BRCA1 and *BRCA2* mutations in a South American population

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Abstract

A sample of 64 high-risk breast and/or ovarian cancer families from Chile were screened for germ-line mutations in the coding sequences and exon–intron boundaries of *BRCA1* (MIN no. 113705) and *BRCA2* (MIN no. 600185) genes using conformation-sensitive gel electrophoresis, and the mutations found were confirmed with direct sequencing. Seven families (10.9%) were found to carry *BRCA1* mutations and three families (4.7%) had *BRCA2* mutations. Six different pathogenic mutations were detected in *BRCA1*, four that had been reported previously (c.187_188delAG; c.300T → G, c.3450_3453delCAAG and IVS17-1G → A) and two novel mutations (c.2605_2606delTT and c.4185_4188delCAAG). In *BRCA2*, we found three different pathogenic mutations, two previously described (c.6174delT and c.6503_6504delTT) and one novel mutation (c.5667delT). We also identified nine variants of unknown significance (five in *BRCA1* and four in *BRCA2*). These findings indicate that the Chilean population has a heterogeneous spectrum of prevalent *BRCA* mutations. Given the results obtained in our study, the screening of the entire *BRCA1* and *BRCA2* coding regions is necessary for the molecular genetic testing of Chilean high-risk breast/ovarian cancer patients. To our knowledge, this is the first genetic study of *BRCA* gene mutations conducted in Chile. The Chilean population has a well-known admixed Amerindian-Caucasian ratio and, therefore, our findings are not only important per se, but they constitute the basis for improved and more specific genetic counselling, as well as to support for preventive campaigns geared toward the Chilean population.

1. Introduction

Breast cancer is the most common cancer in women. Every year, one million new cases are reported world wide, representing 18% of the total number of cancer in women [1]. It has been established that one out of eight women will develop breast cancer at some point in her life [2,3]. In Chile, the mortality rate in women due to breast cancer has been increasing steadily, from 9.5 per 100,000 in 1985 to 12.8 per 100,000 in 1995 [4], actually representing the second-highest mortality rate due to cancer in women (13.2/100,000 women) after gall bladder cancer (16/100,000). Its rate is now higher than cancer of the cervix/uterus (9/100,000 women). The incidence rates of breast cancer have increased concomitantly from 16.2/100,000 in 1998 up to 26,2/100,000 in 2002 [5,6].

In 5–10% of breast cancer cases, the disease is the result of a hereditary predisposition, and a significant part of this percentage can be attributed to mutations in either of two tumor suppressor genes, *BRCA1* [7] (MIN no. 113705) and *BRCA2* [7] (MIN no. 600185), which confer high susceptibility to familial breast and/or ovarian cancer [8,9]. The Breast Cancer Information Core Internet Website (BIC) [10] describes hundreds of mutations in *BRCA1* and *BRCA2* found in families with hereditary breast and ovarian cancer (http://www.nhgri.nih.gov/Intramural_research/Lab-tranfer/Bic/index.html). The proportion of high-risk families with breast and/or ovarian cancer cases caused by *BRCA1* or *BRCA2* mutations varies widely among populations. In some groups, only one or a few founder mutations can explain the majority of inherited breast and ovarian cancer cases, whereas in other populations, a wide spectrum of different mutations throughout the sequence of both genes is present with no evidence of a founder effect [11,12].

The accumulated knowledge about the prevalence and nature of *BRCA1* and *BRCA2* mutations in specific populations may facilitate the genetic analysis of patients who are at risk for breast cancer. Data on the contribution of germline *BRCA1/BRCA2* mutations to breast cancer in South American populations are scarce, and information about the contribution of these mutations toward the incidence of breast cancer in the Chilean population is even more scarce. In this study, we describe the results of germline mutations in *BRCA1* and *BRCA2* in a group of 64 high-risk Chilean families selected on the basis of their family history of breast and ovarian cancer. The genetic diagnosis of germline *BRCA* mutations will help develop a more personalized approach for treating these patients and their relatives.

2. Materials and methods

2.1. Families

In this study, 64 high-risk families were selected from the files of the Servicio de Salud del Area Metropolitana de Santiago, Corporación Nacional del Cáncer (CONAC), and the Fundación Arturo López Pérez. The selection criteria and clinical characteristics of the families included in this study are listed in Table 1. 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 breast cancer, such as Li-Fraumeni syndrome, ataxia-telangiectasia, or Cowden disease. 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.

In the sample of selected families, 12.5% (8/64) had bilateral breast cancer; 7.8% (5/64) had both breast and ovarian cancer, and 1.6% (1/64) presented male breast cancer. From the 64 families, 69 women with breast cancer and 1 woman with ovarian cancer participated in this study. From these women, 31.9% (22/69) had early-age onset ($\leq$40 years) of diagnosis, with a mean age of 32 years. There was no upper or lower age cut-off for breast cancer cases. Breast and ovarian cancer for all cases included in the study were verified by means of the original report of the pathologist.

2.2. BRCA1 *and* BRCA2 *mutation analysis*

Genomic DNA was extracted from peripherical blood lymphocytes of 69 index cases and 104 healthy relatives belonging to the 64 high-risk selected families. Samples were obtained according to the method described by Chomczynsky and Sacchi [13]. The whole coding sequences and exon–intron boundaries of the *BRCA1* and *BRCA2* genes were amplified by polymerase chain reaction using previously described primers [14–16]. The fragments obtained were analyzed for sequence variants using conformational

Table 1
Selection criteria and clinical characteristics of the families included in this study

Selection criteria	Family no.
2 family members with breast cancer	6
2 family members with breast cancer, onset before age 40 in one	7
$\geq$2 family members with breast cancer, bilateral in one	7
$\geq$3 family members with breast cancer	17
$\geq$3 family members with breast cancer, at least one with onset before age 40	18
3 family members with breast cancer, one male cancer	1
$\geq$3 family members with a combination of breast and ovarian cancer	2
$\geq$2 family members with breast cancer, one with both breast and ovarian cancer	2
5 family members with breast cancer, bilateral in one and ovarian cancer in other	1
Single affected individual with breast cancer $<$age 31	3
Total	64

sensitive gel electrophoresis. Amplified samples were denatured at 95°C for 5 minutes and at 65°C for 1 hour to generate heteroduplex. The products were diluted 1:2 in sucrose buffer and loaded in a partially denaturing mutation detection enhancement gel at constant power of 7 W during different time periods, depending on the size of the fragment. Gels were silver-stained and dried on a vacuum gel dryer.

