Biol Res 45: 117-130, 2012

BRCA1 and *BRCA2* mutations in breast cancer patients from Venezuela

Karlena Lara[1], Nigmet Consigliere[1], Jorge Pérez[2], Antonietta Porco[1]

[1] Universidad Simón Bolívar, Departamento de Biología Celular, Laboratorio de Genética Molecular Humana B, Caracas;
[2] Centro Clínico de Estereotaxia CECLINES, Caracas.

ABSTRACT

A sample of 58 familial breast cancer patients from Venezuela were screened for germline mutations in the coding sequences and exon–intron boundaries of *BRCA1* (MIM no. 113705) and *BRCA2* (MIM no. 600185) genes by using conformation-sensitive gel electrophoresis. Ashkenazi Jewish founder mutations were not found in any of the samples. We identified 6 (10.3%) and 4 (6.9%) patients carrying germline mutations in *BRCA1* and *BRCA2*, respectively. Four pathogenic mutations were found in *BRCA1*, one is a novel mutation (c.951_952insA), while the other three had been previously reported (c.1129_1135insA, c.4603G>T and IVS20+1G>A). We also found 4 pathogenic mutations in *BRCA2*, two novel mutations (c.2732_2733insA and c.3870_3873delG) and two that have been already reported (c.3036_3039delACAA and c.6024_6025_delTA). In addition, 17 variants of unknown significance (6 *BRCA1* variants and 11 *BRCA2* variants), 5 *BRCA2* variants with no clinical importance and 22 polymorphisms (12 in *BRCA1* and10 in *BRCA2*) were also identified. This is the first genetic study on *BRCA* gene mutations conducted in breast cancer patients from Venezuela. The ethnicity of our population, as well as the heterogeneous and broad spectrum of *BRCA* genes mutations, must be considered to optimize genetic counseling and disease prevention in affected families.

Key words: *BRCA* genes, Breast cancer, screening mutations

INTRODUCTION

Deleterious germline mutations in the *BRCA1* (MIM no. 113705) and *BRCA2* (MIM no. 600185) tumor suppressor genes have been significantly associated with elevated risk of developing breast and ovarian cancer. Lifetime risk of breast cancer is as high as 80% among women with mutations in these genes, and for ovarian cancer the risk is greater than 40% and 20% for carriers of *BRCA1* and *BRCA2* mutations, respectively (King et al., 2003).

The *BRCA*1 gene is mapped to chromosome 17q21, spanning more than 80 kb distributed in 22 exons, and encodes for a protein of 1,863 amino acids. The *BRCA*2 gene is located at locus 13q12, comprises 10.4 kb organized in 27 exons, and encodes for a protein of 3,418 amino acids (Wooster et al., 1994; Wooster et al., 1995). More than 1600 mutations in *BRCA*1 and 1800 mutations in *BRCA*2 have been described throughout both genes according to the Breast Cancer Information Core website (BIC).

Little is known about the contribution of *BRCA1* and *BRCA2* mutations to hereditary breast and/or ovarian cancer in Hispanic populations. In the last few years, important contributions have been made by different groups that have reported the prevalence of *BRCA1* and *BRCA2* mutations in different populations from South America, such as Chile (Jara et al., 2006; Gallardo et al., 2006), Colombia (Torres et al., 2007), Brazil (Dufloth et al., 2005; Gomes et al., 2007) and Mexico (Ruiz-Flores et al., 2002). All these studies have in common the finding that the spectrum of mutations is quite different among the Latin-American populations investigated.

Ethnicity plays an important role in hereditary breast cancer given that specific founder mutations have been associated with the development of this pathology in various ethnic groups, such as the mutations found in Ashkenazi Jews (Warner et al., 1999). More recently, a study conducted in 53 breast/ovarian cancer families from Colombia reported three recurrent mutations (two in *BRCA1* and one in *BRCA2*) with possible founder effects (Torres et al., 2007). These findings highlight the importance of molecular diagnosis of mutations in the *BRCA1* and *BRCA2* genes in different populations. In addition, the identification of mutations in these genes allows the screening, counseling, testing, and clinical management of high risk families and also contributes to estimating the prevalence of these mutations in a specific population.

Breast cancer is the second most common cancer and second most common cause of cancer death among women in Venezuela (Capote, 2006). By 2008, breast cancer had an incidence rate of 42.5 cases every 100,000 women (incidence cases of 5,404) and a mortality rate of 13.7% (http://globocan.iarc.fr/factsheets/cancers/breast.asp).To date, there is no data on the participation of germline *BRCA* mutations in breast cancer patients from Venezuela. In this study, we performed a mutational analysis of *BRCA1* and *BRCA2* genes using conformation-sensitive gel electrophoresis (CSGE), in order to establish a genetic profile in a sample of Venezuelan breast cancer patients.

METHODS

Patients

The study included 58 breast cancer patients diagnosed at the "Centro Clínico de Estereotaxia" (CECLINES), Caracas, Venezuela. Peripheral blood was acquired from the subjects

Corresponding author: Prof. Dr. A. Porco, Laboratorio de Genética Molecular Humana B. Departamento de Biología Celular. Universidad Simón Bolívar. Apartado 89000. Caracas 1080-A, Venezuela. e-mail: aporco@usb.ve.; Tel/fax: +58-212-9064217.

Received: April 18, 2011. In Revised Form: August 5, 2011. Accepted: September 15, 2011.

after obtaining their signed consent. Blood collection was carried out between May 2006 and November 2009. The patients were identified as high risk individuals with *BRCA* gene mutations because they met at least one of the following criteria according to the *Breast Cancer Linkage Consortium* (1997): early onset (less than 45 years of age) and/or bilaterality; more than three cases of breast cancer and more than one case of ovarian cancer in the family; more than two first-degree relatives affected; and male breast cancer.

DNA Extraction

Genomic DNA was extracted from peripheral blood samples according to Bowen and Keeney (2003). Ethanol precipitated DNA samples were resuspended in sterile water and frozen at -20°C until further use.

