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RESEARCH ARTICLE

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

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We screened index cases from 410 Spanish breast/ovarian cancer families and 214 patients (19 of them males) with breast cancer for germ-line mutations in the *BRCA1* and *BRCA2* genes, using SSCP, PTT, CSGE, DGGE, and direct sequencing. We identified 60 mutations in *BRCA1* and 53 in *BRCA2*. Of the 53 distinct mutations observed, 11 are novel and 12 have been reported only in Spanish families (41.5%). The prevalence of mutations in this set of families was 26.3%, but the percentage was higher in the families with breast and ovarian cancer (52.1%). The lowest proportion of mutations was found in the site-specific female breast cancer families (15.4%). Of the families with male breast cancer cases, 59.1% presented mutations in the *BRCA2* gene. We found a higher frequency of ovarian cancer associated with mutations localized in the 5′ end of the *BRCA1* gene, but there was no association between the prevalence of this type of cancer and mutations situated in the ovarian cancer cluster region (OCCR) region of exon 11 of the *BRCA2* gene. The mutations 187_188delAG, 330A>G, 5236G>A, 5242C>A, and 589_590del (numbered after GenBank U14680) account for 46.6% of *BRCA1* detected mutations whereas 3036_3039del, 6857_6858del, 9254_9258del, and 9538_9539del (numbered after GenBank U43746) account for 56.6% of the *BRCA2* mutations. The *BRCA1* 330A>G has a Galician origin (northwest Spain), and *BRCA2* 6857_6858del and 9254_9258del probably originated in Catalonia (northeast Spain). Knowledge of the spectrum of mutations and their geographical distribution in Spain will allow a more effective detection strategy in countries with large Spanish populations. Hum Mutat 22:301–312, 2003. © **2003 Wiley-Liss, Inc.**

KEY WORDS: *BRCA1*; *BRCA2*; breast cancer, hereditary; cancer; Spanish

DATABASES:
BRCA1 – OMIM: 113705; GenBank: U14680
BRCA2 – OMIM: 600185; GenBank: U43746
www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/ (Breast Cancer Information Core)

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INTRODUCTION

Breast cancer is a major disease affecting women in industrialized countries. It is currently estimated that 5–10% of all breast cancers are hereditary and attributable to mutations in several highly penetrant susceptibility genes of which only two have been identified: *BRCA1* (MIM# 113705) and *BRCA2* (MIM# 600185).

Earlier estimates suggest that *BRCA1* mutations are responsible for 45% of site-specific breast cancer families and the majority of breast and ovarian cancer families [Easton et al., 1993]. These studies also suggest that *BRCA2* mutations might be responsible for 25–35% of site-specific breast cancer and could be implicated in the majority of male breast cancer families [Stratton et al., 1994]. However, recent data show that these percentages could have been overestimated and that the proportion of affected families due to mutations in *BRCA1* or *BRCA2* is much lower and depends on the population analyzed [Szabo and King, 1997] and on the specific characteristics of the families selected [Schubert et al., 1997; Serova et al., 1997; Ford et al., 1998; Malone et al., 1998; Shih et al., 2002].

We report the results of the genetic analyses of 410 familial and 214 sporadic breast cancer cases. Our study, which is the largest conducted to date in a Spanish population, provides comprehensive data on the type and distribution of *BRCA1* and *BRCA2* mutations and allelic variants. This information facilitates *BRCA1* and *BRCA2* mutational screening in Spanish populations considerably.

SUBJECTS AND METHODS

Families and Patients

Seven centers from Spain have contributed to this study: Hospital de la Santa Creu i Sant Pau in Barcelona (SP), Centro Nacional de Investigaciones Oncológicas in Madrid (C), Instituto de Biología y Genética Molecular at the University of Valladolid in Valladolid (Vd), Instituto de Investigaciones Citológicas and Hospital Clínico Universitario in Valencia (V), Hospital Clínico San Carlos in Madrid (SC), Centro de Investigación del Cáncer-Universidad de Salamanca in Salamanca (Sal), and Hospital de Conxo-Universidad de Santiago de Compostela in Santiago de Compostela (G). The families had been investigated for a number of reasons, including research, directed screening of case series of female or male breast cancer, or attendance at cancer genetic clinics. Some of these families had been included in earlier works [Díez et al., 1999a; Osorio et al., 2000; Campos et al., 2001; de la Hoya et al., 2001, 2002; Vega et al., 2002].

The sample studied consisted of 410 families, who were divided into three groups: Group A comprised 96 families with one or more breast cancer cases (at least one case diagnosed before age 50) and one or more ovarian cancer cases; Group B consisted of 292 site-specific female breast cancer families with two or more cases (at least one diagnosed at <50 years of age); and Group C was made up of 22 families with at least one case of breast cancer or ovarian cancer in addition to at least one male breast cancer case. The number of breast cancer cases included affected family members among first- and second-degree relatives of the screened case. The study also included patients without family history, classified into two categories: Group D was made up of 188 women with breast cancer (108 of them diagnosed before 50 years of age) and seven with breast and ovarian cancer; and Group E consisted of 19 males with breast cancer. The characteristics of the families and patients are given 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 (the male case or the youngest female case).

TABLE 1. Classification of Families, Patients, and Frequencies of Mutations in *BRCA1* and *BRCA2* Genes

	Families/ patients N	*BRCA1* mut		*BRCA2* mut		*BRCA1/2* mut	
		n	(%)	n	(%)	(%)	(95% CI)
Group A							
≥3 BC+OC	79	27	(34.2)	13	(16.4)	(50.6)	(39.1–62.1)
Total (≥2BC+OC)	96	34	(35.4)	16	(16.7)	(52.1)	(41.6–62.4)
Group B							
≥3 BC	206	16	(7.8)	19	(9.2)	(17.0)	(11.9–22.1)
Total (≥2BC)	292	23	(7.9)	22	(7.5)	(15.4)	(11.3–19.6)
Group C							
≥1 male BC +≥1 BC+≥1 OC	5	–		3	(60.0)	(60.0)	(14.7–94.7)
≥1 male BC +≥1 BC	17	–		10	(58.8)	(58.8)	(32.9–81.6)
Total	22	–		13	(59.1)	(59.1)	(36.4–79.3)
Total families	410	57	(13.9)	51	(12.4)	(26.3)	(22.1–30.6)
Group D							
1 BC	188	3	(1.6)	1	(0.5)	(2.1)	(0.6–5.3)
1 (BC+OC)	7	–		1	(14.2)	(14.3)	(0.4–57.9)
Total	195	3	(1.5)	2	(2.6)	(2.6)	(0.8–5.9)
Group E							
1 male BC	19	–		–		–	
Total		60		53			

BC, breast cancer; OC, ovarian cancer; mut, mutation; 95% CI, 95% confidence interval.