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, Foster City, CA) and a rhodamine dye terminator system (Perkin Elmer, Wellesley, MA). Any mutation found was confirmed on a second DNA sample isolated from a duplicate tube of blood followed by sequencing in both forward and reverse directions.

In addition to deleterious mutations, we detected several polymorphisms and variants of unknown significance. All the missense mutations that had not been previously demonstrated to have deleterious effects were considered variants of unknown significance. Some of them were novel or had been reported in the BIC database as unclassified variants. We included as polymorphisms those variants that are not predicted to significantly affect exon splicing, variants that do not change amino acids, or variants that change amino acids but appear in the BIC database as polymorphisms.

2.3. Mutation nomenclature

Mutations are described at the cDNA level according to the human *BRCA1* reference sequence (accession no. U14680) and the human *BRCA2* reference sequence (accession no. U43746) from the GenBank sequence database. The *BRCA1/BRCA2* numbering for the traditional mutation nomenclature used in BIC database is based on the reference sequences stated above, where the A of the ATG translation initiation codon is at the position 120 of *BRCA1* and

at the position 229 of *BRCA2*. The approved systematic nomenclature four each mutation is indicated in Tables 2–4. The Human Genome Organization approved systematic nomenclature for the description of sequence variants in DNA and protein sequences [17–19] follows the rule in which the A of the ATG translation initiation codon is +1.

3. Results

Germline *BRCA1* or *BRCA2* mutations were found in 9 (14%) of the 64 Chilean families. We detected a total of nine pathogenic mutations (six for *BRCA1* and three for *BRCA2*; Table 2). Furthermore, we identified 9 variants of unknown significance (5 in *BRCA1* and 4 in *BRCA2*) (Table 3) and 22 polymorphisms (14 in *BRCA1* and 8 in *BRCA2*; Table 4). All were named according to the practice of the BIC database and the systematic nomenclature according to GenBank entries U14680 (*BRCA1*) and U43746 (*BRCA2*).

The c.187_188delAG mutation was found in 2 of the 64 BRCA families (3.1%). The remaining mutations, including *BRCA1* c.300T→G, c.2605_2606delTT, c.3450_3453delCAAG, c.4185_4188delCAAG, and c.IVS17-1G→A, as well as *BRCA2* c.5667delT, c.6174delT, and c.6503_6504delTT, were unique (i.e., each one was detected only in a specific family; Table 2). These results show that the spectrum of *BRCA* gene mutations is heterogeneous and broad in the Chilean population.

3.1. BRCA1 *mutations*

BRCA1 mutations were identified in seven families (10.9%) of our cohort. Six of these were families with site-specific breast cancer, and only one was a breast/ovarian cancer family. From the seven families with *BRCA1* mutations, six had three or more breast cancer cases and three presented bilateral breast cancer cases. In addition, all the positive families for *BRCA1* mutations had one or more cases of breast cancer, with age at diagnosis being below 45 years (Table 2).

Six distinct BRCA1 mutations were identified in our study (Table 2). Four of the mutations lead to premature termination of the protein translation (three frameshift and one nonsense mutations). The other two were missense mutations, with one affecting the splice site. Four of these mutations have been reported previously in the BIC database by other authors. Nevertheless, it is important to emphasize that one of the previously reported mutations, IVS17-1G→A, has only one record in the BIC database. The other two pathogenic mutations had not been reported in neither the BIC nor the Human Gene Mutation Database [20] by January 2005 and are considered novel.

The IVS17-1G→A mutation was identified in a Chilean breast cancer family (F65) containing four cases of female breast cancer at ages 44, 50, 60, and 60 (one of these with bilateral breast cancer; Table 2). We obtained family DNA samples from the index case and from one of her healthy sisters. The mutation was present only in the index case.

This patient also presented one variant of unknown significance c.3399T→A (Table 3) and five polymorphisms (c.3260A→T, c.3232A→C, IVS7 + 35del14, IVS7 + 49del15, and IVS17-53C→T). Her healthy sister was negative for the mutation but was carrier of all the other sequence alterations.

The novel *BRCA1* c.2605_2606delTT mutation was identified in a breast cancer family (F43). The mutation is located in exon 11 and generates a nonsense codon at nucleotide 829, deleting 55% of the protein. It is therefore considered pathogenic because it generates a truncated protein. This family presented five breast cancer cases, two of them diagnosed under the age of 50, as well as eight other types of cancer (Table 2). The index case and one sister presented bilateral breast cancer below the age of 50. In this family, the index case carried the c.2605_2606delTT mutation and two previously described polymorphisms, c.2430T→C and c.3667A→G [10]. We also studied two healthy sisters, who were negative for these mutations.

The other novel mutation in *BRCA1*, c.4185_4188del4, generates a frameshift similar the one produced by the *BRCA1* c.4184_4187del4 mutation, resulting in the same stop codon at codon 1364. The c.4185del4 mutation was found in family F21, which only presented three cases of breast cancer at ages 47, 45, and 49. The mutation was found in the index case but not in her healthy sister. Sequencing of the amplified DNA revealed heterozygosity for the c.4185del4 mutation in the index case and the presence of normal alleles in her healthy sister. We could not obtain DNA from the other healthy sister of the index case but the fact that she is presently 74 years old suggests that she probably has normal alleles.

In addition, five *BRCA1* variants of the missense type — c.465G→C, c.3399T→A, c.3867G→A, c.4861A→T, and c.4864A→C — were detected in four different families (Table 3). It has not yet been demonstrated whether these variants have pathogenic consequences.

Most of the families with mutations in *BRCA1* (5/7) have also presented polymorphisms in *BRCA1* or *BRCA2*. Specifically, families F4, F21, and F43 are all carriers of the polymorphisms c.2430T→C and c.3667A→G, both in heterozygote conditions, in addition to the mutations in *BRCA1*. Family F13 was carrier of polymorphism p.Lys1132Lys in *BRCA2*. Morever, one family (F65) presented five polymorphisms and a variant of unknown significance. This family carries two exonic and three intronic polymorphisms in *BRCA1* and a missense *BRCA1* mutation, c.3399T→A, which is considered a variant of unknown significance.