Multiplex PCR amplification and screening for Ashkenazi Jewish founder mutations

Fragments possibly containing the Ashkenazi Jewish founder mutations c.185_186delAG and c.5382insC in *BRCA1* and c.6174delT in *BRCA2* were amplified by multiplex PCR using three sets of primers and the conditions previously described by Kuperstein et al. (2000), with the exception that the primers lacked the 5'-Cy5 modification. Products were diluted 1:1 in formamide buffer (98% formamide, 10 mM EDTA and 0.05% bromophenol blue) and screened for mutations using a modification of the fluorescent multiplex-PCR analysis (FMPA) technique (Kuperstein et al., 2000). The fragments were analyzed on an 8% denaturing polyacrylamide gel containing 7 M urea, and separated at 10º C, 2700 V, 100 mA and 80 Watts for 90 minutes in 1 X TBE buffer. Gels were revealed by silver staining.

Multiplex amplification and screening of the BRCA1 and BRCA2 genes by CSGE

BRCA1 and *BRCA2* screening was performed by PCR amplification of the entire coding regions, including the flanking splice sites and 3' UTR, using 88 sets of primers (38 for *BRCA1* and 50 for *BRCA2*). Tables I and II summarized the sequences of all primers that were employed. The primers were grouped into 35 multiplex sets (15 for *BRCA1*

TABLE I

PCR primer sequences for amplification of *BRCA1* gene

Exon	Primer Sequence (5′ – 3′)
1	**Fwd:** TAG CCC CTT GGT TTC CGT G[a] **Rev:** TCA CAA CGC CTT ACG CCT C[a]
2	**Fwd:** GAA GTTG TCA TTT TAT AAA CCT TT[b] **Rev:** TGT CTT TTC TTC CCT AGT ATG T[b]
3	**Fwd:** TCC TGA CAC AGC AGA CAT TTA[b] **Rev:** TTG GAT TTT TCG TTC TCA CTT A[b]
5	**Fwd:** CTC TTA AGG GCA GTT GTG AG[b] **Rev:** TTC CTA CTG TGG TTG CTT CC[b]
6	**Fwd:** ATG ATG TAT TGA TTA TAG AG[c] **Rev:** GAT TAC AGA TAC AGA ACT AA[c]
7	**Fwd:** TGC CAC TTA CAT TGT TGG TGT C[c] **Rev:** AAC ATG ATG AAA CCC CGT CTC[c]
8	**Fwd:** TGT TAG CTG ACT GAT GAT GGT[c] **Rev:** ATC CAG CAA TTA TTA TTA AAT AC[c]
9	**Fwd:** CCA CAG TAG ATG CTC AGT AAA TA[1] **Rev:** TAG GAA AAT ACC AGC TTC ATA GA[a]
10	**Fwd:** TGG TCA GCT TTC TGT AAT CG[a] **Rev:** GTA TCT ACC CAC TCT CTT TT CAG[a]
11-1	**Fwd:** CCA AGG TGT ATG AAG TAT GT[b] **Rev:** GAT CAG CAT TCA GAT CTA CC[b]
11-2	**Fwd:** CTC ACT AAA GAC AGA ATG[b] **Rev:** CTT TCT GAA TGC TGC TAT[b]
11-3	**Fwd:** CAG AAA CTG CCA TGC TC AGA[b] **Rev:** AGG CTT GCC TTC TTC CGA TA[b]
11-4	**Fwd:** GTT CAC TCC AAA TCA GTA GAG AG[b] **Rev:** CAG CTT TCG TTT TGA AAG CAG[b]
11-5	**Fwd:** CCT AAC CCA ATA GAA TCA CTC G[b] **Rev:** GAA CCA GGT GCA TTT GTT AAC TTC[b]

TABLE I. Continuation

Exon	Primer Sequence (5′ – 3′)
11-6	**Fwd:** CAG CGA TAC TTT CCC AGA GC[b] **Rev:** GTC CCT TGG GGT TTT CAA A[b]
11-7	**Fwd:** CTG GAA GTT AGC ACT CTA GG[b] **Rev:** GTT GCA CAT TCC TCT TCT GC[b]
11-8	**Fwd:** CCG TTT TCA AAT CCA GGA AA[b] **Rev:** TGA TGG GAA AAA GTG GTG GT[b]
11-9	**Fwd:** GAG GCA ACG AAA CTG GAC TCA[b] **Rev:** CTC AGG TTG CAA AAC CCC TA[b]
11-10	**Fwd:** AAC AGA GGG CCA AAA TTG AA[b] **Rev:** GGG TGA AAG GGC TAG GAC TC[b]
11-11	**Fwd:** AAA GCG TCC AGA AAG GAG AGC[b] **Rev:** GCC TTT GCC AAT ATT ACC TGG[b]
11-12	**Fwd:** CAT TGA AGA ATA GCT TAA ATG[b] **Rev:** CCT GGT TCC AAT ACC TAA GTT[b]
12	**Fwd:** GTC CTG CCA ATG AGA AGA AA[a] **Rev:** TGT CAG CAA ACC TAA GAA TGT[a]
13	**Fwd:** AAT GGA AAG CTT CTC AAA GTA[a] **Rev:** ATG TTG GAG CTA GGT CCT TAC[a]
14	**Fwd:** CTA ACC TGA ATT ATC ACT ATC A[a] **Rev:** GTG TAT AAA TGC CTG TAT GCA[a]
15	**Fwd:** CCA GCA AGT ATG ATT TGT C[c] **Rev:** AAC CAG AAT ATC TTT ATG TAG GA[a]
16	**Fwd:** AAT TCT TAA CAG AGA CCA GAA[a] **Rev:** AAC TCT TTC CAG AAT GTT GT[c]
17	**Fwd:** GTG TAG AAC GTG CAG GAT TG[a] **Rev:** TCG CCT CAT GTG GTT TTA[a]
18	**Fwd:** GGC TCT TTA GCT TCT TAG GAC[a] **Rev:** GAG ACC CAT TTT CCC AGC ATC[a]
19	**Fwd:** CTG TCA TTC TTC CTG TGC TC[a] **Rev:** CAT TGT TAA GGA AAG TGG TGC[a]
20	**Fwd:** ATA TGA CGT GTC TGC TCC AC[a] **Rev:** GGG AAT CCA AAT TAC ACA GC[a]
21	**Fwd:** TCT TCC TTT TTG AAA GTC T[c] **Rev:** GTA GAG AAA TAG AAT AGC CTC T[a]
22	**Fwd:** CAT TGA GAG GTC TTG CTA T[c] **Rev:** GAG AAG ACT TCT GAG GCT AC[a]
23	**Fwd:** CAG AGC AAG ACC CTG TCT C[a] **Rev:** ACT GTG CTA CTC AAG CAC CA[a]
24	**Fwd:** ATG AAT TGA CAC TAA TCT CTG C[a] **Rev:** GTA GCC AGG ACA GTA GAA GGA[a]
24-1	**Fwd:** ATG AGC TTA CAA AGT GGC CT[c] **Rev:** AGA AGT AAA CTT AGG GAA ACC A[c]
24-2	**Fwd:** CTG GAA GCA CAG AGT GGC TT[c] **Rev:** TTA GCC ACC TGA GTA GCT GG[c]
24-c	**Fwd:** ACA GAA ATT AGC CGG TCA T[c] **Rev:** GGA ATG GAT TAT ATA CCAGAG C[c]
24-4	**Fwd:** AGT AAT AAG TAA AAT GTT TA[c] **Rev:** CTA GGA GGT AGA TAC TAT C[c]

