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Molecular analysis of the eighteen most frequent mutations in the BRCA1 gene in 63 Chilean breast cancer families

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ABSTRACT

BRCA1 gene mutations account for nearly all families with multiple cases of both early onset breast and/or ovarian cancer and about 30% of hereditary breast cancer. Although to date more than 1,237 distinct mutations, polymorphisms, and variants have been described, several mutations have been found to be recurrent in this gene. We have analyzed 63 Chilean breast/ovarian cancer families for eighteen frequent *BRCA1* mutations. The analysis of the five exons and two introns in which these mutations are located was made using mismatch PCR assay, ASO hybridization assay, restriction fragment analysis, allele specific PCR assay and direct sequentiation techniques. Two *BRCA1* mutations (185delAG and C61G) and one variant of unknown significance (E1250K) were found in four of these families. Also, a new mutation (4185delCAAG) and one previously described polymorphism (E1038G) were found in two other families. The 185delAG was found in a 3.17 % of the families and the others were present only in one of the families of this cohort. Therefore these mutations are not prominent in the Chilean population. The variant of unknown significance and the polymorphism detected could represent a founder effect of Spanish origin.

Key terms: *BRCA1* gene; frequent mutations; polymorphism; 4185delCAAG; new mutation; Chilean population.

INTRODUCTION

Breast cancer is the second most common malignancy among women in developing countries (Parkin, 1994). In Chile its actual incidence has not been accurately established given that the obligation to notify of occurrences of this disease is yet not mandatory. Recent studies in Chile have established that breast cancer constitutes the second highest mortality rate after stomach cancer, showing even higher mortality rates than cervical cancer, according to the Instituto Nacional de Estadísticas de Chile, (National Institute of Statistics, 1999). Female mortality rates due to breast cancer in Chile have increased steadily and as of 1995 had reached 11.7 per 100.000 (Peralta et al., 1995).

A family history of breast and/or ovarian cancer is one of the main risk factors for the development of these diseases (Lynch, 1981). It has been estimated that 5-10 % of all breast cancers are hereditary and attributable to mutations in several highly penetrant susceptibility genes from which only two have been identified: *BRCA1* (OMIM 113705) and *BRCA2* (OMIM 600185).

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The mutational spectrum in *BRCA1* is very broad, with hundreds of different mutations reported worldwide. Since its isolation, more than 1,237 distinct germ-line mutations, polymorphisms, and variants have been described in the Breast Cancer Information Core Database (BIC). Although there is a large number of distinct mutations, the proportion of families with mutations in *BRCA1* and *BRCA2* strongly depends upon the populations analyzed (Szabo and King, 1997) and the specific characteristics of the selected families (Serova et al., 1997; Schubert et al., 1997; Malone et al., 1998; Ford et al., 1998, Nathanson et al., 2001). *BRCA1* mutation studies have been reported in different ethnic groups (Panguluri et al., 1999; Ruiz-Flores et al., 2002; Saxena et al., 2002; Zhi et al., 2002; Manguoglu et al., 2003). There have been extensive molecular analyses of the *BRCA1* mutations performed in Caucasian populations in countries such as Canada, USA, UK, Sweden, Netherlands, Belgium, Norway, and Spain (Simard et al., 1994; Gayther et al., 1995; Shattuck-Eidens et al., 1995; Johannsson et al., 1996; Peelen et al., 1997; Claes et al., 1999; Borg et al., 1999; Neuhausen., 2000; Osorio et al., 2000; Llort et al., 2002; Vega et al., 2002; Durán et al., 2003; de Sanjose et al., 2003; Díez et al., 2003). *BRCA1* mutations have been reported in Japanese (Inoue et al., 1995; Matsushima et al., 1995; Katagiri et al., 1996; Miki et al., 1996), Taiwanese (Li et al., 1999), other Asian populations (BIC), and in African Americans (Panguluri et al., 1999).

It is of clinical and epidemiological importance to characterize the profile of the mutations in the *BRCA1* and *BRCA2* genes in populations originating from outside the extensively studied European, North American, and Australian gene pool. Knowledge of the *BRCA1* and *BRCA2* gene mutations in the Chilean population is relatively scant.