None of the families met the strict criteria for other known syndromes involving breast cancer, such as Li-Fraumeni, ataxia-telangiectasia, or Cowden disease. This study was approved by the corresponding institutional ethical committees and informed consent was obtained from all the participants.

BRCA1 and BRCA2 Mutation Analysis

The whole coding sequences and exon–intron boundaries of the *BRCA1* and *BRCA2* genes were polymerase chain reaction (PCR)-amplified using previously described primers [Friedman et al., 1994; Miki et al., 1996; Van Orsouw et al., 1999]. The fragments obtained were analyzed for sequence variants using a combination of different methods: single strand conformational polymorphism analysis (SSCP) and protein truncation test (PTT) at the SP, C, and V centers; denaturing gradient gel electrophoresis (DGGE) at the SC center; and conformational sensitive gel electrophoresis (CSGE) at the C, Sal, SC, and Vd centers. The G center analyzed BRCA genes by completely sequencing each exon. In some centers, different techniques were applied in the course of the study. Overall, 172 families were analyzed by CSGE, 149 by SSCP/PTT, 61 by DGGE, and 28 by direct sequencing. A total of 114 patients with no family history were screened by SSCP/PTT, 77 by CSGE, and 23 by DGGE.

SSCP. Amplified samples were diluted in formamide buffer, held at 95°C for 5 minutes, and then on ice for 5 minutes. Samples were loaded onto a nondenaturing polyacrylamide SSCP gel and run at different conditions depending on the fragment analyzed. Gels were silver-stained and dried on a vacuum gel dryer.

PTT. Primer pairs published by Hogervorst et al. [1995] were used to amplify three overlapping fragments of exon 11 in *BRCA1*, and exon 10 and four overlapping fragments of exon 11 in *BRCA2*. Transcription and translation was performed on the PCR product in a reaction using the TNT/T7 coupled reticulocyte lysate system (Promega, Madison, WI). ^{35}S-methionine (Amersham, Aylesburg, UK) was included for labeling purposes. Labeled protein products were size-separated using 12% SDS PAGE. Thereafter, the gels were fixed, dried, and exposed for 18–48 hr to Biomax film (Kodak, Rochester, NY). The sizes of the truncated products were used to estimate the positions of the mutations within each segment.

DGGE. Analysis was performed in a DGGE System-2000 (C.B.S. Scientific Co., Del Mar, CA). Aliquots of PCR products were loaded onto a 10% Acrylamide/Bis-Acrylamide (37.5:1) gel (0–60% or 20–80% urea-formamide chemical gradient according to melting profiles of each PCR fragment) in 1× TAE (40 mM Tris-base, 20 mM NaAC, 1 mM EDTA, pH 8) for 16 hr at 80 V and 58°C. The gel was stained in a solution of ethidium bromide, and the DNA was photographed under ultraviolet light.

CSGE. Amplified samples were held at 95°C for 5 minutes and at 65°C for 1 hr to generate heteroduplexes. The products were diluted 1:2 in sucrose buffer and loaded in a partially denaturing MDE gel at constant power of 7 W during different times depending on the fragment size. Gels were silver-stained and dried on a vacuum gel dryer.

Any fragment showing a mobility shift using the screening techniques was directly sequenced in order to identify the variant. Sequencing was performed in an ABI Prism 310 automated fluorescence-based cycle sequencer and a Rhodamine dye terminator system (Perkin Elmer, Foster City, CA).

In addition to deleterious mutations, we detected several polymorphisms and variants of unknown significance. All the missense mutations or intron variants that have not been demonstrated to have a deleterious effect were considered variants of unknown significance. They were novel or had been reported in the BIC database (www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/) as unclassified variants and were not found in the Spanish control populations. We included as polymorphisms silent variants that are not predicted to significantly affect exon splicing, and amino acid changes with a >1% frequency in the Spanish populations or, despite having lower frequencies, appearing in the BIC database as polymorphisms. In order to assess the frequency of variants in control populations, 100 unrelated Spanish individuals obtained from blood donors were analyzed.

Statistical methods. Comparison of sex ratios and proportions were performed using a contingency table and statistical signification was evaluated using a Chi square test or Fisher exact test when necessary. EPi info ver. 6.0 (Center for Disease Control, Atlanta, GA) was used to perform the statistical analysis.

Mutation nomenclature. Nucleotides are numbered from the start of the GenBank reference sequences for *BRCA1* (U14680) and *BRCA2* (U43746).

RESULTS

We detected a total of 113 pathogenic mutations (60 for *BRCA1* and 53 for *BRCA2*) (Tables 2 and 3, respectively). Furthermore, we identified 63 unclassified variants (28 in *BRCA1* and 35 in *BRCA2*) (Tables 4 and 5, respectively) and 43 polymorphisms (20 for *BRCA1* and 23 for *BRCA2*) (Tables 6 and 7, respectively). All were named according to the practice of the BIC database, and according to the GenBank entries U14680 (*BRCA1*) and U43746 (*BRCA2*).

For screening purposes and patient counseling, it is imperative to determine whether inheritance of these variants causes an increased risk of developing cancer. Truncating mutations may be assumed to cause disease, despite rare exceptions, but the pathogenicity involving unclassified variants is more equivocal because of insufficient information concerning protein function. For this reason, although some of these variants could have a pathogenic effect, we calculated the relative frequencies considering only the clearly deleterious mutations.

The majority (101/113, 89%) of the distinct mutations found lead to premature termination of the protein translation. This number included 26 frameshift mutations, six nonsense mutations, and 17 splice-site mutations in the *BRCA1* gene (Table 2), and 47 frameshift, five nonsense, and one splice-site mutation for *BRCA2* (Table 3). The frameshift mutations included both small deletions and insertions, which ranged from 4 to 23 base-pairs (bp). The splice variants were generated by base substitutions, which either create or destroy splice acceptor and donor sites of the genes.

