole coding sequences and exon-intron boundaries of the *BRCA1* and *BRCA2* genes were analyzed for sequence variants using conformational-sensitive gel electrophoresis according to described protocols [9]. Any fragment showing a mobility shift was directly sequenced in both directions. Sequencing was performed in an ABI prism 3100 automated fluorescence-based cycle sequencer (Applied Biosystems, USA) and a rhodamine dye terminator system (Applied Biosystems, USA).

Multiplex Ligation-Dependent probe Assay (MLPA) was performed for screening LGRs in a subgroup of 56 index cases negative for *BRCA1/2* point mutations. MLPA analysis was performed according to manufacturer instructions (MRC Holland, Holland) using the SALSA MLPA Kit P002-B1 for *BRCA1* and SALSA MLPA Kit P045-B1 for *BRCA2*. The fragments obtained were analyzed by capillary electrophoresis using an ABI3130-xl Genetic Analyzer (Applied Biosystems, USA) with a 36cm capillary array and POP-7 polymer (Applied Biosystems, USA). GeneMapper 4.0 software (Applied Biosystems, USA) was used for fragment analysis and the results (peak size, height and areas) were exported to an Excel data sheet to calculate the relative peak area. Normalization was achieved by dividing the intra-normalized peak area by the average intra-normalized probe ratio of all analyzed samples in the same round of analysis.

Mutation nomenclature

Mutations are described at the cDNA level according to the Human *BRCA1* reference sequence: accession U14680; and the Human *BRCA2* reference sequence: accession U43746; from GenBank sequence database (http://www.ncbi.nlm.nih.gov/genbank/). The *BRCA1* and *BRCA2* numbering for the BIC traditional mutation nomenclature is based on reference sequences as stated above where the A of the ATG translation initiation codon is at position 120 of *BRCA1* and position 229 of *BRCA2*. The Human Genome Organization (HUGO) systematic nomenclature for the description of sequence variants in DNA and protein sequences [12] follows the rule in which the A of the ATG translation initiation codon is +1.

RESULTS

The *BRCA1* and *BRCA2* genes of 362 index cases belonging to 326 high-risk unrelated families were scanned for mutations. We identified a total of fourteen point pathogenic mutations (seven in *BRCA1* and seven in *BRCA2*) (Tables 3 and 4). Furthermore, we identified 14 variants of unknown significance (6 in *BRCA1* and 8 in *BRCA2*) (Table 5) and 28 polymorphisms (17 in *BRCA1* and 11 in *BRCA2*) (Table 6). According to the type of mutation, small deletions leading to frameshifts were more frequent (n=11), followed by splicing mutations (n=1), insertions (n=1) and a missense pathogenic mutation. No large deletions or duplications involving *BRCA1*/*2* were identified in a subgroup of 56 index cases negative for *BRCA1/2* point mutations.

BRCA1/2 pathogenic point mutations

Germline *BRCA1*/*2* point mutations were found in 23 (7.1%) of the 326 Chilean families (Table 2). Despite the low frequency of pathogenic point mutations detected in this series, the families that had at least 3 BC and/or OC cases showed the highest frequency of mutations (15.9%). The frequency was lower for families with 2 BC cases (6.6%), and two out of the nine families (22.2%) with one BC case and one OC case were carriers of pathogenic mutations. No *BRCA1/2* pathogenic mutations were identified in the group of 57 (17.5%) early-onset BC patients (<35 years) reporting no first or second degree relatives affected with BC or OC (Table 2). It is probable that in this group BC is due to moderate or low penetrance genes and not to *BRCA1/2* mutations.