The contemporary Chilean population stems from the admixture of Amerindian peoples (Mongoloid) with the Spanish invaders (European Caucasian) 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) and are restricted to the specific locations of the country where they settled (Encina, 1983; Cruz-Coke, 1976; Valenzuela and Harb, 1977). The relationship between ethnicity, Amerindian admixture, genetic markers, and socioeconomic strata has been extensively studied in Chile (Valenzuela and Harb, 1977; Valenzuela et al., 1987; Valenzuela, 1988; Palomino et al., 1990; Palomino et al., 1991). These reports have demonstrated that the degree of Amerindian admixture can be determined by the frequencies of the alleles of ABO and Rh loci.

In the present study, we have analyzed 63 Chilean families at high-risk for hereditary breast cancer in order to screen eighteen *BRCA1* mutations. The mutations were selected because: a) they are present in the Amerindian and/or in the Spanish settlers which gave origin to the actual admixed Chilean population, and b) the high number of records of the selected mutations in the BIC database. As *BRCA1* is associated with an increase in risk for breast and other types of cancer, the identification of mutations in this gene has significant implications for the medical management of breast cancer patients and their family members.

METHODS

Subjects

In this study, 63 high-risk families were selected from the files of the Central Metropolitan Health Service of Santiago, Corporación Nacional del Cáncer (CONAC), and the Arturo López Pérez Foundation (Table I). The families included were required to comply with at least one of the following criteria: 1) families with at least two first degree relatives with breast and/or ovarian cancer diagnosed at any age, 2) families with at least two first or second degree relatives with breast cancer diagnosed before age 50, 3) families with at least three first or second degree relatives with breast cancer, at least one of whom was diagnosed before age 40, 4) families with at least one member having a cancer

TABLE I

Chilean breast cancer families at high risk for breast cancer predisposing mutations

Case category for selection	Mean age at diagnosis of individual (years)[b]	Number of families (% total families)
Multiple case families (≥ 4[a])	48.81	16 (25.4 %)
Multiple-case families (3[a])	51.12	25 (39.6 %)
Multiple-case families (2[a])	46.13	19 (30.2 %)
Early onset (≤ 30 years) breast cancer[c]	28.33	3 (4.8 %)
Total	43.60	63 (100 %)

a: Number of breast cancer cases per family, including first-degree, second-degree, and distant relatives.
b: Mean age at onset of all individuals in the family affected with breast and/or ovarian cancer (whether sampled or not).
c: Families with one case of breast cancer diagnosed before age 30 (criteria Nº7 Methods)

diagnosed before age 50 and at least one member with ovarian cancer diagnosed at any age, 5) families with at least one case of male breast cancer diagnosed at any age and at least one case of female breast cancer diagnosed at any age, 6) families with three or more different cancers (female or male breast cancer, ovarian, prostate, pancreas and larynx in non-smoking individuals), 7) families with at least one breast cancer diagnosed before age 30, and 8) families with at least one case of bilateral breast cancer.

In the selected families 9.5 % (6/63) had bilateral breast cancer; 3.2 % (2/63) had both breast and ovarian cancer and 1.6 % (1/63) presented male breast cancer. Of the 63 families, 70 women affected with breast cancer (probands) participated in this study. From these women, 31.43 % (22/70) had early age onset (≤ 40 years) of diagnosis with a mean age of 31.9 years. There was no upper or lower age cut-off for breast cancer cases. Breast cancer was verified by the original pathology report for all probands, but not for all affected relatives.

Genomic DNA (gDNA) was extracted from peripherical blood lymphocytes of 70 probands and 98 healthy relatives belonging to the cohort of the 63 families. Samples were obtained according to the method described by Chomczynsky and Sacchi (1987). The DNA samples were screened for the 18 most frequent germ-line *BRCA1* mutations (Table II) which were selected using two criteria: a) the mutations are present in the Amerindian and/or in the Spanish settlers which originated the actual admixed Chilean population, and b) the high number of records of these mutations in the BIC database. The methods used were mismatch polymerase chain reaction (mismatch PCR); allele-specific oligonucleotide (ASO) hybridization; restriction fragment analysis, allele specific PCR assay and direct sequencing. In each mutation, we used molecular weight markers and mutated samples as positive controls, which were kindly provided by Dr. Sabine Pages, Curie Institute, Paris, France, and Dr. Larry Brody, NIH, USA.