3.2. BRCA2 *mutations*

BRCA2 mutations were detected in three families (4.7%). Two of these were breast cancer families and one was a breast/ovarian cancer family. All of them had three

Table 2
BRCA1 and *BRCA2* germline mutations detected in Chilean high-risk breast/ovarian cancer families

E/I no.	BIC nomenclature[a]	Systematic nomeclature[b]	Effect on amino acid	Mut. type	Family	Family type	Index case status (age)	Family history (Br and Ov Ca) (ages)	Other cancers (age)
BRCA1									
E2	c.187_188delAG	c.68_69delAG	p.Glu23fs (Stop39)	F	F4	Br	BrCa (38)	2 Br Ca (38, 62)	Prostate (82), uterine (50)
E2	c.187_188delAG	c.68_69delAG	p.Glu23fs (Stop39)	F	F46	Br/Ov	BrCa (42)	3 Br Ca (42, 80, 70) 3 Ov Ca (34, 41, 55)	Colon (60); pancreatic (42, 65); testicular (38); melanoma (54)
E5	c.300T→G	c.181T→G	p.Cys61Gly	M	F13	Br	BrCa (37)	4 Br Ca (37, 37, 37, 50)	Lung (U); kidney (U) bone (U); stomach (35) prostate (U)
E11	c.2605_2606delTT	c.2486_2487delTT	p.Phe829Stop	N	F43	Br	BrCa (45)	5 Br Ca (45, 49, 51, 51, U) Bil	2 Uterine (U); 6 stomach (U)
E11	c.3450_3453 delCAAG	c.3331_3334 delCAAG	p.Gln1111fs (Stop1115)	F	F36	Br	BrCa (27)	5 Br Ca (27, 37, 37, 52, 79) Bil	3 Uterine (U); esophagus (U) stomach (U)
E11	c.4185_4188 delCAAG	c.4066_4069 delCAAG	p.Gln1356fs (Stop1364)	F	F21	Br	BrCa (49)	3 Br Ca (45, 47, 49)	–
I17	IVS17-1G→A	c.5075-1G→A	affected splicing[?] r.5194_5294del[?]	S	F65	Br	BrCa (44)	4 Br Ca (44, 50, 60, 60) Bil	3 Stomach (36, 36, 50); 2 lung (88, 50); liver (50) colon (65); brain (U)
BRCA2									
E11	c.5667delT	c.5439delT	p.Leu1813fs (Stop1814)	F	F37	Br	BrCa (43)	3 Br Ca (26, 43, 45)	Colon (U)
E11	c.6174delT	c.5946delT	p.Ser1982fs (Stop 2003)	F	F46	Br/Ov	BrCa (42)	3 Br Ca (42, 80, 70) 3 Ov Ca (34, 41, 55)	Colon (60); pancreatic (42, 65); testicular (38); melanoma (54)
E11	c.6503_6504delTT	c.6275_6276delTT	p.Leu2092fs (Stop2098)	F	F39	Br	BrCa (60)	3 Breast (60, 67, 70)	Other cancer (U); myeloma (U)

Abbreviations: E/I no., exon/intron number; Br, breast; Ov, ovarian; Ca, cancer; bil, bilateral breast cancer; U, unknown age; F, frameshift, M, missense; N, nonsense; S, splice.

[a] BIC traditional nomenclature: +1 is 120 bases before the A of the ATG translation initiation codon, based on mRNA *BRCA1*, RefSeq U14680; +1 is 229 bases before the A of the ATG translation initiation codon, based on mRNA *BRCA2*, RefSeq U43746.

[b] Approved systematic nomenclature: the A of the ATG translation initiation codon is +1, based on mRNA *BRCA1*, RefSeq U14680; the A of the ATG translation initiation codon is +1, based on mRNA *BRCA2*, RefSeq U43746.

[?] Probable effect.

Table 3
BRCA1 and *BRCA2* unknown variants detected in Chilean high risk breast/ovarian cancer families

E/I no.	BIC nomenclature[a]	Systematic nomeclature[b]	Effect on amino acid	Mut. type	Family	Family type	Index case status (age)	Family history (Br and Ov Ca; age)	Other cancers (age)
BRCA1									
E7	c.465G→C	c.346G→C	p.Glu116Gln	M	F16	Br/Ov	BrCa (48)	3 Br Ca (48, 53, U) 3 Ov Ca (70, 76, U)	Pancreatic (80); lung (66, U); larynx (77); melanoma (72,53); bladder (50)
E11	c.3399T→A	c.3280T→A	p.Tyr1094Asn	M	F65	Br	BrCa (44)	4 Br Ca (44, 50, 60, 60)	3 Stomach (36, 36, 50); 2 lung (88, 50); liver (50) colon (65); brain (U)
E11	c.3867G→A	c.3747G→A	p.Glu1250Lys	M	F14	Br	Unaffected	3 Br Ca (30, 36, 47)	Uterine (U); stomach (U)
E16	c.4861A→T	c.4742A→T	p.Glu1581Val	M	F38	Br	BrCa (52)	3 Br Ca (52, 55, 60)	Stomach (U); 2 lung (U)
E16	c.4864A→C	c.4745A→C	p.Asp1582Ala	M	F38	Br	BrCa (52)	3 Br Ca (52, 55, 60)	Stomach (U); 2 lung (U)
BRCA2									
E4	c.568C→T	c.340C→T	p.His114Tyr	M	F1	Br	BrCa (47)	4 Br Ca (47, 49, 50, 53)	Leukemia (U); ganglionar (U)
E11	c.5015A→C	c.4787A→C	p.Asn1596Ser	M	F16	Br/Ov	BrCa (48)	2 Br Ca (48, 53) 3 Ov Ca (70, 76, ?)	Lung (66); melanoma (72, 53)
E11	c.5972C→T	c.5744C→T	p.Thr1915Met	M	F40	Br	BrCa (39)	4 Br Ca (38, 39, 39, 40)	Gynecological (U); stomach (U)
E22	c.9023A→C	c.8795A→Cc.	p.His2932Pro	M	F10	Br	BrCa (60)	3 Br Ca (46, 50, 60)	Thyroid (43)

Abbreviations: E/I no., exon/intron number; M, missense; Br, breast; Ov, ovarian; Ca, cancer; U, unknown age.