We also found an inframe deletion of three nucleotides (5197_5199del) and missense mutations 5236G>C, 5236G>A, 5242C>A, and 5263G>A in the *BRCA1* gene. We considered these mutations pathogenic for a number of reasons: their localization in critical domains of the protein; their absence in the control population; the loss of heterozygosity observed in tumor tissue [Osorio et al., 2002]; and in some of them (5242C>A and 5263G>A) their inactivation of the transcription activity of *BRCA1* seen in experimental models [Vallon-Christersson et al., 2001].

Of the distinct mutations, 13 (41.9%) *BRCA1* and nine (40.9%) *BRCA2* mutations had not been reported

TABLE 2. *BRCA1* Germ-Line Mutations in Spanish Breast/Ovarian Cancer Families/Patients

Family/ patient	Exon/ intron	Mutation	Predicted effect	BC at age <50 years (bilateral)	BC at age ≥50 years (bilateral)	Male BC	OC (OC+BC)	Other cancer(s)
C8	2	187_188delAG	p.Q23fs	1	–	–	2(1)	Liver
C38	2	187_188delAG	p.Q23fs	–	2	–	1(1)	–
C97	2	187_188delAG	p.Q23fs	2	–	–	1	–
C102	2	187_188delAG	p.Q23fs	–	–	–	5	Melanoma, leukemia
C121	2	187_188delAG	p.Q23fs	2(1)	1	–	2	Esophagus, lung
C170	2	187_188delAG	p.Q23fs	3	–	–	–	–
SP47[Gy]	2	187_188delAG	p.Q23fs	2(1)	–	–	1(1)	–
SPj2[Gy]	2	187_188delAG	p.Q23fs	?	?	?	1(1)	?
SP67	2	187_188delAG	p.Q23fs	2	–	–	–	–
SP217	2	187_188delAG	p.Q23fs	2	2	–	1(1)	Liver
SP14	2	189_192dupTGTC[a]	p.P25fs	6	1	–	2 (1)	Prostate
C73	3	236_237delTG	p.C39X	2	–	–	4(2)	–
SP5	3	243delA[a]	p.I42fs	1	–	–	1(1)	
SP104	3	243delA[a]	p.I42fs	1(1)	–	–	3	Liver, liver
G4834[Gal]	5	330A>G	r.310_331del[ed]	1(1)	–	–	1	–
G5285[Gal]	5	330A>G	r.310_331del[ed]	1	–	–	2	–
G7071[Gal]	5	330A>G	r.310_331del[ed]	3(1)	–	–	–	–
G7410[Gal]	5	330A>G	r.310_331del[ed]	2	1	–	3	Lung
SP81[Gal]	5	330A>G	r.310_331del[ed]	1(1)	1	–	–	Colon, colon
Vd9	5	330A>G	r.310_331del[ed]	2	1	–	–	–
Vd106	5	330A>G	r.310_331del[ed]	2	–	–	–	
Vd44	IVS5	331+1G>A	r.?	–	1	–	1	Bladder
SC3	8	589_590delCT	p.S157X	1(1)	2	–	2(1)	–
SC42	8	589_590delCT	p.S157X	2	–	–	2(1)	Prostate
Sal1	8	589_590delCT	p.S157X	1	3	–	–	Esophagus
G3	11	910_913delGTTC[b]	p.S264fs	1	–	–	1	–
Vd3	11	1135_1136insA	p.L339fs	1	–	–	–	Uterus
SP44	11	1240_1242delinsT	p.T374fs	2	1	–	–	–
SP1e	11	1452G>T[a]	p.E445X	1	2	–	–	–
SP95	11	2031delG[b]	p.Q638fs	–	1	–	3	–
Sal2	11	2031delG[b]	p.Q638fs	1	2	–	1	–
V15	11	2080delA	p.L654fs	1	–	–	1	
SP79	11	3600_3610del11	p.A1161fs	3(2)	1	–	1	Bowel
G4	11	3958_3962delins4[a]	p.S1280fs	–	–	–	2	Uterus, bowel
Vd116	11	3958_3962delins4 [a]	p.S1280fs	–	2	–	2(1)	Colon, rectum
SC5	12	4230_4231insATCT[a]	p.G1371fs	3	–	–	1	–
SC144	12	4284_4285dupAG[a]	p.S1389fs	1	–	–	–	–
SPE18	13	4314_4315delAC	p.T1399fs	1	1(1)	–	–	Brain, liver
SP149	18	5197_5199del3	r.5194_5271del[ed]	2	–	–	2	Pancreas
V23	18	5236G>C	p.G1706A	1	1	–	1	–
SP111	18	5236G>C	p.G1706A	1	1	–	2	–
C14	18	5236G>A	p.G1706E	2	–	–	–	–
C72	18	5236G>A	p.G1706E	3(2)	1	–	2(2)	–
Vd23	18	5236G>A	p.G1706E	1	1	–	2	Colon, pancreas
C81	18	5242C>A	p.A1708E	4	–	–	–	Astrocitoma, matrix
SC61	18	5242C>A	p.A1708E	4	–	–	2	Prostate, liver, colon
SC7	18	5242C>A	p.A1708E	5(2)	–	–	–	–
SC121	18	5242C>A	p.A1708E	4	–	–	–	Prostate
Vd54	18	5242C>A	p.A1708E	6	3	–	–	Uterus
SP19	18	5263G>A	p.S1715N	2	1	–	–	Bladder
SP92	IVS18	5271+5G>A[b]	r.5194_5271del[ed]	3	–	–	–	–
SC4	IVS18	5272–1G>C	r.?	–	1	–	3	Prostate
C36	IVS18	5272–1G>A	r.?	6	2	–	–	–
Vd117	IVS18	5272–1G>A	r.?	–	3	–	–	Skin, liver
C134	IVS20	5396+1G>A	r.?	1(1)	2	–	–	Stomach, leukemia
Vd85	IVS20	5396+1G>A	r.?	1	1	–	–	–
SC2	IVS20	5397–1G>C[b]	r.?	4(1)	–	–	1	–
V37	21	5451G>T[b]	r.?	2	–	–	–	Colon, lung
SP12	23	5538delA[b]	p.I1806fs	4	–	–	1(1)	–
SP107	24	5625G>T[a]	p.E1836X	1(1)	–	–	–	–

Nucleotides are numbered from the start of the *BRCA1* GenBank reference sequence U14680.
[a]Unique to Spain.
[b]Novel.
BC, breast cancer; OC, ovarian cancer;[Gy], Gypsy;[Gal], Galician; r?, effect on RNA-level unknown but expected; [ed], experimentally determined by RNA analysis.