Mismatch PCR assay

The *BRCA1* 185delAG (Exon 2), 1675delA (Exon 11b), E1250X (Exon 11d), R1443X (Exon 13) and 5382insC (Exon 20) mutations, were identified by a mismatch PCR assay using primers shown in Table IIIA. This assay introduces a mismatch into the primers of each of the mutations analyzed. As a result, the PCR product of the normal allele acquires a restriction site that the mutant allele does not have. The acquired restriction site allows us to confirm the presence of the mutant alleles. For the

analysis of the 185delAG and 5382insC mutations we used the primers and conditions described by Abeliovich et al., (1997) and Backe et al., (1998) respectively. For the other mutations, amplifications were in 50 μl reactions containing 200ng of gDNA, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.2 mM of dNTPs, 40 pmoles of primers (Table IIIA) and 1.5 U Taq DNA polymerase (Promega). PCR conditions were 95ºC for 5 min followed by 30 cycles of 1 min at 94ºC, 1 min at 55ºC, 1 min at 72ºC, and 10 min at 72ºC. The PCR products were digested with restriction endonucleases following the conditions described by suppliers (New England Biolabs) and the fragments were resolved on agarose gels, and/or 5 % denaturing sequence gels and silver staining. Table IIIA also shows the restriction enzymes and the sizes of the PCR products after digestion with the restriction enzyme used in each case.

TABLE II

Most Frequent *BRCA1* Mutations[a]

Exon	NT	Codon	Base Change	AA Change	Designation	Mutation Type	Mutation Effect
2	185	23	delAG	Stop 39	185delAG	F	F
5	300	61	T to G	Cys to Gly	C61G	M	M
5	331+1	intron	G to A	–	331+1G>A	S	S
11a	1135	339	InsA	Stop 345	1135insA	F	F
11a	1294	392	del40	Stop 397	1294del40	F	F
11b	1675	519	delA	Stop 531	1675delA	F	F
11b	1806	563	C to T	Gln to Stop	Q563X	N	N
11b	2457	780	C to T	Gln to Stop	Q780X	N	N
11c	2800	894	delAA	Stop 901	2800delAA	F	F
11c	2804	895	delAA	Stop 901	2804delAA	F	F
11c	3166	1016	insTGAGA	Stop 1025	3166ins5	F	F
11d	3600	1161	delGAAGATACTAG	Stop 1163	3600del11	F	F
11d	3867	1250	G to T	Glu to Stop	E1250X	N	N
11d	3875	1252	delGTCT	Stop 1262	3875del4	F	F
11d	4184	1355	delTCAA	Stop 1364	4184del4	F	F
12	-	intron	del3835	–	IVS12-1632del3835	IVS	UV
13	4446	1443	C to T	Arg to Stop	R1443X	N	N
20	5382	1756	insC	Stop 1829	5382insC	F	F

a: Most frequent *BRCA1* mutations listed in the BIC database
F: Frameshift; **M**: Missense mutation; **N**: Nonsense mutation; **UV**: Unclassified Variant; **IVS**: Intron Variant Sequence; **S**: Splicing mutation.

TABLE III

BRCA1 Primers and Conditions of Mismatch PCR, ASO Hibridization Assay and Restriction Analysis

A. Mismatch PCR

Mutation	Forward	Reverse	Restriction Enzime	Size (bp)[d] Normal	Mutant
185delAG[a]	5' GAAGTTGTCATTTTATAAACCTTT	5'TGACTTACCAGATGGGAGAC	Hinf I	150-20	168
1675delA[c]	5' TTCATCCTGAGGATTTTATCTA	5' CATGAGTTGTAGGTTTCTGCTG	Xba I	454-19	473
E1250X[c]	5'AGGCATAGCACCGTTGCTTC	5' TCTTCCAATTCACTGCACTG	Hpy188 I	167-21	188
R1443X[c]	5'TTCTGCCCTTGAGGACCCG	5'ATGTTGGAGCTAGGTCCTTAC	BstUI	123-19	142
5382insC[b]	5'CCAAAGCGAGCAAGAGAATCTC	5'GGGAATCCAAATTACACAGC	DdeI	214-36-20	234-36-20

a.Primers and conditions described by Abeliovich et al. (1997)
b.Primers and conditions described by Backe et al. (1998)
c.Primers designed by authors using *BRCA1* genomic sequence available in Gen Bank (Accession number L78833)
d.Size of the PCR products after digested with the restriction enzyme.