[a] Friedman et al. 1994
[b] Dufloth et al. 2005
[c] Present study

TABLE II

PCR primer sequences for amplification of *BRCA2* gene

Exon	Primer Sequence (5′ – 3′)
1	**Fwd**: CGC GAG CTT CTG AAA CTA GG[a] **Rev**: CCC ACT ACC ACC ACC ACT AAC[a]
2	**Fwd**: GCG CTT CTG AGT TTT ACC TC[a] **Rev**: GTC AAT ACC TGC TTT GTT GC[a]
3	**Fwd:** TCA CAA ATT TGT CTG TCA C[a] **Rev:** ACC ATA TTG CAT TAC TTA CC[a]
4	**Fwd:** ATT GTA CTG TTT CAG GAA GGA ATG[a] **Rev:** GCC AAA ATA TTA GCA TAA AAA TCA G[a]
5	**Fwd:** GCC AGT TTT TTA AAA TAA CCT AAG[a] **Rev:** AAA AGC ATT GTT TTT AAT CAT ACC[a]
6	**Fwd:** CCT TTT TTT ACC CCC AGT G[a] **Rev:** GGC AAA GGT ATA ACG CTA TTG[a]
7	**Fwd:** GGT CGT CAG ACA CCA AAA C[a] **Rev:** TCA ACC TCA TCT GCT CTT TC[a]
8	**Fwd:** TGT GCT TTT TGA TGT CTG AC[a] **Rev:** CAT GAA TAG GGG ACT ACG G[a]
9	**Fwd:** ACC ATG GAT AAG GGG GGA C[a] **Rev:** GGG TGA CAG AGC AAG ACT CC[a]
10 – 1	**Fwd:** GTG CTT CTG TTT TAT ACT TT[b] **Rev:** CTA CAT TTG AAT CTA ATG G[a]
10 – 2	**Fwd:** CTC ATT TGT ATC TGA AGT GG[b] **Rev:** GCT GCT CTT CAT CTC TCT T[a]
10 – 3	**Fwd:** GCC ATT AAA TGA GGA AAC AG[b] **Rev:** GCC AGC TTC CAT TAT CAA T[a]
10 – 4	**Fwd:** CTG TTT GCT CAC AGA AGG AG[b] **Rev:** CAG AGG TAC CTG AAT CAG CA[a]
11 – 1	**Fwd:** AGT GAA TGT GAT TGA TGG TAC[b] **Rev:** CAT GCT GCA GCC AAG ACC TCT[b]
11 – 2	**Fwd:** GAA GGA CAG TGT GAA AAT G[b] **Rev:** CCT TTC TTG AAG GTG ATG C[b]
11 – 3	**Fwd:** AAG ATG TAT GTG CTT TAA ATG[b] **Rev:** CTC CTC TGC AAG AAC ATA AAC[b]
11 – 4	**Fwd:** AGA CAC AGG TGA TAA ACA AG[b] **Rev:** CAA GGT ATT TAC AAT TTC AA[b]
11 - 5	**Fwd:** GCT CTC TGA ACA TAA CAT TAA G[b] **Rev:** CAT TAT GAC ATG AAG ATC AG[b]
11 – 6	**Fwd:** TAT CTT AAA GAC CAC TTC TG[b] **Rev:** TGA AAC AAC AGA ATC ATG AC[b]
11 – 7	**Fwd:** CTT CTG CAG AGG TAC ATC[b] **Rev:** CAG TAA ATA GCA AGT CCG[b]
11 – 8	**Fwd:** TTT GAT GGC AGT GAT TCA AG[b] **Rev:** CTT ATG TCA GAA TGT AAT TC[b]
11 – 9	**Fwd:** ATC AGA AAC CAG AAG AAT TG[b] **Rev:** ATC TCA ATG GTC TCA CAT GC[b]
11 – 10	**Fwd:** CAG AGA GGC CTG TAA AGA C[b] **Rev:** GAA GTC TGA CTC ACA GAA G[b]
11 – 11	**Fwd:** TGA AAA TTC AGC CTT AGC[b] **Rev:** GCA TCT TTT ACA TTG GAT[b]
11 – 12	**Fwd:** GTA TTG AGC CAG TAT TGA AG[b] **Rev:** TGC CTC GTA ACA ACC TGC CAT[b]