in the BIC database by April 2003 and are considered novel, or were in the BIC database, but had been described only in Spanish families. With the exception of a few common mutations, sequence alterations were found throughout the whole coding region of *BRCA1* and *BRCA2*. Only 10 *BRCA1* (17%) and 27 *BRCA2*

TABLE 3. *BRCA2* Germ-Line Mutations in Spanish Breast/Ovarian Cancer Families/Patients

Family/ patient	Exon/ intron	Mutation	Predicted effect	BC at age <50 years (bilateral)	BC at age ≥50 years (bilateral)	Male BC	OC (OC+BC)	Other cancer(s)
V1	IVS2	295+2T>C	r.?	1	–	–	1	–
Vd13	10	1538_1541del4	p.K437fs	2	1	–	–	Stomach
Vd14	10	1538_1541del4	p.K437fs	4	3	–	–	Bladder, stomach
SP94	10	1825delA[b]	p.T533fs	3(1)	–	–	–	–
SP113	10	1825delA[b]	p.T533fs	2(1+ut)	–	–	–	Prostate, uterus, stomach
C21	11	3036_3039del4	p.K936fs	4(2)	1(+pan)	–	2	Bowel, lung, liver
C59	11	3036_3039del4	p.K936fs	4	–	–	2	–
C87	11	3036_3039del4	p.K936fs	1	–	–	1(1)	Pancreas
C93	11	3036_3039del4	p.K936fs	5	–	–	–	Colon, sarcoma
C128	11	3036_3039del4	p.K936fs	2	–	–	–	Lymphoma
SC110	11	3036_3039del4	p.K936fs	3	–	1	–	–
SP121	11	3036_3039del4	p.K936fs	2	–	–	1	–
SP122	11	3036_3039del4	p.K936fs	3	1	–	–	Lung
Vd48	11	3036_3039del4	p.K936fs	3	2	–	–	–
Vd79	11	3036_3039del4	p.K936fs	2(1)	–	–	–	Stomach
Vd89	11	3036_3039del4	p.K936fs	4	–	–	–	–
Vd123	11	3036_3039del4	p.K936fs	2	1	–	–	–
Sal8	11	3036_3039del4	p.K936fs	1	4	–	–	–
Sal9	11	3036_3039del4	p.K936fs	1	4	–	–	–
SPB71	11	3374del A[a]	p.N1049fs	1(1)	1	1	–	Brain
SC126	11	3492dupT	p.P1088fs	1	4(1)	1	1	Stomach, liver
Vd12	11	3492dupT	p.P1088fs	3	1	–	1	Uterus, colon
SP120	11	4088delA	p.N1287fs	–	–	–	1(1)	Leukemia, uterus
Vd141	11	4150G>T	p.E1308X	4	1	–	–	–
SC16	11	5804_5807del4	p.I1859fs	3(1)	–	1(1)	–	Prostate, 5 others
SP127	11	6079_6082del4	p.S1951fs	3	–	–	–	–
V70	11	6208C>T	p.Q1994X	1	–	–	–	Lymphoma
SC12	11	6503_6504delTT	p.L2092fs	1	1	–	1	Colon
SC121	11	6503_6504delTT	p.L2092fs	4	–	–	–	Prostate
SP23[Cat]	11	6857_6858delAA[a]	p.E2210fs	5	1	–	–	Rectum, stomach
SP148[Cat]	11	6857_6858delAA[a]	p.E2210fs	1	–	–	1	–
SP54c[Cat]	11	6857_6858delAA[a]	p.E2210fs	1(+lung)	–	1	–	–
C24	14	7337_7338delAA[a]	p.K2370fs	–	–	1	3	–
SC8	18	8240_8262del23[a]	p.A2671fs	1	5(1)	–	–	–
SC139	18	8293dupTT[b]	p.C2689fs	1	–	1	–	–
SP76	18	8298_8299dupTT[b]	p.S2691fs	3	–	–	1(1)	Prostate (2), neck
V78	23	9206_9219del14[b]	p.S2993fs	3	1	–	–	–
V120	23	9246C>A[b]	p.Y3006X	1	–	–	1	–
V3	23	9246C>A[b]	p.Y3006X	–	2	–	1	–
C22	23	9254_9258del5	p.Y3009fs	3(3)	1	3	–	Stomach, bone, uterus
V127[Cat]	23	9254_9258del5	p.Y3009fs	1	2	–	–	Lung
V145[Cat]	23	9254_9258del5	p.Y3009fs	1	–	1	–	Stomach, colon
SP4[Cat]	23	9254_9258del5	p.Y3009fs	1	1	–	1	Prostate
SP37[Cat]	23	9254_9258del5	p.Y3009fs	2	–	1	–	–
SP119[Cat]	23	9254_9258del5	p.Y3009fs	4	–	–	1	–
SP123[Cat]	23	9254_9258del5	p.Y3009fs	2	–	2	–	Lung, colon, bladder
SP162[Cat]	23	9254_9258del5	p.Y3009fs	1	1	–	1(1)	Liver, stomach
SP178[Cat]	23	9254_9258del5	p.Y3009fs	5	–	1	–	Pancreas (2)
SP33	25	9514G>T	p.E3096X	4(2)	–	–	2(1)	–
C45	25	9538_9539delAA	p.K3104fs	8	3	1	1(1)	–
SP105	25	9538_9539delAA	p.K3104fs	2	1	–	–	–
Vd59	25	9538_9539delAA	p.K3104fs	2	–	–	1(1)	Larynx
Vd138	25	9538_9539delAA	p.K3104fs	2	1	–	–	Pancreas, bladder

Nucleotides are numbered from the start of the *BRCA2* GenBank reference sequence U43746.
[a]Unique to Spain.
[b]Novel.
BC, breast cancer; OC, ovarian cancer; [Cat], Catalan; r?, effect on RNA-level unknown but expected.

mutations (51%) were found in exon 11 of each gene, respectively, including the most common mutation in *BRCA2* (3036_3039del), which was found 14 times.

The three most common *BRCA1* mutations were 187_188del (commonly referred to as 185delAG), 330A>G, and 5242C>A, which were identified in 10, seven, and five families, respectively. This group of mutations accounted for 37% of mutations identified in *BRCA1*. When adding the 589_590del and 5236G>A mutations, each one found in three families, the percentage increased to 47%. Three recurrent mutations were found in *BRCA2*: 3036_3039del, 9254_9258del, and 9538_9539del, found in 14, nine, and four unrelated families, respectively. Including the 6857_6858del, found in three families, these four mutations accounted for 57% of all mutations identified in the 53 *BRCA2* families.