B. Allele Specific Oligonucleotides Hibridization Assay[a]

Mutation	Normal Oligonucleotides	Mutant Oligonucleotides	Tm (mutant oligonucleotides)
331+1 G→A	5'ATAACCAAAAGGTATATAATTTGG	5'ATAACCAAAAGATATATAATTTGG	58ºC
1135insA	5' CAGCACAGAAAAAAAGG	5' CAGCACAGAAAAAAAAGG	50ºC
1294del40	5'TGATGAACTGTTAGGT	5'TGATGAACAAATGCCAA	46ºC
2800delAA	5'CCTTAAAGAAACAAAG	5'CCTTAAAGACAAAGTCCA	50ºC
2804delAA	5'AGAAACAAAGTCCAAA	5'TAAAGAAACAGTCCAAAAGT	52ºC
3166ins5	5' ATGAGAACATTCCAAGT	5' ATGAGATGAGAACATTCC	50ºC
3600del11	5' TAAAGGAAGATACTAGTTTTG	5'GTGAAATAAAGTTTTGCTGA	52ºC
3875del4	5'GAGTGTCTGTCTAAGAA	5'GAGTGTCTAAGAACACA	48ºC
4184del4	5'AGAAAATAATCAAGAAGAG	5'AGAAAATAAGAAGAGCAAAG	52ºC

a: From Friedman et al. (1994)

C. Restriction Fragments Analysis

Mutation	Forward	Reverse	Restriction Enzime	Size (bp)[d] Normal	Mutant
C61G[a]	5' CTCTTAAGGGCAGTTGTGAG	5'ATGGTTTTATAGGAACGCTATG	HpyCH4 III	154-74-50	228-50
Q563X[b]	5' ATGATAAATCAGGGAACTAACC	5' CATGAGTTGTAGGTTTCTGCTG	Hpy188 I	320-98	418
Q780X[a]	5'CACCTAAAAGAATAGGCTGAG	5' AGTAATGAGTCCAGTTTCGTTG	Hpy188 III	525-380-183	708-380

a: From Friedman et al. (1994)
b: Primers designed by authors using *BRCA1* genomic sequence available in Gen Bank (Accession number L78833)
c: Size of the fragments after restriction enzyme assay

Allele-specific oligonucleotide (ASO) hybridization assay

The *BRCA1* 331+1G→A (Exon 5), 1135insA (Exon 11a), 1294del40 (Exon 11a), 2800delAA (Exon 11c), 2804delAA (Exon 11c), 3166ins5 (Exon 11c), 3600del11 (Exon 11d), 3875del4 (Exon 11d) and 4184del4 (Exon 11d) mutations were identified by ASO hybridization assay using the conditions previously described by Friedman et al., (1995). Ten picomoles of oligonucleotides specific to each mutation and its wild-type sequence were 3'-end labeling with digoxigenin-11-ddUTP using a digoxigenin-oligonucleotide-labeling kit (Roche Applied Science) according to manufacturer's instructions. ASOs for known mutations are given in Table IIIB. Dot blot filters carrying amplified DNA were pre-hybridized with 20ml/100cm^2 of pre-hybridization buffer 5x SSC (1xSSC: 0.15 M NaCl and 0.015 M Na-citrate, pH 7.0), 1 % of casein, 0.1 % N-laurylsarcosine, and 0.02 % SDS at 5-10ºC below oligonucleotide-melting temperature (Th) for at least 1 hour and hybridized overnight with 20 ml of hybridization buffer (pre-hybridization buffer with non-isotopic oligonucleotide) at the same temperature (Th). The filters were washed twice with 20 ml of 0.3 M NaCl, 30mM Na Citrate, pH 7.0 for 5 min at room temperature and twice with 75 mM NaCl, 7.5 mM Na Citrate pH 7.0 for 15 min at room temperature. The filters were manipulated exclusively with tweezers and were not allowed to dry following the hybridization step.

Chemiluminescent detection

The filters were washed with 20 ml of buffer 1 (100mM Maleic acid, 150mM NaCl, 0.3 % Tween 20, pH 7.5) for one min at room temperature. It was then transferred to a new recipient and incubated with 20 ml of Buffer 2 (Buffer 1 with 1 % (w/v) casein) for 30 min at room temperature. The anti-digoxigenin antibody (Roche Applied Science) was diluted 1:10,000 in 20 ml of Buffer 2 five min before the end of the incubation with Buffer 2. Following incubation for 30 min, Buffer 2 was replaced by the diluted antibody solution, and the filter was left for 30 min at room temperature. The antibody solution was then removed and the membrane washed with 20 ml of Buffer 1. The filter was transferred to a new recipient and incubated with 20 ml of Buffer 1 containing 0.3 % Tween 20 for 15 min at room temperature. Buffer 1 was then replaced by 20 ml of Buffer 3 (100 mM Tris-HCl, pH 9.5, 100 mM NaCl) and incubated for 5 min at room temperature. The filter was placed between two cellulose acetate sheets, and 0.5 ml/100 cm2 of CSPD diluted 1:100 in Buffer 3 was added to the filter, between the acetate sheets. After careful removal of the air bubbles, the acetate sheets were heat sealed, and the filter was incubated for 15 min at 37ºC. Finally, the filter was exposed on Kodak X-OMAT ray film for 2 h.