TABLE II. Continuation

Exon	Primer Sequence (5′ – 3′)
11 – 13	**Fwd:** GTT TCA GTA AAG TAA TTA AG[b] **Rev:** AGA TTT TCC ACT TGC TGT GC[b]
11 – 14	**Fwd:** AAG TCA GTC TCA TCT GCA A[b] **Rev:** GAA ACT TGC TTT CCA CTT G[b]
11 – 15	**Fwd:** CAT CTG CTT TCT CTG GAT TTA[b] **Rev:** ATG TTC TCA ACA AGT GAC ACT[b]
11 – 16	**Fwd:** ATG TTG AAG GTG GTT CTT CAG[b] **Rev:** GTG ATT GGC AAC ACG AAA GG[b]
12	**Fwd:** CTT TAG CTT TAA AAA AAT GG[a] **Rev:** TAC CTA TAG AGG GAG AAC AG[a]
13	**Fwd:** TCA CTG AAA ATT GTA AAG CC[a] **Rev:** AAA CAT GTC TTA CCG AAA GG[a]
14 – 1	**Fwd:** CCA TTG CAG CAC AAC TAA G[a] **Rev:** AAA TGG ATG TCC TGA AAC TG[a]
14 – 2	**Fwd:** TCA AGC AAT TTA GCA GTT TC[a] **Rev:** AGG CAA AAA TTC ATC ACA C[a]
15	**Fwd:** ACA CCT GGC TAC TTT TGT G[a] **Rev:** GTT TAT GAG AAC ACG CAG AG[a]
16	**Fwd:** TAG CAG GAG GCG TAT AAA CG[a] **Rev:** TAA ACC CCA GGA CAA ACA GC[a]
17	**Fwd:** CTT TTA TTT GTT CAG GGC TC[a] **Rev:** TCT CTT AAA TGG GGG CTA G[a]
18	**Fwd:** ACG GAA ATT GAT AGA AGC AG[a] **Rev:** CAA GAG GTG TAC AGG CAT C[a]
19	**Fwd:** ATATTTATTAATTTGTCCAG[a] **Rev:** GTAAGTTTCAAGAATACATC[a]
20	**Fwd:** TGT GTG TAA CAC ATT ATT AC[a] **Rev:** AGA CTT TGT TCT CAT ATT AG[a]
21	**Fwd:** TTA GAA AAC ACA ACA AAA CC[a] **Rev:** TCT CAC CTT GAA TAA TCA TC[a]
22	**Fwd:** ATA TCT TAA ATG GTC ACA GG[a] **Rev:** AAA ACT GAT AAA AAC AAA GC[a]
23	**Fwd:** AAA TGA TAA TCA CTT CTT CC[a] **Rev:** TCC ATA AAC TAA CAA GCA C[a]
24	**Fwd:** TGA ATT TTT GTT TTG TTT TC[a] **Rev:** TGC ATT ACC TGT TTT TTT C[a]
25	**Fwd:** TTT TCC ATT CTA GGA CTT GC[a] **Rev:** AAA ATG TGT GGT GAT GCT G[a]
26	**Fwd:** TTC TCT GTT CCC CTC TCC CT[a] **Rev:** GGA AAG TGT GCA CCC AGA GT[a]
27	**Fwd:** CGT TTT CAT TTT TTT ATC AG[a] **Rev:** TTT TCT TTA TGG GTG TTT C[a]
27-1	**Fwd:** GTT AGT CCC ATT TGT ACA TT[a] **Rev:** TTA GTT GTA ATT GTG TCC TG[a]
27-2	**Fwd:** GAT TAT CTC AGA CTG AAA CG[a] **Rev:** CAA AGA TGT TTT TCT TGA TT[a]
27-3	**Fwd:** ATT GGT ATA CTT TTG CTT CA[a] **Rev:** TAT CTG CAT CAA AAT AAC TG[a]
27-4	**Fwd:** TTG TGT CAT TAA ATG GAA TG[a] **Rev:** CCA ATT TGA AAG CAA GAT AT[a]

[a] Present study
[b] Dufloth et al. 2005

and 20 for *BRCA2*), according to the PCR product size and annealing temperature (Table III). Some exons were amplified as uniplexes and then mixed for further analysis. Individual and Multiplex PCR (MPCR) amplifications included an initial amplification for 5 min at 95°C, followed by 35 cycles at 95°C for 1 min, annealing at temperatures indicated in Table III for 1 min and 1 min at 72°C. A final elongation step was done at 72°C for 10 min. The PCR reactions were carried out on a Mastercycler Gradiente Thermal Cycler (Eppendorf) using 0.2, 0.4, 0.6 and 0.8 mM of dNTP's (according to primers conforming the MPCR: 1, 2, 3 and 4 pairs, respectively), 0.075 U of Platinum ®*Taq* DNA Polymerase (Invitrogen, São Paulo, Brazil), 1 X of Platinum ®*Taq* DNA Polymerase Buffer (200 mM Tris-HCl pH 8.4 and 500 mM KCl), 0.75 μM of each primer and approximately 200 ng of genomic DNA. The final concentration of $MgCl_2$ used for these amplification reactions are also indicated in Table III. The MPCR products were screened for mutations in a mildly denaturing CSGE

TABLE III

PCR conditions for multiplex amplification of *BRCA1* and *BRCA2* genes

Gene	MPCR Group	Exon/Primers grouped	Size (bp)	Annealing Temperature (°C)	Final concentration of $MgCl_2$ (mM)
BRAC1	1	16/6/14/10	448/350/310/241	53	4,5
	2	15 & 23 & 9	331/255/211	55	1,5
	3	1/12	316/265	55	3,0
	4	18/22/19	352/294/249	55	3,0
	5	13/17	320/263	55	3,0
	6	24-3/8 & 24-2	419/350/267	55 & 60	3,0
	7	24-1/24	359/280	64	3,0
	8	11-5/21	422/294	53	2,5
	9	11-4/11-10 & 2	415/361/258	55	3,0 & 2,5
	10	24-4/11-2	494/271	50	2,5
	11	20/3/11-7	401/339/296	53	2,5
	12	11-3/11-11/5	347/301/235	53	2,5
	13	11-6/11-12	362/326	53	2,5
	14	11-1/11-9/11-8	442/401/349	53	3,0
	15	7	649	60	1,5
BRCA2	1	11-11/27/12/24	399/315/230/172	53	4,0
	2	3/23/20/21	323/270/220/178	53	4,5
	3	11-4 & 19	361/200	53 & 48	1,5
	4	18/17/2/5	462/349/192/120	55	5,0
	5	27-3/13/6 & 9	349/280/195/129	57	3,5
	6	27-2/22	375/251	53	2,5
	7	8/25/1/14-1	357/291/205/148	60	4,0
	8	27-4/4/7	425/223/162	56	4,0
	9	15/14-2	437/358	55	2,5
	10	11-5/27-1/11-1	451/398/358	53	2,5
	11	11-8/11-7	407/327	53	2,0
	12	10-1/11-14	343/301	50	2,0
	13	11-2/11-10/10-4	406/348/288	53	3,0
	14	11-3/10-2/10-3	407/362/320	53	2,5
	15	11-16/11-9/11-13	469/403/350	53	3,5
	16	11-12	377	53	1,5
	17	11-15	376	53	1,5
	18	11-6	364	53	1,5
	19	16	618	58	1,5
	20	26	565	61	1,0

gel containing 12.5% polyacrylamide as previously described (Ganguly et al., 1993).