TABLE 4. *BRCA1* Unclassified Variants in Spanish Breast/Ovarian Cancer Families/Patients

Family/ patient	Exon/ intron	Variant	Predicted effect	BC at age <50 years (bilateral)	BC at age ≥50 years (bilateral)	Male BC	OC (OC+BC)	Other cancer(s)
SC142	IVS2	200–14C>T	Non coding	2	2	–	–	–
V75	5	318G>T	p.D67Y	1	1	–	–	Lung
SC19	IVS5	332–16A>G[a]	Non coding	1	–	–	1(1)	Stomach, basal.cell
SPj1	6	411G>C[a]	p.G98R	1	1	–	–	–
V119	7	433A>G	p.Y105C	1	–	–	–	Liver
Vd129	8	655A>G	p.Y179C	2	1	–	–	Skin, liver
SP46	11	855T>G	p.L246V	2	1	–	–	–
Vd129	11	1575T>C	p.F486L	2	1	–	–	Skin, liver
G2	11	2121C>T	p.L668F	–	3	–	–	–
V23	11	2640C>T	p.R841W	1	1	–	1	–
SC23	11	3827T>G	p.N1236K	4(1)	–	–	–	Stomach, thyroid
Sal5	11	3867G>A	p.E1250K	1	–	–	–	–
Sal4	11	3970A>G[b]	p.H1284R	–	2	–	–	–
Sal3	11	4158A>G	p.R1347G	1	1	–	–	Prostate
SC116	IVS13	4477–10C>T	Non coding	1	–	–	–	–
SC120	IVS13	4477–10C>T	Non coding	4	1	–	–	Uterus, brain
SC136	IVS13	4477–10C>T	Non coding	1	–	–	–	Prostate, stomach
V131	15	4654G>T	p.S1512I	1	–	–	–	Uterus
Vd84	15	4654G>T	p.S1512I	1	2	–	–	Liver
Vd120	15	4654G>T	p.S1512I	1	–	–	–	Leukemia
SC90	15	4654G>T	p.S1512I	4(2)	1	–	–	Lung
SC137	15	4654G>T	p.S1512I	–	–	1	–	Prostate (2)
Sal6	16	5075G>A	p.M1652I	–	2	–	–	–
Sal7	16	5075G>A	p.M1652I	–	4	–	–	Pancreas, esophagus
SC8	19	5277A>G	p.T1720A	1	5(1)	–	–	–
G1	23	5530T>A	p.V1804D	1	1	–	–	Esophagus
V128	23	5553C>G[b]	p.P1812A	1	3	–	2(1)	Prostate
SPj2	24	5657G>A[a]	p.Q1846Q	1	–	–	1	–

Nucleotides are numbered from the start of the *BRCA1* GenBank reference sequence U14680.
[a]Unique to Spain.
[b]Novel.
BC, breast cancer; OC, ovarian cancer.

The prevalence of mutations that were presumably disease-associated in Spanish breast/ovarian cancer families was 26.3% (108/410; 95% CI, 22.1–30.6). Nevertheless, the percentage depended on the number of breast cancer cases in the family, the presence of ovarian cancer, and on the presence of male breast cancer. The proportion of families and patients with detected mutations in the different phenotypic groups are summarized in Table 1. As has been reported in other populations, the frequency of mutations was higher in Group A, the breast and ovarian cancer group (52.1%; 95% CI, 41.6–62.4). The proportion increased with the number of cancer cases in the family, and the highest frequency (68.2%; 95% CI, 45.1–86.1) was found in families with five or more cases of breast and ovarian cancer (n = 22, 10 with *BRCA1* and five with *BRCA2* mutations). A high percentage of mutations (57.1%; 95% CI, 28.9–82.3) was also obtained in families with only one breast cancer and one ovarian cancer. The lowest proportions of mutations were found in Group B, the group of site-specific female breast cancer families, even in families with three or more cancer cases (17.0%; 95% CI, 11.9–22.1). The group of families in which *BRCA2* mutations were found more frequently was that with at least one male breast cancer patient (59.1%; 95% CI, 36.4–79.3). Low mutation frequencies were found in Group D: 2.6% (95% CI, 0.8–5.9) for single women with breast cancer and no family history and 14.3% (95% CI, 0.4–57.9) for single women with breast and ovarian cancer and no family history. No mutations were found in single breast cancer–affected males.

We investigated possible correlations between mutation position and phenotypic characteristics. Earlier studies have described a higher proportion of breast cancer incidence related to ovarian cancer in a region between nucleotides 1 and 4447 of *BRCA1* [Gayther et al., 1995]. However, a recent study by the Breast Cancer Linkage Consortium has refined these allelic variations in *BRCA1*, defining a central region of the gene between nucleotides 2401 and 4190 in which the ovarian to breast cancer ratio is higher than that of mutations in other regions of the gene [Thompson et al., 2002]. By dividing the gene into two parts, we found that the breast to ovarian cancer ratio was 1.68 considering mutations between nucleotides 1 and 4427, and 5.28 considering nucleotide 4428 to the end; the difference was statistically significant ($p < 0.01$) and confirmed the view that mutations in the 5′end of the gene are associated with a higher ovarian cancer risk. When dividing the gene into three regions, the differences in the ratios between nucleotides 1–2400 and 2401–4190 (1.63 vs. 2.00) were not statistically significant, probably because of the small number of mutations found in the central part of the *BRCA1* gene.