Restriction Fragments Analysis

The *BRCA1* C61G (Exon 5), Q563X (Exon 11b) and Q780X (Exon 11b) mutations were identified by restriction fragment analysis. Genomic DNA of breast cancer probands was amplified by PCR assay, using primers given in Table IIIC and the conditions previously described in the section mismatch PCR assay. The PCR products were digested with a restriction enzyme (Table IIIC) using the conditions described by suppliers (New England Biolabs) and the products were resolved on agarose gels. Table IIIC shows the size of the normal and mutant alleles after restriction enzyme assay.

Allele specific PCR assay

The IVS12-1643del3835 mutation was identified by allele specific PCR assay. The reaction contained 1.5 mM $MgCl_2$, 400 nM primer 12F (5' TTCTTCAGCACCCCGT TC CA), 400 nM of the deletion specific primer 12Fdel3835 (5' CAATGTGTTCC TGCCCTACT), and 800 nM primer 12R (5' ACACTGGAAGACAACAGATATTAA). PCR conditions were 95ºC for 5 min, (94º for 1 min, 60ºC for 1 min, and 72ºC for 1 min) for 30 cycles, and 72º C for 10 min. The reactions were resolved on 1.5 %

agarose gels, producing a single band of 1243 bp among normal individuals and both 1343 bp and 500 bp in individuals who are heterozygous or homozygous for the IVS12-1643del3835 mutation.

Direct sequencing

All the mutants, unknown significance variants, and polymorphisms found were sequenced in the probands and in the healthy relatives to verify the sequence variants. The DNA isolated from mutant allele carriers was amplified by PCR and the products purified using and ENZA Cycle- pure kit (Labclinic). The purified DNA was subjected to cycle sequencing using an automated fluorescence-based cycle sequencer (ABI Prism 3100, Perkin-Elmer) and dye terminator system.

Human Subjects

All the individuals that agreed to participate in the study signed a written informed consent form. The study was approved by the University of Chile, School of Medicine Review Board.

RESULTS

We have studied 70 probands and 98 healthy relatives of 63 breast and/or ovarian families to screen 18 frequent mutations in five exons (2, 5, 11, 13 and 20) and two introns (5 and 12) in the breast/ovarian cancer susceptibility gene *BRCA1*. Two *BRCA1* frequent mutations and one variant of uncertain significance (BIC) were found in four of the families. Also, one new mutation and one polymorphism previously described (BIC) were found in two other families (Table IV).

In two families of the cohort (3.17 %) (families F4 and F46), the identified mutation was 185delAG in exon 2, which is the most common alteration reported in *BRCA1* (Shattuck-Eidens et al., 1995; Simard et al., 1994; Struewing et al., 1995). The reported families segregating this mutation are usually of Ashkenazi Jewish origin (Tonin et al., 1995). Families F4 and F46 did not self-report any Ashkenazi ancestors. Family F4 (Fig 1A) presented two cases of women affected with breast cancer at ages 62 (II-3) and 38 (III-3), one case of uterine cancer and one case of prostate cancer. The 185delAG mutation was detected in the proband and in her healthy sister, who is now 48 years old. Family F46 (Fig 1B) contained three cases of women with breast cancer at ages 80 (III-6), 70 (III-10), and 42 (IV-2), three cases of ovarian cancer and five more cases affected with other cancers (Fig. 1B, Table IV). The mutation was found in the proband and in her healthy 18 year-old-niece (V-1). The mother of V-I died of ovarian cancer when she was 34 years old, and she was probably a carrier of 185delAG mutation or other breast-ovarian cancer susceptibility mutation.