In the sample of 326 Chilean families, our group identified 14 point pathogenic mutations. Nine have been previously reported by our group [9], four by other groups, and one was a new mutation not previously described. Nevertheless, it is important to emphasize that two of the previously reported mutations c.3977_3980delTGAG (*BRCA1*) and c.8296delGT (*BRCA2*) have only two and one records in the BIC database, respectively. The frameshift c. 3977_3980delTGAG was identified in a Chilean family (F203), which only presented two cases of female BC diagnosed at age 36 (index case) and at age 40 (bilateral BC). The father of the index case was diagnosed with prostate cancer at age 50 (Table 3). We obtained a DNA sample only from the index case who was carrier of the aforementioned mutation. This mutation has only two records in the BIC database, one of them in an Asian population. The c.8292delGT mutation has only one record in the BIC database. This mutation was found in family F93, a BC/OC cancer family containing two BC cases diagnosed at ages 26 (index case) and 32, and one OC at 60. We only analyzed the index case and four healthy relatives (a sister, a brother and two cousins), and only the index case was a carrier of the c.8296delGT mutation.

Four of the 14 distinct pathogenic mutations found by our group were novel. To our knowledge, these have not been reported in the BIC database or elsewhere. Three of them were reported in our previous study and to date have not been reported in other studies (c.2605_2606delTT [*BRCA1*]; c.4185_4188delCAAG [*BRCA1*]; c.5667delT [*BRCA2*]) [9], and c.9490delG (*BRCA2*) is a new novel mutation. The *BRCA2* c.9490delG mutation was identified in only one BC/OC family (F211). The family presented three BC cases, two of them diagnosed under the age of 50, and one OC/BC diagnosed at age 42/50. This mutation was found in the index case (OC/BC) and in her sister affected with BC at age 38. This mutation is located in exon 25 and generates a stop at codon 3088, deleting 9% of the protein. The deleted segment includes a DNA binding domain with unique structural features for binding ssDNA and dsDNA.

Three recurrent mutations in *BRCA1* (c.187_188delAG, c.2605_2606delTT, and c.3450_3453delCAAG) and three in *BRCA2* (c.4969_4970insTG, c.5374_5377delTATG, and c.6503_6504delTT) contributed to 63.6% and 66.7% of all deleterious mutations of each gene, which may reflect the presence of region-specific founder effects. The most frequent mutation of *BRCA1* was c.3450_3453delCAAG, found in three *BRCA1*(+) families (27.3%), whereas c.5374_5377delTATG was the most

prevalent alteration in *BRCA2* (found in four families, 33.3%). Taken together, *BRCA1/2* recurrent point mutations account for 65.2% (15/23) of the *BRCA1/2*(+) families.

Variants of unknown significance

Six BRCA1 variants of the missense type were detected in five different families (Table 5). Of these, five were described in our previous report [9]. In BRCA2 we found eight variants of unknown significance. Seven correspond to missense variants, some of which are described in our previous report [9]. In 12 of the 13 families, the variants of unknown significance were present in the BC cases; in only one family (F14) the variant was detected in a healthy relative. In 9 of the 13 families, we obtained a DNA sample from healthy relatives only. In family F239, the mother with BC was a carrier of variant c.8381T>C, but her daughter who also had BC was not a carrier. The families who were carriers of variants of unknown significance did not present pathogenic mutations in the BRCA1/2 genes. The only exception was F65, which presented the c.IVS17-1G>A mutation (BRCA1). In this family, it is likely that the BC was a result of the pathogenic mutation c.IVS17-1G>A. The most interesting variant is c.4747_4749delATT, which produces the deletion of codon 1583, and corresponds to a novel variant with no BIC records and not described by other authors to date. This variant produces the deletion of isoleucine at position 1583 of the BRCA2 protein, which was found in a BC case diagnosed at age 32 with no family history of BC or OC, and was not detected in her healthy sister. Nevertheless, in her maternal lineage there are relatives with gallbladder cancer, early onset gastric cancer, leukemia and lung cancer cases. We cannot exclude the possibility that this mutation could be a pathogenic point mutation.

DISCUSSION

In the present study we conducted a screening for point mutations in the complete coding sequence of *BRCA1* and *BRCA2* genes in a group of 326 high risk families. Additionally, we analyzed LGR in both genes in a sample of 56 high-risk BC cases. Although there are some previous studies conducted in Hispanic American populations such as Mexico [13], Colombia [14], and Chile [10], this is the largest study conducted in high risk families in an Hispanic American population.