With respect to the *BRCA2* gene, some authors [Gayther et al., 1997; Thompson et al., 2001] have

TABLE 5. *BRCA2* Unclassified Variants in Spanish Breast/Ovarian Cancer Families/Patients

Family/ patient	Exon/ intron	Variant	Predicted effect	BC at age <50 years (bilateral)	BC at age ≥50 years (bilateral)	Male BC	OC (OC+BC)	Other cancer(s)
SC10	IVS1	218C>T	Non coding	1	2	–	–	Brain
SP29	IVS1	154C>G[a]	Non coding	2	3	–	–	–
V54	3	451G>C	A75P	1	1	–	–	Liver
SC10	IVS4	654–84T>C	Non coding	1	2	–	–	Brain
SC39	IVS4	654–84T>C	Non coding	–	4	–	–	Lung (2)
SC116	IVS4	654–84T>C	Non coding	1	–	–	–	–
Vd7	IVS6	745–19C>T	Non coding	1	1	–	–	–
Vd100	10	1379C>T	S384F	1	–	–	–	–
Sal10	10	1411T>G[b]	W395G	1	–	–	–	Colon (2)
Vd8	IVS10	2137+12delT	Non coding	1	2	–	–	–
SC90	11	3398A>G[b]	K1057R	4(2)	1	–	–	Lung
Sal11	11	6328C>T	R2034C	–	3	–	–	–
V70	11	6359G>C	G2044A	1	–	–	–	Lymphoma
V123	11	6359G>C	G2044A	1	1	–	–	Stomach
V49	11	6515C>G	P2096R	1	–	–	–	–
Vd111	11	6518C>T	T2097M	2	–	–	–	–
V122	11	6551G>A	R2108H	1	–	–	–	Uterus, liver
SC97	15	7675A>G	S2483G	–	3(1)	–	1	Lung
SP9	15	7772C>G	T2515I	1	2	–	–	–
SC90	IVS15	7829+7C>T	Non coding	4(2)	1	–	–	Lung
Sal12	18	8377G>T	A2717S	–	1	–	–	Lung
V57	18	8410G>A	V2728I	1	2	–	–	Leukemia, colon
SC26	18	8410G>C	V2728L	2(1)	1	–	–	Prostate
SC142	18	8410G>C	V2728L	2	2	–	–	–
V134	18	8410G>C	V2728L	3	2(1)	–	–	Pancreas, leukemia, prostate
Vd4	22	9078G>T	K2950N	3	–	–	–	Prostate
Vd22	22	9078G>T	K2950N	–	–	1	–	–
Vd128	22	9078G>T	K2950N	1	–	–	–	Colon
SC143	22	9079G>A	A2951T	3	2	–	–	–
Sp81	23	9266C>T	T3013I	1(1)	1	–	–	Colon (2)
SP151	23	9266C>T	T3013I	1	2	–	–	–
Vd193	23	9266C>T	T3013I	2	–	–	–	Liver
SP52	IVS25	9729+9A>C	Non coding	–	2	–	–	–
C68	27	10273A>G	T3349A	1	1	–	–	–
SP8	27	10338G>A	R3370R	4(1)	–	–	–	–

Nucleotides are numbered from the start of the *BRCA2* GenBank reference sequence U43746.
[a]Unique to Spain.
[b]Novel.
BC, breast cancer; OC, ovarian cancer.

reported that the *BRCA2* truncating mutations in families with a high proportion of ovarian cancer appear to be clustered in a 3.3-kb region in exon 11, between nucleotides 3035 and 6629. Data from our families did not support this clustering in the absence of a higher incidence of ovarian cases relative to breast cancer cases associated with the ovarian cancer cluster region (OCCR) of exon 11. The 10 families with mutations in this 3.3-kb region contained 32 breast cancer cases (three of them in males and one bilateral) and four ovarian cancer cases, compared with 152 breast cancer cases (13 of them in males) and 21 ovarian cases in 42 families with mutations elsewhere. Likewise, as regards the 20 positive families with ovarian cancer, only four presented a mutation in the OCCR region. The 3036_3039del mutation is on the 5′ border of the OCCR and its inclusion in the OCCR had no effect on the analysis. There were no differences in the mean ages of breast cancer or ovarian cancer diagnosis between carriers of *BRCA1* mutations located upstream or downstream of the 4447 limit. The comparison of the age at diagnosis of the *BRCA2* breast cancer cases in accordance with the location relative to OCCR yielded no significant differences. The mean age at diagnosis was 43.7 and 44.1 years inside and outside this region, respectively. Likewise, there were no differences between the average age of diagnosis of the breast cancer cases in *BRCA1* families (43.6 years) and that of the patients in families with *BRCA2* mutations (42.8 years).

Nearly all the families in our study also manifested malignancies such as prostate, colon, liver, stomach, lung, and uterus. Although some of these malignancies have been reported to be frequent features in families carrying mutations, we found no correlation between their presence or absence and the distribution or type of *BRCA* mutations.

DISCUSSION

As regards the 410 breast/ovarian cancer families, marked differences in the proportions of mutations were found when considering the distinct phenotypic groups (Table 1). The highest percentage of mutations were found in the breast and ovarian cancer group and the male breast cancer group, associated with mutations in

TABLE 6. *BRCA1* Polymorphisms in Spanish Breast/Ovarian Cancer Families/Patients

Exon/ intron	Variation	Predicted effect	Frequency
IVS7	561–34C>T	Non coding	T 27 %
IVS8	561_58delT	Non coding	delT 30%
9	710C>T	p.C197C	T 3%
11	1186A>G	p.Q356R	G 7%
11	2196G>A	p.D693N	A 8%
11	2201C>T	p.S694S	T 40%
11	2430T>C	p.L771L	C 40%
11	2731C>T	p.P871L	C 40%
11	3232A>G	p.E1038G	G 35%
11	3238G>A	p.S1040N	A 5%
11	3667A>G	p.K1183R	G 40%
13	4364A>G	p.E1415E	
13	4427C>T	p.S1436S	T 30%
IVS14	4604–63G>C	Non coding	C 25%
16	4956A>G	p.S1613G	G 22%
16	5075G>A	p.M1652I	A 2%
IVS16	5106–68G>A	Non coding	G 23%
IVS16	5106–92G>A	Non coding	G 23%
IVS18	5272+66G>A	Non coding	A 32%
IVS20	5396+48_59dup12	Non coding	

Nucleotides are numbered from the start of the *BRCA1* GenBank reference sequence U14680.