TABLE IV

Germline *BRCA1* mutations

Family	Female	Average	Ovarian	Average	Male	Cancer at	BRCA1	Exon	Effect
F4	2	50	-	-	0	Prost, Ut	185delAG	2	Stop 39
F46	3	64	3	43.3	0	Pan, Test, Co, Melan	185delAG	2	Stop 39
F13	4	40.25	-	-	0	Prost, St, Kid, Lu, Bo	C61G	5	Cys to Gly
F14	3	38	-	-	0	Ut, St	E1250K	11	Glu to Lys
F21	3	47	-	-	0	-	4185del4	11	Stop 1364
F25	3	45.6	-	-	0	Ut, Pan, St, Melan	E1038G	11	Polymorphism

F: Family; **Prost**: Prostate cancer; **Ut**: Uterine cancer; **Co**: Colon cancer; **Pan**: Pancreatic cancer; **Melan**: Melanoma; **Test**: Testicular cancer; **St**: Stomach cancer; **Kid**: Kidney cancer; **Lu**: Lung cancer; **Bo**: Bone cancer.
* Average age of female breast cancer cases per family; ** Average age of ovarian cancer cases per family.

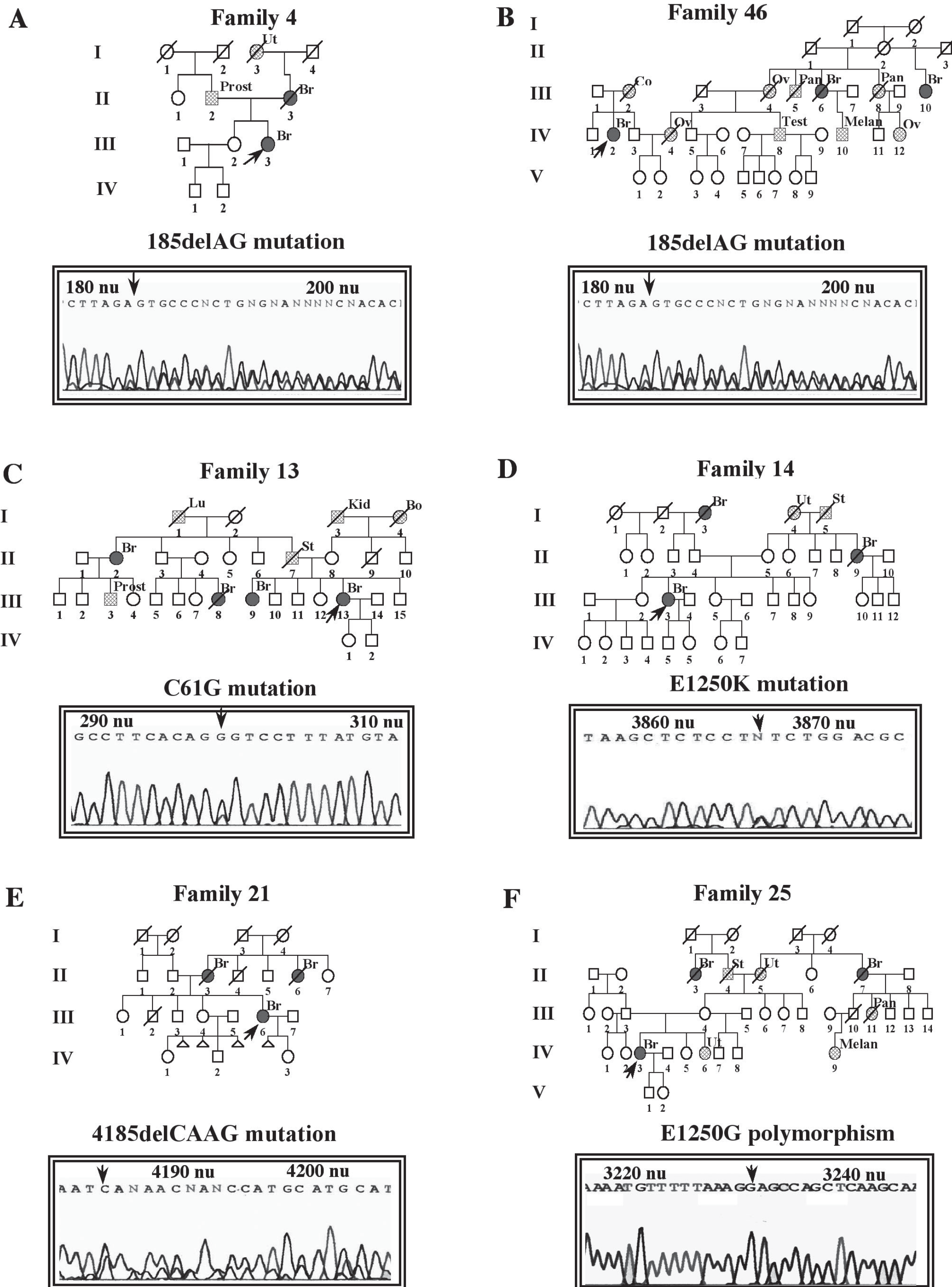

Figure 1: Pedigrees of families F4 (**A**), F46 (**B**), F13 (**C**), F14 (**D**), F21 (**E**) and F25 (**F**). *Lower panel*, electropherograms showing the mutations that were found in the families. **F:** Family; **Br**: Breast cancer; **Ov**: Ovarian cancer; **Prost**: Prostate cancer; **Ut**: Uterine cancer; **Co**: Colon cancer; **Pan**: Pancreatic cancer; **Melan**: Melanoma; **Test**: Testicular cancer; **St**: Stomach cancer; **Kid**: Kidney cancer; **Lu**: Lung cancer; **Bo**: Bone Cancer; **nu**: nucleotide
↗ :proband

Another frequent mutation was C61G in exon 5. This mutation was found in only one family studied, (F13) (1.59 %). Family F13 (fig. 1C) contained four cases of female breast cancer at ages 50 (II-2), 37 (III-8), 37 (III-9) and 37 (III-13), one case of prostate cancer, and four cases with other cancers (Table IV). In this family we obtained DNA samples from the following women III-7, III-12, and III-13. The mutation was detected in the proband and in her healthy sister (III-12) (Fig 1c), but it was not found in her healthy cousin (III-7).

Unexpectedly during the screening for E1250X mutation, we identified the E1250K variant, given the technique used allows the detection of any change of G base. The E1250K variant, previously reported although of unknown significance, corresponds to a change of G to A in nucleotide 3867 in exon 11. This variant was found in family F14 (Fig. 1D). This family contained three cases of female breast cancer at ages 36 (I-3), 30 (II-9), and 47 (III-4), one