Evidences from different studies show that 3 - 50% of familiar BC cases are accounted for by mutations in *BRCA1/2* [15]. Prevalence of *BRCA1/2* mutations among high-risk cancer patients may vary by ethnicity and study inclusion criteria. In this study the frequency of *BRCA1/2* point mutations in the high-risk families was low (7.1%). This percentage depends on the number of BC cancer in the family, the presence of OC, and on the presence of male BC. The highest frequency of *BRCA1/2* mutations was found in families (n=88) with 3 or more cases of BC or OC (15.9%, 95%CI [8.3 – 23.6]), and 22.2% of the families (n=9) with one BC and one OC were carriers of *BRCA1/2* mutations. Our results agree with those reported by other groups in Spanish and in Hispanic American populations. Diez et al. [16] reported 17% (95%CI 11.9 – 22.1) of *BRCA1/2* mutation in the group of families with 3 or more BC cases (n=206). A study conducted in Chilean population by Gallardo et al. [10] reported a 25% mutation frequency in families with 3 or more BC cases (5/20 [95%CI 6.0 – 44.0]) [14], and in Colombian population a 22.2% (95%CI 0 – 49.4) mutation frequency was found in families with 3 BC cases (n=9). In Mexico, Ruiz-Flores et al. [13] reported that three of 17 (18% [95%CI 0 – 35.8]) site-specific BC families were carriers of *BRCA1/2* mutations.

No *BRCA1/2* pathogenic mutations were found in the group of early-onset BC patients (<35 years), which suggests that this group of families are likely associated with other susceptibility genes. Previously, our group reported that in Chilean BC cases, the *RAD51*-135G>C polymorphism and the combined genotypes Thr/Met – E/G (*XRCC3*-T241M – *RAD51D*-E233G) were 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. These findings also increased BC risk for BC cases diagnosed before 35 years and

with no family history of BC and/or OC [17]. Also, Diez et al. [16] described low frequencies (2.6%) of *BRCA1/2* mutation for single women with BC and no family history.

We report four novel pathogenic mutations (two in *BRCA1* and two in *BRCA2*). Three of them were reported in our previous study [9], and the *BRCA2* c.9490delG mutation was described for the first time. The new mutation was found in the BC/OC index case of a family that included other three BC cases and one prostate cancer case. The mutation was found in the index case who presented OC at age 42 and BC at age 50 and in her sister affected with BC at age 38. We considered c.9490delG mutation to be a pathogenic mutation because it generates a truncated protein at codon 3088, deleting 330 aminoacids at its C-terminus. The C-terminal aminoacids are critical because the nuclear localization signals (NLSs) of *BRCA2* reside within the final 156 residues of *BRCA2* [18]. The smallest known cancer-associated deletion of *BRCA2* is predicted to remove 224 aminoacids (7% of the coding sequence) from its C-terminus [19]. Previous reports have indicated that all of the known disease linked truncation of *BRCA2* are likely to be non-functional because they do not translocate to the nucleus [18]. Therefore, the c.9490delG is probably pathogenic and disease associated.

Recurrent mutations

In our first screening of *BRCA1/2* mutations in a sample of 64 high-risk BC and/or OC families from Chile we conclude that the BRCA gene mutation spectrum was heterogeneous and broad [9]. The present study found three recurrent mutations in *BRCA1* (c.187_188delAG, c.2605_2606delTT, and c.3450_3453delCAAG) and three in *BRCA2* (c.4969_4970insTG, c.5374_5377delTATG, c.6503_6504delTT), which contributed to 63.6% and 66.7% of all deleterious mutations in each gene respectively, that may reflect the presence of region-specific founder effects.