TABLE 7. *BRCA2* Polymorphisms in Spanish Breast/Ovarian Cancer Families/Patients

Exon/ intron	Variation	Predicted effect	Frequency
IVS1	203G>A	Non coding	A 28%
IVS1	203G>C	Non coding	C 31%
4	550A>C	p.N108H	
IVS8	909+56C>T	Non coding	T 7%
10	1093A>C	p.N289H	C 4%
10	1342A>C	p.N372H	C 20%
10	1593A>G	p.S455S	
11	2166C>T	p.S646S	
11	2457T>C	p.H743H	C 7%
11	3199A>G	p.N991D	G 4%
11	3624A>G	p.K1132K	G 37%
11	4035T>C	pV1269V	C 31%
11	4084A>G	p.K1286E	G 24%
11	4486G>T	p.D1420Y	
11	4812C>T	p.S1528S	T 4%
11	5972C>T	pT1915M	T 5%
11	6328C>T	p.R2034C	
14	7470A>G	p.S2414S	G 25%
14	7625C>T	p.A2466V	
IVS16	8034–14T>C	Non coding	C 35%
22	9079G>A	p.A2951T	
IVS24	9485–16T>C	Non coding	C 6%
27	10462A>G	p.I3412V	

Nucleotides are numbered from the start of the *BRCA2* GenBank reference sequence (GenBank accession no. U43746).

the *BRCA1* and the *BRCA2* genes, respectively. Interestingly, eight out of 14 families with one breast cancer and one ovarian cancer carried a mutation (five in *BRCA1* and three in *BRCA2*). Even though the statistical power for studying certain associations was limited due to the small number of this type of families screened, this result was consistent with growing evidence regarding the contribution of both genes to the overall incidence of ovarian cancer [Risch et al., 2001]. Consequently, although further studies are needed, it appears worthwhile to offer genetic testing to those families with two affected women, with at least one of them having ovarian cancer.

The small proportion of mutations found in the site-specific breast cancer group (15.4%) suggested that most of these families are associated with other susceptibility genes. These results were in agreement with earlier reports of Spanish and other populations [Hakansson et al., 1997; Serova et al., 1997; Osorio et al., 2000; de la Hoya et al., 2001; Meindl et al., 2002].

BRCA1 Mutations

We detected 31 different *BRCA1* mutations in 60 Spanish breast/ovarian cancer cases. The most frequent *BRCA1* alteration found in our population was the 187_188delAG mutation, which has its origins in Jewish populations [Struewing et al., 1995], and has one of the highest prevalences in western European populations. This mutation is present in almost 1.9% of families with high/moderate risk of breast/ovarian cancer [Díez et al., 1999b]. Haplotype studies indicate a common origin of the mutation in Spanish (Sephardic) and Ashkenazi Jewish populations. This mutation has also been detected in three unrelated Gypsy women, two of whom were affected by breast/ovarian cancer (Table 2). These women shared the same marker alleles present in Jewish samples [Díez et al., 1998]. Another frameshift mutation was found in exon 2 (189_192dupTGTC) and two were found in exon 3 (236_237delTG, and 243delA). Two of them appeared to occur only in Spanish families.

The 330A>G substitution (p.C64X) affected the splice-site donor in intron 5. It caused aberrant splicing resulting in a deletion of 22 nucleotides in exon 5 and a terminating translation at codon 64. This mutation was detected in seven families, most of them of known Galician origin. The results of haplotype studies were consistent with 330A>G being a founder *BRCA1* mutation of Spanish origin [Vega et al., 2001]. As reported in the BIC database, the 330A>G mutation has been observed in families of probable Spanish origin in diverse geographical locations in Europe other than Spain (France and the United Kingdom), and in Caribbean and South American families. The 589_590delCT mutation in exon 8 was detected in three families and has been reported twice in the BIC database.

In exon 11, seven frameshift mutations were detected. Four of them have not been reported in populations other than the Spanish population (according to the BIC database) and three were described for the first time in our study. Two of them were complex *BRCA1* mutations, consisting of a combination of a deletion and an insertion (1240_1242delinsT and 3958_3962delins4). In each case, DNA from both parents of the carrier confirmed that both events occurred in the same allele.

The missense mutations 5236G>C and 5236G>A were found in two and three families, respectively. These alterations could be significant for a number of reasons.

They were not found in 100 control chromosomes. Tumor tissue of two patients belonging to two families with the 5236G>A mutation presented loss of the wild-type allele, and in one family the mutation was present in three affected members, two of them showing loss of the wild-type allele in the tumor [Osorio et al., 2002]. These missense mutations resulted in the replacement of a small hydrophobic amino acid (glycine) by a noncharged amino acid (alanine) and by a hydrophilic-charged amino acid (glutamic), respectively. Furthermore, the comparison of human, canine, and murine *BRCA1* sequences in this region [Szabo et al., 1996] shows a series of 36 amino acid residues with a 96% identity (from positions 1680 to 1716), corresponding to the first BRCT domain in the *BRCA1* protein, suggesting that these amino acid changes could play an important role in the *BRCA1* function. Further support for this hypothesis was provided by the presence in the same region of the 5236G>C substitution, which has been shown to cause loss of function in different assays [Vallon-Christersson et al., 2001]. The variant 5242C>A was found in five Spanish families and has been reported to the BIC database 12 times, twice in Latin American and Caribbean families.

Twenty-two families presented 13 different *BRCA1* mutations in the C-terminal region of the protein. Six of them were clustered in exon 18 and its intron boundaries, suggesting that this region, corresponding to the highly conserved BRCT domains of the C-terminal region of the *BRCA1* protein [Scully et al., 1999], could be a hot spot for functional alterations, as evidenced by the presence of missense mutations with associated deleterious effects. The inframe deletion of an alanine (5197_5199delCTG) and the novel mutation g.IVS18+5G>A can be considered as deleterious since they cause skipping of exon 18 [Campos et al., in press]. The missense mutation 5263G>A (p.S1715N) was found in a family with three breast cancer cases. S1715 is an evolutionary conserved residue, and displays loss of activity in functional studies [Vallon-Christersson et al., 2001], suggesting that this is a disease-associated mutation. This observation agrees with the presence of the mutation in the two affected members of this family who were analyzed and the loss of the wild-type allele in one tumor analyzed. Five mutations (g.IVS18–1G>C, g.IVS18–1G>A, g.IVS20+1G>A, g.IVS20–1G>C, and 5451G>T) occurred at exon–intron boundaries and are expected to affect correct splicing of the mature RNA. Two of these splicing site mutations and two frameshift mutations (detected in exons 21 and 23, respectively) have not been reported before.

BRCA2 Mutations

We identified 22 *BRCA2* predisposing mutations in 53 individuals (Table 3). The splice-site mutation g.IVS2+2T>C was detected once in a breast/ovarian cancer family. This mutation had been found in a Latin American/western European family (reported in the BIC database). In the large exon 10, two frameshift mutations (1538_1541del and 1825delA) were detected twice. The 1825delA has not been described before. Exon 11 showed a large diversity of alterations. Two of them may be unique to Spain.