case of stomach cancer and one case of uterine cancer. This variant was studied in the proband (III-4) and in five healthy relatives (II-5, II-6, III-2, III-5 and III-10). This missense mutation was only found in the mother (68 years) (II-5) and in the sister (50 years) (III-2) of the proband, neither of whom were affected with breast and/or ovarian cancer. The proband (47 years) of this family does not present the aforementioned variant.

The new mutation detected is a four base pair deletion of CAAG in nucleotide 4185 in exon 11 and its leads to a premature termination at codon 1364. This mutation was identified during the screening for 4184del4 by direct sequencing. The 4185del4 mutation was found in family F21 (Fig. 1E) which presented three cases of breast cancer at ages of 47 (II-3), 45 (II-6), and 49 (III-6). We obtained DNA samples only from III-4 and III-6 individuals. The mutation was found in the index case (III-6) but not in her healthy sister (III-4). Sequencing of the amplified DNA revealed heterozygosity for the 4185del4 mutation in the proband and the presence of normal alleles in her healthy sister. To our knowledge, this mutation has not been previously described and is not included in the BIC database.

A polymorphic variant previously reported was detected in family F25 (Fig. 1F) during the screening of the 3166ins5 by direct sequencing. This polymorphism corresponds to a change of A→G at 3232 nucleotide in exon 11. This family presented three cases of female breast cancer at ages 46 (II-3), 55 (II-7), and 36 (IV-3), two cases of uterine cancer, and three cases with other cancers (Table IV). In this family we obtained a DNA sample only from the proband. The proband (IV-3) with breast cancer was homozygote for the sequence variant.

DISCUSSION

Germ-line mutations in the *BRCA1* and *BRCA2* genes account for the majority of high-risk breast/ovarian cancer families, depending on the population studied. The BIC database list has recorded the most frequent disease-associated mutations. In this study we analyzed eighteen frequent mutations located in five exons and two introns in the *BRCA1* gene, in 63 Chilean families with breast/ovarian cancer history. *BRCA1* mutations were found in five of our families (7.93 %) but only three of them (4.76 %) had two of the eighteen mutations studied.