DNA sequencing

Samples displaying abnormal CSGE patterns were sequenced using an automated ABI 377 genetic analyzer (Applied Biosystems, Foster City, CA, USA) at the UEGF-IVIC (Caracas, Venezuela). All the nucleotide changes identified were confirmed by repeating the PCR and sequencing reaction using the corresponding forward and reverse primers. Mutation nomenclature was according to den Dunnen y Antonarakis (2001).

RESULTS

In the 58 breast cancer patients that were analyzed in the present study (Table IV), no Ashkenazi Jewish founder mutations were identified. Ten out of these 58 breast cancer patients (17.2%) carried *BRCA* mutations, six (10.3%) in *BRCA1* and four (6.9%) in *BRCA2* (Table V).

TABLE IV

Characteristics of the studied population

Patient	Age	Age at diagnosis	# of cases of BC in the family	Sex	Expression of hormone receptors	Histological type of the BC
CM001	40	36 and 42	2 (sister, aunt)	F	ER+, PR+	NE
CM003	35	35	1 (sister)	F	NE	NE
CM007	62	56	3 (mother, sister, grandmother)	F	NE	NE
CM008	40	34	1 (sister)	F	NE	NE
CM009	47	46	1 (mother)	F	NE	NE
CM012	43	37	2 (mother, grandmother)	F	ER+	NE
CM014	57	55	2 (sister, other (?))	F	NE	NE
CM016	46	39	2 (mother, paternal aunt)	F	ER+, PR+	NE
CM017	57	57	3 (2 sisters, aunt)	F	NE	NE
CM018	54	52	3 (mother, sister, grandmother)	F	NE	NE
CM019	46	46	2 (mother, other (?))	F	NE	NE
CM020	52	51	2 (sister, maternal aunt)	F	ER+, PR+	NE
CM022	35	35	2 (mother, grandmother)	F	ER+	NE
CM023	40	40	1 (mother)	F	NE	NE
CM025	35	35	1 (sister)	F	NE	NE
CM028	50	45	2 (grandmother, male cousin)	F	NE	NE
CM029	48	47	1 (sister)	F	ER-, PR+	NE
CM031	49	NE	1 (grandmother)	F	NE	NE
CM032	55	55	2 (mother, sister)	F	NE	NE
CM033	46	46	2 (aunt, female cousin)	F	NE	NE
CM035	38	38	2 (mother, female cousin)	F	NE	NE
CM037	56	48	1 (sister)	F	NE	NE
CM038	68	NE	3 (mother, sister, niece)	F	NE	NE
CM039	37	36	3 (sister, 2 aunts)	F	ER-, PR-, HER2-	NE
CM040	38	38	At least 2 (mother, aunts)	F	NE	NE
CM043	59	39 and 59	1 (mother)	F	ER+, PR+	NE
CM044	77	NE	2 (mother, sister)	F	NE	NE
CM045	54	53	3 (mother, grandmother, aunt)	F	ER+	NE
CM046	35	35	1 (father)	F	NE	NE
CM047	50	47 and 50	2 (mother, sister)	F	ER+, PR-, HER2-	Invasive Ductal Carcinoma
CM050	37	37	1 (mother)	F	ER+, PR+	NE
CM053	56	56	1 (sister)	F	NE	NE
CM054	65	62	NE	M	NE	NE

TABLE IV. Continuation

Patient	Age	Age at diagnosis	# of cases of BC in the family	Sex	Expression of hormone receptors	Histological type of the BC
CM055	51	48	2 (sister, aunt)	F	ER-, PR-	NE
CM057	48	44	1 (mother)	F	NE	NE
CM058	32	NE	1 (paternal aunt)	F	ER-, EP-, HER2-	NE
CM062	46	NE	3 (mother, 2 maternal aunts)	F	NE	NE
CM065	30	30	1 (aunt)	F	NE	NE
CM066	34	NE	NE	F	NE	NE
CM067	29	NE	NE	F	NE	NE
CM068	54	NE	4 (3 sisters, 1 aunt)	F	ER-	NE
CM069	47	47	1 (mother)	F	ER+, PR+	Bilateral Infiltrating Ductal Carcinoma
CM070	63	58	none	F	NE	NE
CM072	31	27	none	F	NE	NE
CM073	24	24	none	F	NE	NE
CM075	59	42	2 (mother, grandmother)	F	NE	NE
CM076	41	41	1 (mother)	F	NE	NE
CM079	52	52	none	F	NE	NE
CM081	45	38 and 42	3 (father, 2 uncles)	F	ER-	NE
CM083	64	33	At least 4 (sisters)	F	NE	NE
CM084	40	40	2 (mother, paternal aunt)	F	NE	NE
CM087	43	26	1 (paternal aunt)	F	NE	NE
CM088	61	NE	2 (sister, aunt)	F	NE	NE
CM089	53	44	2 (sister, other (?))	F	NE	NE
CM090	53	41	2 (sister, grandmother)	F	NE	NE
CM092	56	43	1 (mother)	F	ER+	NE
CM093	57	47	1 (mother)	F	NE	NE
CM094	59	35 and 45	2 (aunts)	F	NE	NE

Abbreviations: BC, breast cancer; NE, not specified; F, Famale; M, Male;ER, Estrogen Receptor; PR, Progesterone Receptor; HER2, Hepidermal Growth Factor Receptor 2.

The patients analyzed in this study fell into 5 subsets (Table VI). All patients belonging to the subset of families with male breast cancer were carriers of *BRCA2* gene mutations (100%), whereas all those patients belonging to the subset of families with breast/ovarian cancer presented *BRCA1* mutations (100%). Lower frequencies of *BRCA* mutations were found in patients belonging to subsets of families with bilateral breast cancer (37.5%), early age of onset (18.1%) and multiple breast cancer cases in the family (10%).