[a] BIC traditional nomenclature, +1 is 120 bases before the A of the ATG traslation initiation codon, based on mRNA *BRCA1*, RefSeq U14680; +1 is 229 bases before the A of the ATG translation initiation codon, based on mRNA *BRCA2*, RefSeq U43746.

[b] Approved systematic nomenclature, the A of the ATG traslation initiation codon is +1, based on mRNA *BRCA1*, RefSeq U14680; the A of the ATG traslation initiation codon is +1, based on mRNA *BRCA2*, RefSeq U43746.

breast cancer cases and two had one or more cases who were diagnosed before the age of 45 years (Table 2).

Three different *BRCA2* mutations were detected (Table 2). Two of the *BRCA2* mutations have been described previously and one had no record in neither the BIC nor the HGMD database as of January 2005. None of the mutations were identified in more than one family. All the mutations resulted in a truncated protein.

The two previously described mutations detected in our study were c.6174delT and c.6503_6504delTT. The c.6174delT mutation was identified in a breast/ovarian family and the c.6503_6504delTT in a breast cancer family (Table 2).

The novel *BRCA2* mutation c.5667delT was identified in a breast cancer family (F37), which included three breast cancer cases diagnosed before the age of 45 years and one case of colon cancer (Table 2). The index case in this family, a woman who was diagnosed with breast cancer at the age of 43, was a carrier of the aforementioned mutation as well as a homozygote for the c.3667A→G polymorphism. Her healthy daughter is negative for this mutation. This mutation is located in exon 11 and generates a stop codon at position 1814, thus deleting 47% of the protein.

In addition, the following four *BRCA2* missense mutations of unknown significance were detected in four different families: c.568C→T, c.5015A→C, c.5972C→T, and c.9023A→C (Table 3).

4. Discussion

Few studies are available which describe the spectrum of *BRCA1* and *BRCA2* mutations among Latin American populations. In the United States, some studies have analyzed populations of Hispanic origin [21,22]. In Chile, the prevalence and mortality rates of breast cancer have increased in recent years. At this time, to our knowledge, there has been no published work describing mutations of the entire *BRCA1* and *BRCA2* coding regions associated with hereditary breast cancer.

The contemporary Chilean population consists of an admixture of Amerindian peoples (Asians) with Spanish settlers (European Caucasian), which was initiated in the 16th and 17th centuries. Later migrations (19th 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 these groups are restricted to the specific locations of the country where they settled [23,24]. The relationship among ethnicity, Amerindian admixture, genetic markers, and socioeconomic strata has been studied extensively in Chile [25–27].

As previously mentioned, 9 distinct *BRCA1* and *BRCA2* gene mutations were detected in 10 of the 64 Chilean breast/ovarian cancer families. One mutation was found in two families, and the rest were detected in 8 different families. These findings seem to indicate that the *BRCA1/2* gene mutation spectrum is rather broad in the Chilean population. Most of the disease-causing mutations were small deletions resulting in frameshifts that cause premature stop codons. Three of the nine mutations detected are novel (two in *BRCA1* and one in *BRCA2*), and the IVS17-1G→A mutation presents only one record in the BIC database. Therefore, the majority of the mutations have been detected previously in other populations.

Table 4
BRCA1 and *BRCA2* polymorphisms detected in Chilean high-risk breast/ovarian cancer families

E/I no.	BIC nomenclature[a]	Systematic nomeclature[b]	Predicted effect
BRCA1			
I7	c.560+35del14	c.441+35del14	Non coding
I7	c.560+49del15	c.441+49del15	Non coding
I7	c.560+64delT	c.441+64delT	Non coding
I8	c.561-57del7	c.442-57del7	Non coding
E11	c.2430T→C	c.2311T→C	p.Leu771Leu
E11	c.3232A→C	c.3113A→C	p.Glu1038Gly
E11	c.3667A→G	c.3548A→G	p.Lys1183Arg
I17	c.5194-53C→T	c.5075-53C→T	Non coding
I18	c.5271+66G→A	c.5152+66G→A	Non coding
I20	c.5397-57A→C	c.5278-57A→C	Non coding
I20	c.5397-56A→T	c.5278-56A→T	Non coding
I20	c.5397-46C→G	c.5278-46C→G	Non coding
I20	c.5397-45C→A	c.5278-45C→A	Non coding
I20	c.5397-27insC	c.5278-27insC	Non coding
BRCA2			
E2	c.203G→A	UTR	UTR
E10	c.1038A→T	c.810A→T	p.Ser270Ser
E11	c.2895T→C	c.2667T→C	p.Asn889Asn
E11	c.3255C→T	c.3027C→T	p.Phe1009Phe
E11	c.3267A→G	c.3039A→G	p.Ser1013Ser
E11	c.3624A→G	c.3396A→G	p.Lys1132Lys
E22	c.9025A→C	c.8797A→C	p.Arg2933Arg
E22	c.9078G→A	c.8850G→A	p.Lys2950Lys

Abbreviation: E/I no., exon/intron number.

[a] BIC traditional nomenclature, +1 is 120 bases before the A of the ATG traslation initiation codon, based on mRNA *BRCA1*, RefSeq U14680, or +1 is 229 bases before the A of the ATG traslation initiation codon, based on mRNA *BRCA2*, RefSeq U43746.

[b] Approved systematic nomenclature, the A of the ATG traslation initiation codon is +1, based on mRNA *BRCA1*, RefSeq U14680, or the A of the ATG traslation initiation codon is +1, based on mRNA *BRCA2*, RefSeq U43746.