The contemporary Chilean population stems from the admixture of Amerindian peoples (Asians) with the Spanish settlers (European Caucasian) initiated in the 16^{th} and 17^{th} centuries. Later migrations (19^{th} 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 [20]. The relationships among ethnicity, Amerindian admixture, genetic markers, and socioeconomic strata has been extensively studied in Chile [21-23].

The most prevalent mutation in *BRCA1* was c.3450_3453delCAAG. This frameshift mutation deletes the SQ sequences known as SQ-cluster domain (SCDs) that are the preferred sites of ATM phosphorylation. It also deletes the entire C-terminal region that includes two regions known as the BRCT domains. They are involved in DNA repair pathways and bind to many proteins. The c.3450_3453delCAAG mutation was detected in three unrelated families. Two of these families presented five cases of BC and the third family presented only two BC cases but with early age at diagnosis (Table 3). According to data from BIC database, this mutation has been identified in Norwegian, Australian, British, Spanish, African,-American and Colombian populations. Blesa et al. [24] detected this mutation in two (3.9%) out of 51 high-risk families from Eastern Spain (Valencian Community). In Colombia the 3450_3453delCAAG mutation was the most commonly observed mutation in *BRCA1*, detected in 9.4% of the BC/OC cancer families from this country. The Chilean and Colombian populations have a high percentage of Hispanic admixture, and therefore the presence of the c.3450_3453delCAAG in both countries could be explained by a founder effect of Spanish origin.

The 2605_2606delTT was reported first by our group in the Chilean population as a novel *BRCA1* mutation [9]. To date, we have detected this *BRCA1* frameshift mutation in two Chilean families that also included cases with early age at diagnosis (Table 3). No BIC records or other description exist for this mutation. The affected families (F43 and F84) with the c.2605_2606delTT mutation had Chilean ancestry dating to many generations ago. Therefore, this mutation could have been present in the original Amerindian population that inhabited Chile prior to the colonization.

The most prevalent mutation in *BRCA2* was c.5374-5377delTATG. It was found in four families. In addition, it was detected in other two Chilean families in a previous report [10], for a total of six unrelated carrier families in the Chilean population. This 4-pb deletion is also the third most frequent *BRCA2* mutation in Spain [16, 25, 26], and it was found in 6 of 59 (10.2%) families from different geographic areas of this country. Three of these Spaniard families are from Castilla-Leon [27, 28]. Notably, the Spanish settlers that arrived in Chile in the 16th century came mainly from Central Spain, specifically from the former kingdom of Castilla-Leon. Therefore, it is possible that the high prevalence of c.5374-5377delTATG in Chilean families may be a consequence of a founder effect of Spanish origin. The *BRCA2* c.5374_5377delTATG mutation truncates the BRCA protein at codon 1732, disrupting 3 out of 8 BRC repeats that interact with RAD51, and the entire C-terminal region. Previous reports have indicated that *BRCA2* is associated with an older age of diagnosis of BC [29]. On the contrary, in the Chilean families carriers of the c.5374_5377delTATG mutation the average age of BC onset is 42 years. Moreover, one of the positive families presents three cases with prostate cancer (Table 4).

The second recurrent mutation in *BRCA2* is a 2-bp insertion of TG in nucleotide 4969 in exon 11 (c.4969_4970insTG), and it leads to a premature termination at codon 1617. This frameshift mutation is predicted to result in a protein truncation at codon 1617, deleting 53% of the protein. This mutation was previously described by Gallardo et al. [10] in an Italian/Chilean family. We detected this point mutation in two unrelated families. The affected families (F77 and F325, Table 4) had Chilean ancestry dating from many generations ago. Both families had cases with early age at diagnosis ($\leq$ 40 years), indicating a high penetrance of this mutation. Moreover the family F325 presents one case of prostate cancer diagnosed at age 35. To our knowledge this mutation has not been described in other populations.

The spectrum of recurrent mutations identified in Chilean families differed completely from that of Hispanic families of predominantly Mexican origin from Southern California [30]. None of the six recurrent mutations identified in Hispanic families of predominantly Mexican origin in the USA were found in the Chilean families or in Hispanic families from Colombia [14], implying that the mutation spectra among Hispanic populations in South America and the USA may differ.