BRCA1 mutations

Four *BRCA1* truncating mutations were identified (Table V). The c.951_952insA mutation was identified in two unrelated patients and represented a novel mutation with no previous report on the BIC database. The c.4603G>T mutations on exon 14 of *BRCA1* gene, was previously reported and identified on the BIC database as a missense mutation (p.R1495M). However, Ozcelik *et al* (1999) and Yang *et al* (2002) determined that this mutation affects the splicing process by altering the -1 position of the exon 14 donor splice site, which causes the skipping of exon 14 resulting in a frame shift and in consequence leading to a stop codon at amino acid 1462 of the BRCA1 protein.

In addition, six *BRCA1* variants of unknown significance were detected in eight patients, five were missenses and one was an inframe deletion (Table VII). From these variants, the c.179A>C, IVS20-22C>T and g.6002C>T mutations had no precedents on the BIC data base. It has not yet been demonstrated whether these variants have pathogenic consequences.

All of the 58 patients presented at least one polymorphism in *BRCA1* (Table VIII). In particular, the IVS1+101C>G and c.4427T>C, c.6998C>T polymorphisms were reported here for the first time. Another polymorphism detected (IVS7+16(TTC) nTTTTC) was a triplet deletion (TTC) in a $(TTC)_7$TTTTC region on intron 7 of *BRCA1*. Although this polymorphism is not included in the BIC database, it has been previously reported in a Spanish population (Salgado et al., 2008). Three

genotypes were identified: (TTC)7/7, (TTC)7/6 y (TTC)6/6 with frequencies of 41.4%, 48.3% and 10.3%, respectively.

BRCA2 mutations

Four different truncating mutations in the *BRCA2* gene were identified, as shown in Table V. Two of these mutations (c.2732_2733insA and c.3870_3873delG) had not been previously reported in the BIC database. In addition, eleven *BRCA2* mutations of unknown significance were detected in fourteen different patients (Table VII), which included eight missense mutations, one intervening sequence and two 3'UTR variants. From these, mutations c.1282T>C, c.3479G>A, c.3875T>A, c.9799T>C, IVS12-63A>C, c.10594G>T and c.11323T>C had not been previously reported in the BIC database.

In *BRCA2*, ten different polymorphisms (Table VIII) were identified in 55 of the 58 patients. The c.10590A>C and c.11314_11323insT polymorphisms were reported here for the first time.

TABLE V

Germline mutations found in *BRCA1* and *BRCA2*

Patient ID	Exon/Intron	Mutation[a]	Change and/or Effect on protein[b]	Mutation type	# Entries[c]	Age at diagnosis	Family history	Other cancers
			BRCA1					
CM067	E11	c.951_952insA	Stop 286	F	0	29	-	-
CM068	E11	c.951_952insA	Stop 286	F	0		4 BrCa, 1 OvCa	-
CM001	E11	c.1129_1135insA	Stop 345	F	47	37	2 BrCa, 1 OvCa	-
CM055	E11	c.1129_1135insA	Stop 345	F	47	48	2 BrCa, 1 OvCa	-
CM025	E14	c.4603G>T	Arg1495Met (Splice error: loss of Exon 14, Stop 1462)	M	26	35	1 BrCa	-
CM066	I20	IVS20+1G>A	Stop 1737 (Loss of Exon 20) or Stop 1767 (alternative donor site)	S	42	34	-	-
			BRCA2					
CM053	E11	c.2732_2733insA	Stop 837	F	0	56 (Bil)	1 BrCa	-
CM046	E11	c.3036_3039delACAA	Stop 959	F	105	35	1 male BrCa	-
CM054	E11	c.3870_3873delG	Stop 1227	F	0	62	-	PrCa
CM081	E11	c.6024_6025delTA	Stop 1943	F	9	38 (Bil)	3 male BrCa 1 CoCa	-

Abbreviations: Br, breast; Ov, ovarian; Pr, prostate; Co, colon; Ca, cancer; bil, bilateral breast cancer; F, frameshift, M, missense; 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] The amino acid numbering given is for the mature processed protein, as used in the BIC database.
[c] Number of entries at the BIC database.

TABLE VI

BRCA mutations in high-risk patients with breast cancer in Venezuela

Characteristics of the families	Cases tested	Cases with *BRCA1* mutations	Cases with *BRCA2* mutations	Total (%)
Early age of Onset (<45 yr)	33	4 (12.1%)	2 (6%)	6 (18.1%)
Bilateral Breast Cancer	8	1 (12.5%)	2 (25%)	3 (37.5%)
Br/Ov Cancer Families	3	3 (100%)	-	3 (100%)
Multiple-case families (2 to 4 BrCa)	30	3 (10%)	-	3 (10%)
Male BrCa families	3	-	3 (100%)	3 (100%)
Total	58	6 (10.3%)	4 (6.9%)	10 (17.2%)