BRCA1 mutations were found in seven of our families (10.9 %). In *BRCA1*, we detected six mutations, four of which have been described previously in other populations. The first one was the c.187_188delAG (traditionally denominated as 185delAG) mutation in exon 2. This mutation is the most common alteration reported in *BRCA1* and is the first with a high frequency in Ashkenazi Jews [28–31]. In Chile, Trincado et al. did not detect the c.187_188delAG mutation in 55 women affected with breast cancer, 15 of which had a positive family history and 40 had sporadic breast cancer [32]. The study conducted by Jara et al. was the first one to establish a frequency of 0.26% for the c.187_188delAG mutation in a group of 382 healthy Chilean women with at least two relatives with breast cancer [33]. In the present study, this mutation was found in two families of the cohort (3.12%; F4 and F46). These two families did not self-report Ashkenazi ancestors. In family F4, the mutation was detected in the index case and in her healthy sister. The healthy sister is actually 48 years old. In family F46, the mutation was detected in the index case and in her healthy 18 year-old-niece. The index case's sister died of ovarian cancer when she was 34 years old, and she was probably a carrier of the c.187_188delAG mutation or another breast-ovarian cancer susceptibility mutation. Ah Mew et al. recently identified the c.187_188delAG mutation in a non-Jewish Chilean family [34]. Epidemiologic studies have recently detected the c.187_188delAG mutation in non-Jewish individuals in different countries [35], including Spaniards of non-Jewish origin [36–38]. Therefore, the existence of this mutation in the actual admixed Chilean population may have been brought by the Spanish settlers. Nevertheless, this mutation has not yet been studied in the few Amerindians who still remain geographically isolated in some regions of the country.

The second previously described *BRCA1* mutation was c.300T→G (exon 5). This mutation has been described in Poland [39,40], Hungary [41], Germany [42], and in other regions of Europe [10], but it is not a recurrent mutation in the Spanish population. The c.300T→G mutation in our group of families was found only in family F13, a breast cancer family with four cases of female breast cancer (Table 2). In this family, we obtained DNA samples from the index case and from two healthy relatives. The mutation was detected in the index case and in her healthy sister, but not in her healthy cousin. This family had no recent European ancestry, and the maternal lineage had only Chilean ancestry dating back several generations. The paternal lineage is Ashkenazi Jewish dating back two generations ago. The c. 300T→G mutation, however, is not frequent in Jewish populations. There is cancer history in both familial lineages, although breast cancer is present only in the paternal lineage. A possible explanation for this finding could be the admixture of this family's Jewish ancestors with Europeans, among whom this mutation is frequent. This mutation probably has a low frequency in the actual Chilean population.

The third previously described mutation was a four–base pair (bp) deletion of CAAG in exon 11 (*BRCA1*), which produced a frameshift mutation, c.3450_3453delCAAG. This frameshift mutation is predicted to result in a protein truncation at codon 1115, putatively deleting 40% of the protein. The c.3450_3453delCAAG mutation has 32 records in the BIC database, and has been reported in Norwegian, Australian, British, Colombian, Spanish, and African-American populations. This mutation was found in one family (F36) that presented five cases of female breast cancer and other different cancers (Table 2). For this family, we obtained DNA samples from the index case and from five healthy relatives. The mutation was found in the index case but all the relatives were negative. Sequencing of the amplified DNA revealed heterozygosity for the c.3450_3453delCAAG mutation in the index case. Family F36 reported recent Spanish ancestors in both lineages. It is possible, therefore, that c.3450_3453delCAAG could represent a founder effect of Spanish origin.

The fourth previously described mutation in *BRCA1* was IVS17-1G→A, which affects the splice site. This mutation

has only one record on the BIC database in the Ashkenazi Jewish population, with a very low frequency (1/737) [43]. Approximately 4% of the genetic variants in the BIC database are reported as splice site alterations. For most of these mutation types, knowledge about their effect at the cDNA level is scarce. Accurate RNA splicing requires the absence of mutations in the *cis*-acting consensus elements known to be involved in RNA splicing (i.e., the conserved sequence motifs at the intron–exon junctions and the branchpoint) [44] showed that a sequence of eight nucleotides is highly conserved at the exon–intron boundary in other vertebrate species, the splice donor or 5' splice site sequence [(A/C)AG// gta/g)agt]. The boundary between an intron and an exon, the acceptor or 3' splice site, also exhibits a highly conserved sequence of four nucleotides, preceded by a pyrimidine-rich region (tyttytytyyyyncag//G, where y represents any pyrimidine and n stands for any nucleotide).

The IVS17-1G→A mutation can lead to an aberrant transcript. A splice site prediction program [45], the Berkeley Drosophila genome project (http://www.fruitfly.org/ seq_tools/splice.html), calculated a score of 0.89 for the normal acceptor around 64781: CAG//atgctg. In this mutation, the normally used splice acceptor was destroyed and four novel alternative acceptor sites were created (novel acceptors: TAG//gaattgc around 64823 with a score of 0.65; CAG//aattgca around 64903 with a score of 0.80; CAG// ataagct around 65024 with a score of 0.77; and TAG//gacctaatct around 65070 with a score of 0.46). Moreover, other alternative acceptor sites correspond to the normal acceptor site in exon 19 around 65359: TAG//gggtgacccag with a score of 0.96. This last acceptor site has the greatest probability of being used, if it occurs it promotes a skip of exon 18. Another possibility is to use the second acceptor site (score 0.80), which produces the deletion of the first 42 base pairs of exon 18. This deletion generates a stop codon eight amino acids downstream. The use of the remaining alternative acceptors could also produce important changes in the BRCA1 protein. Therefore, it is highly probable that the IVS17-1G→A mutation may correspond to a pathogenic mutation. This analysis illustrates the usefulness of such a splice site predictor program for analyzing splice site alterations because it allows the prediction of the activation of cryptic splice sites. For counseling purposes, it is extremely important to differentiate between pathogenic and polymorphic splice site alterations.