Variants of unknown significance

In our screening of the BRCA1/2 genes, we found fourteen variants of unknown significance, six in BRCA1 and eight in BRCA2 (Table 5). In 12 of the 13 families the variants of unknown significance were identified exclusively in healthy relatives, and therefore it was not possible to establish whether they were associated with the cancers present in the families. Only in family F239 the proband with BC was carrier of variant c.8381T>C, but her daughter who also had BC was not a carrier. In this family it is likely that the variant is not the genetic cause of the BC. The most interesting variant was c.4747_4749delATT in exon 11 of BRCA2, which deletes isoleucine at position 1583 of the BRCA2 protein. The BRCA2 protein directly binds to human RAD51 through its eight BRC repeats located at the 5' region of exon 11. The number, sequence and spacing between these motifs is conserved during vertebrate evolution, suggesting its functional relevance [31]. BRC3 and BRC4 (amino acids 1517 – 1551) repeats have the strongest interaction with RAD51 [32]. Pellegrini et al. [33] have solved the crystal structure of the BRC4 peptide sequence bound to the RAD51 protein. This structure demonstrates a series of hydrophobic and hydrophilic interactions involving the hairpin structure of BRC4. Contact between BRC4 and RAD51 is maintained from Leu1521 to Glu1548 of the BRC4 sequence. The c.4747_4749delATT mutation deletes a hydrophobic amino acid (isoleucine) at position 1583, near to BRC4. This mutation was found only in a BC case with no family history of BC or OC cancer but with early age at diagnosis. Considering that this mutation does not result in a protein truncation, it is not possible to classify it as pathogenic. Nevertheless, we cannot exclude the possibility that this mutation could be a pathogenic point mutation. Functional assays are necessary to determine how this mutation could affect critical BRC4 interaction sites.

Large genomic rearrangements

To determine the impact of *BRCA1/2* LGRs in Chilean population, 56 hereditary BC and/or OC Chilean families negative for small mutations were screened using the MLPA technique. This represents the first study to screen for LGRs in *BRCA1/2* in Chile and South America. No LGRs were found in either *BRCA1* or *BRCA2*. In different populations it has been reported that LGRs in *BRCA1* account for 0 – 27% of all disease-causing mutations, while LGRs in *BRCA2* are rare and play a minimal role in BC/OC [34, 35]. The variation in reported percentages may be attributable to ethnicity, inclusion criteria or technique used for LGRs detection. In this study, the mutation analysis for LGRs in *BRCA1* and *BRCA2* was performed using MLPA, which is the most common technique for LGR detection in the BRCA genes. The literature recommends screening for LGRs in *BRCA1* in families with multiple cases of BC/OC and average age at diagnosis of 50 years or less. It is also recommended to screen for LGRs in *BRCA2* in families with at least one case of male BC. In our study only 36 out of the 56 analyzed cases belong to families with at least three BC/OC cases, five of which have OC cases in their families.

With respect to ethnicity, *BRCA1*/2 LGRs play little or no role in hereditary BC in Afrikaner [35], Finnish [36-38], French-Canadian [39], and Iranian [40] populations. The high proportion of LGRs detected in the Hispanic population of the USA can be explained by a single founder deletion of exons 9-12 [41]. In Spain, the frequency of LGRs affecting the *BRCA1/2* genes have been estimated at around 2% in *BRCA1* and 1.5% in *BRCA2* in cases previously testing negative for *BRCA1/2* point mutations [42]. In Asian populations, LGRs account for 3% of case in Singapore [43] and 0.8% in Korea [44]. In the Chilean group screened in this study, no LGRs were found (0/56) in either *BRCA1* or *BRCA2*. Is likely that such mutations play no role in BC/OC or have a low frequency in the actual Chilean population.

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The authors declare that they have no competing interests.

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