TABLE VII

Unknown variants found in *BRCA1* and *BRCA2*

Patient ID	Exon/Intron	Mutation[a]	Change and/or Effect on protein[b]	Mutation type	Clinical importance	# Entries[c]	Age at diagnosis	Family history
				BRCA1				
CM065	E2	c.179A>C	p.K20N	M	U	0	30	1 BrCa
CM039	E11	c.3238G>A	p.S1040N	M	U	45	36	3 BrCa
CM070	E11	c.3238G>A	p.S1040N	M	U	45	58 (Bil)	1 Skin Ca
CM029	E11	c.3827T>G	p.N1236K	M	U	35	47	1 BrCa
CM001	E11	c.4182_4184delAAT	p.N1355del	IFD	U	2	37	2 BrCa, 1 OvCa
CM055	E11	c.4182_4184delAAT	p.N1355del	IFD	U	2	48	2 BrCa, 1 OvCa
CM045	I20	IVS20-22C>T	U	IVS	U	0	53	3 BrCa
CM019	E24(3'UTR)	g.6002C>T	U	M	U	0	≤ 46	2 BrCa
				BRCA2				
CM031	E10	c.1282T>C	p.Y352H	M	U	0	49	1 BrCa, 1EnCa, 1 CoCa
	E11	c.3479G>A	p.S1084N	M	U			
CM053	E11	c.2457T>C	p.H743H	Syn	No	7	56	1 BrCa
CM066	E11	c.3147G>A	p.S973S	Syn	No	1	≤ 34	-
CM054	E11	c.3875T>A	p.F1216Y	M	U	0	62	-
CM032	E11	c.4791G>A	p.L1521L	Syn	No	2	55	2 BrCa
CM045	E11	c.4791G>A	p.L1521L	Syn	No	2	53	3 BrCa
CM009	E11	c.6328C>T	p.R2034C	M	U	97	46	1 BrCa
CM079	E11	c.6328C>T	p.R2034C	M	U	97	52 (Bil)	-
CM032	E11	c.6741G>C	p.V2171V	Syn	No	1	55	2 BrCa
CM045	E11	c.6741G>C	p.V2171V	Syn	No	1	53	3 BrCa
CM029	Intrón 12	IVS12-63A>C	U	IVS	U	0	47	1 BrCa
CM037	Intrón 12	IVS12-63A>C	U	IVS	U	0	48	1 BrCa
CM040	E15	c.7697T>C	p.I2490T	M	U	238	38	≥ 2 BrCa
CM012	E18	c.8237C>T	p.S2670L	M	U	8	37	2 BrCa
CM019	E22	c.9079G>A	p.A2951T	M	No	40	≤ 46	2 BrCa
CM039	E26	c.9799T>C	p.W3191R	M	U	0	36	3 BrCa
CM037	E27	c.10462A>G	p.I3412V	M	U	110	48	1 BrCa
CM069	E27	c.10462A>G	p.I3412V	M	U	110	47 (Bil)	1 BrCa
CM083	E27	c.10462A>G	p.I3412V	M	U	110	33 (Bil)	≥ 4 BrCa
CM054	E27 (3'UTR)	c.10594G>T	U	M	U	0	62	-
CM079	E27 (3'UTR)	c.11323T>C	U	M	U	0	52 (Bil)	-

Abbreviations: Br, breast; Ov, ovarian; Co, colon; En, endometrium; Ca, cancer; Bil, bilateral breast cancer; U, unknown; M, missense; IFD, inframe deletion; IVS, intervening sequence; Syn, synonymous.

[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]The amino acid numbering given is for the mature processed protein, as used in the BIC database.

[c]Number of entries at the BIC database.

TABLE VIII

Polymorphisms found in *BRCA1* and *BRCA2*

Exon/Intron	Polymorphism[a]	Change and/or Effect on protein[b]	# of Patients	# Entries[c]
		BRCA1		
I1	IVS1+101C>G	U	25	0
I7	IVS7+16(TTC)nTTTTC	U	58	0
I7	IVS-34T>C	U	14	9
I9	IVS8-58delT	U	21	8
E11	c.1186A>G	p.Q356R	6	82
E11	c.2430T>C	p.L771L	25 TC and 5 CC	25
E11	c.3232A>G	p.E1038G	25 AG and 5 GG	37
E11	c.3667A>G	p.K1183R	25 AG and 5 GG	33
E13	c.4427T>C	p.S1436S	25 TC and 5 CC	0
E16	c.4956A>G	p.S1613G	25 AG and 5 GG	36
I16	IVS16-68A>G	U	22	1
I16	IVS16-92A>G	U	22	6
I18	IVS18+65G>A	U	25	5
E24 (3′UTR)	c.6998C>T	U	19	0
		BRCA2		
E2 (5′UTR)	c.203G>A	U	12 GG and 1 AA	12
I8	IVS8+56C>T	U	12	3
E10	c.1093A>C	p.N289H	6	82
E10	c.1342C>A	p.H372N	30 CA and 2 AA	9
E10	c.1593A>G	p.S455S	6	7
E11	c.3199A>G	p.N991D	11	6
E14	c.7470A>G	p.S2414S	11	10
E18	c.8381T>C	p.I2718T	12	2
E27 (3′UTR)	c.10590A>C	U	13	0
E27 (3′UTR)	c.11314_11323insT	U	6	0

Abbreviations: U, unknown.
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 The amino acid numbering given is for the mature processed protein, as used in the BIC database.
c Number of entries at the BIC database.

DISCUSSION

Since the identification of *BRCA1* (Friedman et al., 1994; Miki et al., 1994) and *BRCA2* (Wooster et al., 1995) genes, the major genes known to confer high risk of breast and ovarian cancer, several mutations have been identified throughout the entire gene sequence, most of which are nonsense or frame shift mutations that produce truncated proteins (BIC database). The identification of mutations with founder effect in some specific ethnic groups has highlighted the importance of genetic testing in different populations. Despite the high prevalence of breast cancer in the Venezuelan population, studies related to the identification of *BRCA1* and *BRCA2* mutations, among patients with either breast cancer or a high-risk family history, have not been previously conducted.

Mutation screening was performed using the combined approach of CSGE and sequencing analysis. In a previous study developed in our laboratory, this method showed a sensitivity of over 90% for mutation detection (Albánez et al., 2011), besides being simple, rapid and cost-effective for this purpose. In addition, this technique has been shown to detect almost every unique single-base mismatch when it was compared to DGGE and SSCP for *BRCA* mutations detection (Ganguly, 1997). Indeed, we discarded the SSCP method during this investigation since we found only 33% sensitivity to detect the *BRCA* mutations (data not shown), which is in agreement with a previous study where SSCP showed 35% sensitivity to detect mutations in the coagulation factor IX gene (Sarkar et al., 1992)

As previously mentioned, eight distinct germline mutations, 4 in *BRCA*1 and 4 in *BRCA*2, were detected in 10 of the 58 Venezuelan breast cancer patients, representing a frequency of 17.2%. This frequency was similar to that previously reported in the Chilean population (15.6% and 20.3%, Jara et al., 2006; Gallardo et al., 2006, respectively), but higher than that found in the Brazilian population (13%; Dufloth et al., 2005) and lower than that found in 53 families from Colombia (24.5%; Torres et al., 2007). One of the *BRCA* mutations detected in our study was found in two unrelated patients, another was found in two related patients, and the rest were detected in 6 different patients. These findings suggest that the *BRCA1*/2 gene mutation spectrum is rather broad in the Venezuelan population. All the disease-causing mutations were small deletions, insertions or missenses that cause premature stop codons.