BRCA2 mutations were found in three of the families (4.7%). We detected two previously described mutations, c.6174delT and c. 6503_6504delTT. The 6174delT mutation has 846 records in the BIC database; ethnicity is indicated in 603 records. The analysis of these latter data established that 92.4% (557/603) corresponds to Ashkenazi, 0.16% (1/603) to Asian, 0.99% (6/603) to Caucasian, 0.66% (4/603) to central/eastern Europe, 3.15% (19/603) to western Europe, 2.15% (13/603) to Jewish, 0.16% (1/603) to Russia, and 0.16% (1/603) to Latin American/Caribbean populations. The affected family (F46) with the 6174delT

mutation has Russian maternal ancestors and Turkish paternal ancestors in both lineages three generations ago. Nevertheless, the cancer cases in this family appear in the last three generations, which lived in Chile after their ancestors migrated to this country. The 6174delT mutation had not been described in Spanish nor in Turkish populations. As a result, the presence of 6174delT in family F46 could be explained as a mutation inherited from the Russian ancestors of this family. Alternatively, the low penetrance of the *BRCA2* 6174delT mutation would explain that it was not detected in the other families of the cohort. This fact does not allow us to conclude that it is not present in the Chilean population or, alternatively, that this mutation could have a very low frequency in this population.

The other previously described mutation detected in *BRCA2* was 6503delTT in exon 11, which was present only in one of the 64 families. This is a frameshift mutation caused by the outcome of a 2-bp deletion in codon 2092 of exon 11f of the *BRCA2* gene and it creates a termination codon at 2099. The *BRCA2* 6503delTT mutation has been found to recur in geographically diverse populations [10]. This mutation has 82 records in the BIC database, and ethnicity is indicated in 48 records. The analysis of these latter data established that 56.2 % (27/48) correspond to western Europeans, 29.2% (14/48) to Caucasians, 4.2% (2/48) to English, and 2.1% (1/48) to French Canadians, Irish/Scottish, Latin, or Swedish populations. It has also been described in Mauritius [46], in the Swedish population, in male breast cancer cases [47], and in the Spanish population [48]. The affected family (F39) with the 6503delTT mutation had Chilean ancestors many generations ago. Therefore, this mutation could represent a founder effect of Spanish origin, or it could have been present in the original Amerindian population that inhabited Chile.

We report three novel pathogenic mutations (two in *BRCA1* and one in *BRCA2*), each one of them detected in a specific family. The first novel mutation in *BRCA1* was c.2605_2606delTT. It was found in a breast cancer family, which included five breast cancer cases and eight other cancers. This mutation is a 2-bp deletion of TT in nucleotide 2605 in exon 11. We considered c.2605_2606delTT as a pathogenic mutation because it generates a truncated protein. Furthermore, this mutation changes the codon 829, which is inside of the RAD51 interaction domain (758–1064 amino acids) [49]. Therefore, considering the BRCA1 and RAD51 roles in the maintenance of genomic integrity, the carriers of this mutation could display a diminished capacity in the signaling and/or repair of certain forms of DNA damage.

The second new mutation detected is a 4-bp deletion of CAAG in nucleotide 4185 in exon 11 (*BRCA1*) and it leads to a premature termination at codon 1364. This frameshift mutation is predicted to result in a protein truncation at codon 1364, putatively deleting 27% of the protein. The mutation 4185del4 differs from a previously described mutation, c.4184delTCAA, which corresponds to a 4-bp

deletion in nucleotide 4184. Nevertheless, the effect caused by these two mutations is the same, given that both of them generate a stop codon 1364. Therefore, the only difference between the mutations is that the deletion in our case occurs 1 bp after the 4184del4 mutation. To our knowledge, this mutation has not been described previously. This mutation was detected in family F21, in which all the breast cancer cases were diagnosed before age 50. Therefore, the c.4185del4 mutation has high penetrance and it may explain the breast cancer in this family.

The third novel mutation was c.5667delT in exon 11 of *BRCA2* gene. This frameshift mutation is predicted to result in a protein truncation at codon 1814. It has been suggested that the entire exon 11 is functionally relevant and active in RAD51 regulation [50]. Within exon 11 in *BRCA2*, there exists a highly conserved region in vertebrates called the BRC repeat, which includes eight internal repeats. The number sequence and spacing between these motifs is conserved during vertebrate evolution, suggesting its functional relevance [51]. These eight motifs are implicated in RAD51 binding. The c.5667delT mutation is located between BRC5 (amino acids 1649–1735) and BRC6 (amino acids 1822–1914) repeat motifs [52,53]. This mutation is probably pathogenic and disease associated.

Finally, in our scanning of the *BRCA* genes, we found nine unknown variants (Table 3); two were previously described and seven were novel. The families carriers of variants of unknown significance did not present pathogenic mutations in the *BRCA* genes. The only exception was F65, which presented the c. IVS17-1 G→A mutation (*BRCA1*) and the unknown variant c.3399T→A (*BRCA1*). Among the variants of unknown significance, only the c.Glu116Gln variant changes an amino acid by a similar one (Glu by Gln), and this variant is probably not the main cause of the disease in family F16, a breast/ovarian cancer family. In all the other cases, the amino acidic changes are more important because they change the charge or the hydrophobicity of the amino acid (Table 3), and further analyses must be done to find out the true effect of the BRCA1 protein [54]. Finally, the missense c.9023A→C mutation in *BRCA2* results in a change of histidine by proline amino acid. This mutation probably results in a modification of the protein conformation because proline can introduce turns in the protein structure. This mutation is located in the exon 22, in the C-terminal domain (residues 2478–3185), which is a conserved region in vertebrates [51]. Nevertheless, the BRCA2 protein contains no well-defined functional domains. In fact, no *BRCA2* missense mutations have been unequivocally designed as disease-associated because the of paucity of data on functional domains [55].

In summary, we report a study of the entire coding sequence of the *BRCA* genes in Chile. We found a percentage of mutations (14%) among the Chilean population similar to those reported in other countries for breast cancer families. The present study is the first BRCA disease-associated mutations analysis in Chilean families, an admixed South American population. Nevertheless, the *BRCA* gene mutation spectrum is heterogeneous and broad. We propose that the screening of the whole coding region of both *BRCA1* and *BRCA2* is necessary for molecular genetic testing in high-risk Chilean breast/ovarian cancer patients to optimize genetic counselling and disease prevention in affected families. This BRCA analysis should be done in selected high-risk families, with young age and the presence of bilateral breast cancer as the main features.

References

[1] McPherson K, Steel CM, Dixon JM. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. BMJ 2000;321: 624–8.

[2] Surveillance Epidemiology and End Results Cancer Statistics Review 1973–1999. Available at: http://www.seer.cancer.gov/csr/1973_1999/breast.pdf. Accessed January 2005.