The 185delAG was the first mutation detected with a high frequency in Ashkenazi Jews (Friedman et al., 1995; Struewing et al., 1995). Recent epidemiological studies have detected the 185delAG mutation in non-Jewish individuals in populations of other countries (Bar-Sade et al., 1998), including non-Jewish origin Spanish (Osorio et al., 1998; Díez et al., 1998; Díez et al., 1999). Trincado et al., (1999), did not detect the 185delAG mutation in 55 Chilean women affected with breast cancer, 15 of whom had a positive family history and 40 with sporadic breast cancer. The study of Jara et al., (2002) was the first to establish a frequency of 0.26 % for the 185delAG mutation in a group of 382 healthy Chilean women with at least two relatives with breast cancer. In the present study two families

were carriers of the 185delAG mutation, and individuals of these families reported no Jewish ancestors. Recently Ah Mew et al., (2002) identified the 185delAG mutation in a non-Jewish Chilean family. Therefore, the existence of this mutation in the current admixed Chilean population may have been brought by the Spanish settlers. Nevertheless this mutation has not yet been studied in the remnants of the few Amerindians that still remain geographically isolated in some regions of the country.

The second mutation detected was the C61G (exon 5) present in only one of the 63 families. This mutation has been described in Poland (Jakubowska et al., 2001, van Der Looij et al., 2000a), Hungary (van Der Looij et al., 2000b), Germany (Spitzer et al., 2000), and in other regions of Europe (BIC), although it is not a recurrent mutation in the Spanish population. The affected family with the C61G mutation had no recent European ancestry, the maternal lineage having only Chilean ancestry dating back several generations. The paternal lineage is Jewish Ashkenazi dating back two generations; however, the C61G mutation is not frequent in Jewish populations. There is a history of cancer 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 the Jewish ancestors of this family with Europeans where this mutation is frequent.

The third sequence variant found in our sample was in exon 11d and consisted of a change of G to A in nucleotide 3867, which causes a change of an acidic amino acid (glutamic acid) for a basic one (lysine). This missense mutation, designated E1250K, has been classified as a variant of unknown significance (BIC). The E1250K has been reported at least eight times, and five of the records correspond to Western Europe (BIC). It has also been described in Spanish populations (Díez et al., 2003) and is absent in different series of healthy controls. This variant was detected in only one of our 63 families. In this family breast cancer was diagnosed at an early age in the three cases. Therefore, it is more probable that this family presents hereditary breast cancer rather than sporadic breast cancer. The unknown significance variant was not present in the proband, but it was in the two healthy relatives; therefore this family did not allow us to establish the variant significance. A probable explanation could be that in this family another disease-associated mutation is present to account for hereditary breast cancer. The presence of this variant in the Chilean population could represent a founder effect of Spanish origin.

In family F21, a four base pair deletion of CAAG in nucleotide 4185 was detected. This frameshift mutation is predicted to result in a protein truncation at codon 1364, putatively deleting 27 % of the protein. Mutation 4185del4 differs from the previously described 4184delTCAA (BIC), which corresponds to a deletion of 4 bp in nucleotide 4184. Nevertheless these two mutations produce the same effect, given that both generate a stop codon 1364. Therefore the only difference between them is that in our case, the deletion occurs one base pair after the 4184del4 mutation. To our knowledge, this mutation has not been previously described. In family F21 all the breast cancer cases were diagnosed prior to age 50. Therefore the 4185del4 mutation has high penetrance and it may explain the breast cancer in this family. One of the healthy sisters of the proband (III-4) had normal alleles. We could not obtain DNA from the proband's other healthy sister, however, since she is now 74 years old, she probably has normal alleles.

Our last finding corresponds to a polymorphic variant in the nucleotide 3232 designated E1038G (BIC). This polymorphism has been described in Germany, Italy, USA, Sweden, Greece, and in the Spanish population (BIC). It has also been described in Chinese Malaysians and Brazil (BIC). The highest number of records in BIC database corresponds to cases in the Spanish population. It is therefore likely that the presence of this polymorphism in the Chilean population also represents a founder effect of Spanish origin.

Since the present Chilean population stems from the admixture of Amerindian (Mongoloids) and the Spanish (Caucasians),

some of the mutations associated with hereditary breast cancer in Chileans may be the same mutations that were present in the ancestors. Other, new mutations may be generated following the Amerindian and Spanish admixture, which have still not been described. The results of the present study show that four of the sequence variants detected in Chilean breast cancer families correspond to previously described variants and one corresponds to a new mutation with no BIC record. However, none of the mutations detected showed a high frequency in the group of families studied.

The identification of BRCA1 mutations could facilitate the setting up of a methodological strategy that could be less expensive and less time-consuming for the BRCA1 mutation detection in Chileans.

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