Four recurrent mutations were found in *BRCA2*: 3036_3039delACAA, 6857_6858delAA, 9254_9258del ATCAT, and 9538_9539delAA, which account for 56.6% of all pathogenic mutations identified in the 53 *BRCA2* families. The most common mutation in *BRCA2* (3036_3039del), which was present in 14 families, has been found in many western European countries (e.g., Belgium, France, Italy, and Switzerland) and in the United States and Canada. Analysis of microsatellite markers has shown that the origin may be unique [Neuhausen et al., 1998] 80 generations ago (95% CI, 46–134). The estimated age of this mutation is more than two times that for the 6174delT mutation, which is common to Ashkenazi Jews. This is consistent with the possibility of an earlier and more extensive dispersion of this mutation across Europe, including the Iberian Peninsula.

The 6857_6858del in exon 11 was identified in our study in three Catalan families, who share the same haplotype flanking the *BRCA2* gene (data not shown). It has recently been reported in one Chilean family [Carvallo et al., 2002], which also had its origin in Catalonia, suggesting a common ancestor with the mutation.

The second most frequent mutation in *BRCA2* was the 9254_9258del, identified in nine families in this study. This mutation has previously been reported in three other families of Spanish origin [Neuhausen et al., 1998]. All the families came from the northeast of Spain (Catalonia-Valencia) and shared a common haplotype over a 2-cm region spanning the *BRCA2* locus, indicating a unique origin [Campos et al., 2003, in press]. This mutation seems to be associated with a high risk of male breast cancer. Among the 13 *BRCA2* families with male breast cancer cases, the 9254_9258del mutation was present in five families (38.46%), but only in four out of the 40 (10%) *BRCA2* families with female breast cancer cases (OR=5.63; 95% CI, 0.99–33.85; p=0.033). Finally, the 9538_9539del mutation was detected in four high-risk families, and had been described in the BIC database once before.

Families and Patients With no *BRCA1* and *BRCA2* Mutations Detected

Despite extensive mutation screening, no *BRCA1* or *BRCA2* mutations were detected in 337 female breast cancer families and in nine families containing male breast cancer. These results were in agreement with earlier studies showed a much lower percentage of mutations than predicted by the initial linkage studies [Serova et al., 1997; Ford et al., 1998; Nathanson and Weber, 2001; Antoniou et al., 2002]. These findings also pointed to other susceptibility genes.

However, since the screening methods used in this study were not completely sensitive, some mutations may

have been missed. Regulatory sequences and large genomic deletions or insertions were beyond the scope of this study, although this kind of alteration may account for as many as 15% of abnormalities in *BRCA1* and *BRCA2* [Unger et al., 2000] and several were recurrent [Petrij-Bosch et al., 1997]. There are currently no data for such chromosomal rearrangements in a Spanish population. It goes without saying that the mutation frequency reported by most screening studies, including our own, is a conservative estimate. On the other hand, only one individual per family was analyzed, and theirs could have been a sporadic case. This possibility is demonstrated by the existence of noncarrier affected relatives in some families.

The mutation frequencies in Group D were much lower than those observed in other series; this was probably due to the inclusion of women with sporadic cancer diagnosed after 50 years of age in the selection criteria. Interestingly, all the mutation carriers without family history were women diagnosed between 23 and 36 years of age, with the exception of one *BRCA2* mutation (4088delA), which was detected in a woman who had had breast cancer at 48 years of age and ovarian cancer at 61 years of age. At present, general population–based screening for these mutations has not been recommended given the low prevalence of these mutations even in early-onset breast cancer patients.

In summary, our study confirmed that the proportion of mutations in *BRCA1* and *BRCA2* genes in breast cancer families is low, and that the presence of at least one case of ovarian cancer or male breast cancer strongly predicts the occurrence of mutations in *BRCA1* and *BRCA2*, respectively. Furthermore, it is likely that a high proportion of families in our series could be attributed to susceptibility genes other than *BRCA1* and *BRCA2*.

Geographic Distribution

In the present study, carried out with the largest series of Spanish patients to date, there were no marked differences between the percentages of deleterious mutations in *BRCA1* and *BRCA2* genes (53% and 47%, respectively). However, there were some differences due to the geographic origin of the families, with a higher proportion of *BRCA1* findings in families from northwest Spain. It has been suggested that the differences found in Galicia [Vega et al., 2002] or Catalonia [Campos et al., 2003], compared with those in other parts of Spain, could be due in part to the presence of founder effects. Alternatively, the lower prevalence of *BRCA2* mutations in these families and patients could be due to fewer mutations and to lower penetrance, and/or to later age of onset of *BRCA2* breast cancer. Similarly, the frequencies of variants of unknown biological significance and polymorphisms could also vary according to the geographical origin of the control individuals studied. Additional research into control populations is needed to compare information regarding *BRCA1* and *BRCA2* genetic diversity in various subpopulations in Spain.

Some *BRCA1* and *BRCA2* mutations identified in our population have been found in European countries. However, 13 *BRCA1* and nine *BRCA2* distinct deleterious mutations (22/53, 41.5%) appear to be unique to Spaniards. The high number of country-specific mutations, and especially the recurrent mutations associated with the geographical origin of the families, could facilitate the setting up of a methodological strategy that could be less expensive and less time-consuming for mutation detection in Spain. The families included in the study could be regarded as representative of the Spanish population. However, patients from the Basque country (well known for its genetic and linguistic isolation and distinctiveness) were underrepresented, and the mutations potentially specific to the Basques may have been missed.

Many European mutations have been observed in the United States or Canada, reflecting European migrations to North America. Similarly, in many cases, Latin families from America can trace their origins to the period of Spanish colonization, and in the last century, to migratory movements from diverse regions of Spain. An example of this is the appearance of the 330A>G *BRCA1* mutation among Latin/South American/Caribbean families with Galician ancestors (from the BIC database and personal communications), and the 6857_6858del *BRCA2* mutation in one Chilean family with Catalan origins. Therefore, the occurrence in Latin America of founder mutations that originated in Spain will be useful in establishing a cost-effective mutational analysis in such populations. Thus, a stepwise mutation test for the *BRCA1* (exons 2, 5, 8, and 18) and *BRCA2* genes (5′ and 3′ ends of exon 11, exon 23, and exon 25) should be devised, bearing in mind the frequencies of recurrent mutations as well as the familial background of the patients counseled.

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