[3] Medina E, Kaempffer AM. Cancer mortality in Chile: epidemiological considerations. Rev Med Chil 2001;129:1195–202.

[4] Peralta O, Jorquera A, Rencoret C, Castillo CD, Solé J, Campodónico Y. Cáncer de Mama, Resultados del Programa de Pesquisa y Tratamiento del Servicio de Salud Central. Rev Chil Obstet Ginecol 1995;60:417.

[5] INE (Instituto Nacional de Estadísticas de Chile). Available at: http://www.ine.cl. Accessed January 2005.

[6] MINSAL (Ministerio de Salud de Chile). Available at: http://www.minsal.cl. Accessed January 2005.

[7] MIN Online Mendelian Inheritance in Man. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM. Accessed December 2004.

[8] Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, Skolnick MH. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. Science 1994;266:66–71.

[9] Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995;378:789–92.

[10] Breast Cancer Information Core-Database (BIC). Available at: http://www.nhgri.nih.gov/Intramural_research/Lab_tranfer/Bic/. Accessed January 28, 2005.

[11] Szabo CI, King MC. Population genetics of *BRCA1* and *BRCA2*. Am J Hum Genet 1997;60:1013–20.

[12] Neuhausen SL. Ethnic differences in cancer risk resulting from genetic variation. Cancer 1999;86(11 Suppl):2575–82.

[13] Chomczynsky P, Sacchi N. Single step method of RNA isolation by guanidium thiocyanate-phenol-cloroform extraction. Anal Biochem 1987;162:156–8.

[14] Friedman LS, Ostermeyer EA, Szabo CI, Dowd P, Lynch ED, Rowell SE, King MC. Confirmation of *BRCA1* by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nat Genet 1994;8:399–404.

[15] Miki Y, Katagiri T, Kasumi F, Yoshimoto T, Nakamura Y. Mutation analysis in the *BRCA2* gene in primary breast cancers. Nat Genet 1996;13:245–7.

[16] van Orsouw NJ, Dhanda RK, Elhaji Y, Narod SA, Li FP, Eng C, Vijg J. A highly accurate, low cost test for *BRCA1* mutations. J Med Genet 1999;36:747–53.

[17] Antonarakis SE. Recommendations for a nomenclature system for human gene mutations. Nomenclature Working Group. Hum Mutat 1998;11:1–3.

[18] den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat 2000;15:7–12.

[19] den Dunnen JT, Paalman MH. Standardizing mutation nomenclature: why bother? Hum Mutat 2003;22:181–2.

[20] Human Gene Mutation Database. Available at: http://www.archive.uwcm.ac.uk/uwcm/mg. Accessed December 2004.

[21] Schubert EL, Lee MK, Mefford HC, Argonza RH, Morrow JE, Hull J, Dann JL, King MC. *BRCA2* in American families with four or more cases of breast or ovarian cancer: recurrent and novel mutations, variable expression, penetrance, and the possibility of families whose cancer is not attributable to *BRCA1* or *BRCA2*. Am J Hum Genet 1997;60:1031–40.

[22] Mullineaux LG, Castellano TM, Shaw J, Axell L, Wood ME, Diab S, Klein C, Sitarik M, Deffenbaugh AM, Graw SL. Identification of germline 185delAG *BRCA1* mutations in non-Jewish Americans of Spanish ancestry from the San Luis Valley, Colorado. Cancer 2003;98:597–602.

[23] Encina F. Historia de Chile. Santiago, Chile: Editorial Ercilla; 1983: 51–9.

[24] Cruz-Coke R. Origen y evolución étnica de la población Chilena. Rev Med Chile 1976;101:365–8.

[25] Valenzuela CY, Harb Z. Socioeconomic assortative mating in Santiago, Chile: as demonstrated using stochastic matrices of mother–child relationships applied to ABO blood groups. Soc Biol 1977;24: 225–33.

[26] Valenzuela CY, Acuña M, Harb Z. Gradiente sociogenético en la población chilena. Rev Med Chile 1987;115:295–9.

[27] Valenzuela CY. On sociogenetic clines. Ethol Sociobiol 1988;9: 259–68.

[28] Shattuck-Eidens D, McClure M, Simard J, Labrie F, Narod S, Couch F, Hoskins K, Weber B, Castilla L, Erdos M, Brody L, Friedman L, Ostermeyer E, Szabo C, King MC, Jhanwar S, Offit K, Norton L, Gilewski T, Lubin M, Osborne M, Black D, Boyd M, Steel M, Ingles S, Haile R, Lindblom A, Olsson H, Borg A, Bishop DT, Solomon E, Radice P, Spatti G, Gayther S, Ponder B, Warren W, Stratton M, Liu Q, Fujimura F, Lewis C, Skolnick MH, Goldgar DE. A collaborative survey of 80 mutations in the *BRCA1* breast and ovarian cancer susceptibility gene. JAMA 1995;273:535–41.

[29] Simard J, Tonin P, Durocher F, Morgan K, Rommens J, Gingras S, Samson C, Leblanc JF, Belanger C, Dion F, Liu Q, Skolnick M, Goldgar D, Shattuck-Eidens D, Labrie F, SA Narod. Common origin of *BRCA1* mutations in Canadian breast and ovarian cancer families. Nat Genet 1994;8:392–8.

[30] Struewing JP, Abeliovich D, Peretz T, Avishai N, Kaback MM, Collins FS, Brody LC. The carrier frequency of the *BRCA1* 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. Nat Genet 1995;11:198–200.

[31] Friedman LS, Szabo CI, Ostermeyer EA, Dowd P, Butler L, Park T, Lee MK, Goode EL, Rowell SE, King MC. Novel inherited mutations and variable expressivity of *BRCA1* alleles, including the founder mutation 185delAG in Ashkenazi Jewish families. Am J Hum Genet 1995;57:284–97.

[32] Trincado P, Fardella C, Mayerson D, Montero L, O'Brien A, Barrueto K, Gallegos R. Prevalencia de la deleción 185AGdel gen *BRCA1* en mujeres Chilenas con cáncer de mama. Rev Med Chile 1999;127:19–22.

[33] Jara L, Ampuero S, Seccia L, Bustamante M, Blanco R, Santibáñez E, Reyes JM, Ojeda JM. Frecuencia de la mutación 185delAG en el gen *BRCA1* en mujeres Chilenas sanas con antecedentes familiares de cáncer de mama. Rev Med Chile 2002;130: 1113–23.

[34] Ah Mew N, Hamel N, Galvez M, Al-Saffar M, Foulkes WD. Haplotype analysis of a *BRCA1*: 185delAG mutation in a Chilean family supports its Ashkenazi origins. Clin Genet 2002;62:151–6.

[35] Bar-Sade RB, Kruglikova A, Modan B, Gak E, Hirsh-Yechezkel G, Theodor L, Novikov I, Gershoni-Baruch R, Risel S, Papa MZ, Ben-Baruch G, Friedman E. The 185delAG *BRCA1* mutation originated before the dispersion of Jews in the Diaspora and is not limited to Ashkenazim. Hum Mol Genet 1998;7:801–5.

[36] Diez O, Domenech M, Alonso MC, Brunet J, Sanz J Cortes J, del Rio E, Baiget M. Identification of the 185delAG *BRCA1* mutation in a Spanish Gypsy population. Hum Genet 1998;103:707–8.

[37] Diez O, Osorio A, Robledo M, Barroso A, Domenech M, Cortes J, Albertos J, Sanz J, Brunet J, San Ramón JM, Alonso MC, Baiget M, Benitez J. Prevalence of *BRCA1* and *BRCA2* Jewish mutations in Spanish breast cancer patients. Br J Cancer 1999;79:1302–3.

[38] Osorio A, Robledo M, Albertos J, Diez O, Alonso C, Baiget M, Benitez J. Molecular analysis of the six most recurrent mutations in the *BRCA1* gene in 87 Spanish breast/ovarian cancer families. Cancer Lett 1998;123:153–8.

[39] Jakubowska A, Gorski B, Byrski T, Huzarski T, Gronwald J, Menkiszak J, Cybulski C, Debniak T, Hadaczek P, Scott RJ, Lubinski J. Detection of germline mutations in the *BRCA1* gene by RNA-based sequencing. Hum Mutat 2001;18:149–56.

[40] van Der Looij M, Wysocka B, Brozek I, Jassem J, Limon J, Olah E. Founder *BRCA1* mutations and two novel germline *BRCA2* mutations in breast and/or ovarian cancer families from northeastern Poland. Hum Mutat 2000;15:480–1.

[41] van Der Looij M, Szabo C, Besznyak I, Liszka G, Csokay B, Pulay T, Toth J, Devilee P, King MC, Olah E. Prevalence of founder *BRCA1* and *BRCA2* mutations among breast and ovarian cancer patients in Hungary. Int J Cancer 2000;86:737–40.

[42] Spitzer E, Abbaszadegan MR, Schmidt F, Hauser A, Buwitt U, Lauter FR, Potschick K, Krocker J, Elling D, Grosse R. Detection of *BRCA1* and *BRCA2* mutations in breast cancer families by a comprehensive two-stage screening procedure. Int J Cancer 2000;85: 474–81.

[43] Phelan CM, Kwan E, Jack E, Li S, Morgan C, Aube J, Hanna D, Narod SA. A low frequency of non-founder *BRCA1* mutations in Ashkenazi Jewish breast-ovarian cancer families. Hum Mutat 2002;20:352–7.

[44] Shapiro MB, Sehapathy P. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucleic Acids Res 1987;15:7155–74.

[45] Reese M. Splice site prediction by neural network. Available at: http://www.fruitfly.org/seq_tools/splice.html. Accessed December 2004.

[46] Khittoo G, Manning A, Mustun H, Appadoo J, Venkatasamy S, Fagoonee I, Ghadirian P, Tonin PN. Mutation analysis of a Mauritian hereditary breast cancer family reveals the *BRCA2* 6503deITT mutation previously found to recur in different ethnic populations. Hum Hered 2001;52:55–8.

[47] Haraldsson K, Loman N, Zhang QX, Johannsson O, Olsson H, Borg A. *BRCA2* germ-line mutations are frequent in male breast cancer patients without a family history of the disease. Cancer Res 1998;58:1367–71.

[48] Diez O, Osorio A, Duran M, Martinez-Ferrandis JI, de la Hoya M, Salazar R, Vega A, Campos B, Rodriguez-Lopez R, Velasco E,

Chaves J, Diaz-Rubio E, Jesus Cruz J, Torres M, Esteban E, Cervantes A, Alonso C, San Roman JM, Gonzalez-Sarmiento R, Miner C, Carracedo A, Eugenia Armengod M, Caldes T, Benitez J, Baiget M. Analysis of *BRCA1* and *BRCA2* genes in Spanish breast/ ovarian cancer patients: a high proportion of mutations unique to Spain and evidence of founder effects. Hum Mutat 2003;22:301–12.

[49] Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston DM. Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell 1997;88:265–75.

[50] Pellegrini L, Venkitaraman A. Emerging functions of *BRCA2* in DNA recombination. Trends Biochem Sci 2004;29:310–6.

[51] Shivji MK, Venkitaraman AR. DNA recombination, chromosomal stability and carcinogenesis: insights into the role of BRCA2. DNA Repair (Amst) 2004;3:835–43.

[52] Wong AK, Pero R, Ormonde PA, Tavtigian SV, Bartel PL. RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene *BRCA2*. J Biol Chem 1997;272: 31941–4.

[53] Chen PL, Chen CF, Chen Y, Xiao J, Sharp ZD, Lee WH. The BRC repeats in *BRCA2* are critical for RAD51 binding and resistance to methyl methanesulfonate treatment. Proc Natl Acad Sci USA 1998;95:5287–92.

[54] Figge MA, Blankenship L. Missense mutations in the BRCT domain of BRCA-1 from high-risk women frequently perturb strongly hydrophobic amino acids conserved among mammals. Cancer Epidemiol Biomarkers Prev 2004;13:1037–41.

[55] Nathanson KL, Wooster R, Weber BL, Nathanson KN. Breast cancer genetics: what we know and what we need. Nat Med 2001;7:552–6.