There are no reports of the *BRCA*1 mutation c.951_952insA on exon 11 in the BIC database and therefore its possible pathological effect is suggested by the absence of important sequences in the altered protein, such as the NLS and BRCT domains, and could be supported by the fact that this was the only mutation identified in two unrelated patients with breast cancer. One of these patients developed breast cancer at 29 years of age, while the other has three second-degree and one third-degree relatives with breast cancer; one of the second-degree relatives was also affected with ovarian cancer. All these are characteristics of the presence of mutations in the *BRCA1* gene (Ford et al., 1998). The other three *BRCA1* mutations (c.1129_1135insA, c.4603G>T and IVS20+1G>A) found in this study have been previously reported in the BIC database as mutations with clinical importance. The c.1129_1135insA was identified in two related patients (sisters) with family history of breast/ovarian cancer, indicating that this mutation may be segregating with the pathology in this family. The mutation c.4603G>T was identified in a patient with early-onset of breast cancer (35 year) and with a sister affected with this pathology. These two mutations (c.1129_1135insA and c.4603G>T) have been reported mostly in populations from Western Europe (BIC database), and it is not surprising to find these mutations in the Venezuelan population, which is characterized by a high ethnic heterogeneity and possesses an important European influence (Rodríguez-Larralde et al., 2001). Finally, the IVS20+1G>A mutation was identified in a patient that developed breast cancer at the age of 34. This variant can affect the splicing process in two ways, by promoting the skip of exon 20 generating a truncated protein of 1737 amino acids or by promoting the retention of part of intron 20, which in this case generates a truncated protein of 1767 residues (Tesoriero et al., 2005). In both cases the resulted protein lacks important functional domains and therefore appears to produce loss of BRCA1 function and is expected to be associated with the same breast cancer risk as other protein truncating mutations (Brose et al., 2004).

Of the *BRCA2* mutations, the c.3036_3039delACAA mutation has 105 records in the BIC database, most of which correspond to European populations, mainly Italian and Spanish, both having influence on the ethnic background of the Venezuelan population (Rodríguez-Larralde et al., 2001). This mutation was detected in a patient with early-onset of breast cancer (35 years) whose father was affected with the pathology as well. Previous investigations have suggested that male breast cancer is one of the hallmarks for the presence of *BRCA2* mutations (Honrado et al., 2005). The other previously described mutation detected in *BRCA2* (c.6024_6025_delTA), which was identified in a patient who developed breast cancer at an early age (38 years), has bilaterality and a family history of the pathology in three male relatives. Although we were not able to test the male relatives to determine if they were carriers of this mutation, the evidence of the relation of *BRCA2* mutation and male breast cancer (Honrado et al., 2005) indicate that this mutation is responsible for the increased risk of developing breast cancer in this family. One of the novel mutations in *BRCA2*, c.2732_2733insA was found in a bilateral breast cancer patient whose sister was affected with this pathology. The other novel mutation (c.3870_3873delG) was identified in the only male breast cancer patient included in this study. We considered these novel mutations as pathogenic because they generate truncated proteins of 837 and 1227 amino acids, respectively, that affect regions before and inside the conserved BRC repeats in the BRCA2 protein, which is well known to participate in the interaction between BRCA2 and RAD51 (Mitchell, 2002). Therefore, considering the role in the maintenance of genomic integrity of BRCA2 and RAD51, the carriers of these mutations could display a diminished capacity in the signaling and/or repair of certain forms of DNA damage.

It has been established that tumors arising in carries of *BRCA1* and *BRCA2* gene mutations differ morphologically and histopathologically from sporadic breast cancer of age-matched controls (Honrado et al., 2005). Breast cancers in patients with *BRCA1* germline mutations are more often negative for estrogen receptor (ER-), progesterone receptor (PR-), and HER-2 (HER-2-) compared to controls, whereas *BRCA2* tumors do not show a significant difference in the expression of any of these proteins (Lakhani et al., 2002). In agreement with this (Table IV), the tumors from the c.951_952insA, c.1129_1135insA

(*BRCA1*) and c.6024_6025delTA (*BRCA2*) mutations carriers have been shown to be ER-, ER-/PR- and ER-, respectively. On the other hand, the tumor developed in the carrier of the c.3036_3039delACAA *BRCA2* mutation was an invasive ductal carcinoma, which is the most common histological type in all forms of hereditary breast cancer and seems to be significantly more frequent in *BRCA1*- and *BRCA2*-mutation carriers than in non-carriers (Chappuis et al., 2000).

Among the variants of unknown significance indentified in the present study, c.179A>C (p.K20N), c.3238G>A (p.S1040N) and c.3827T>G (p.N1236K) in *BRCA1* and c.1282T>C (p.Y352H), c.3479G>A (p.S1084N) and c.6328C>T (p.R2034C) in *BRCA2* generate biochemically similar changes in amino acids. Therefore these variants are probably not the main cause of the disease in the carrier patients. The novel c.4182_4184delAAT variant found in *BRCA1* was identified in the two previously mentioned patients that were related and also carried the pathogenic mutations c.1129_1135insA. Thus, it is not possible to establish whether the c.4182_4184delAAT variant is also pathological. However, if it is determined that this variant is not in the same chromosome as the c.1129_1135insA mutation, any clinical significance could be discarded, since it is well known that an individual with both *BRCA* alleles mutated is not viable (McCarthy et al., 2003; Reid et al., 2008). This could be established by analyzing the genotype for both mutations in each parent. If each of these variants is identified in a different progenitor, it will mean that both variants are indeed in different *BRCA* alleles in these patients. Other novel variants with unknown significance found in this study are IVS-22C>T in intron 20 of *BRCA1* and c.IVS12-63A>C in intron 12 of *BRCA2*. According to the BIC database, approximately 4% of the genetic variants are reported as splice-site alterations and the knowledge about their effect at the cDNA level is scarce. It is well known that accurate RNA splicing requires that the conserved sequence motifs at the intron–exon junctions and the branch point must be mutation free (Shapiro and Senapathy, 1987). Among these conserved sequences are a highly conserved eight-nucleotide sequence at the exon–intron boundary, the splice donor or 5′ splice site sequence [(A/C) AG//gta/g)agt] and the acceptor or 3′ splice site, preceded by a pyrimidine-rich region (tyttytytyyyyncag//G, where y represents any pyrimidine and n represents any nucleotide). Therefore, the IVS-22C>T mutation could lead to an aberrant transcript since it is located inside the pyrimidine-rich region. Similarly, the c.IVS12-63A>C variant can affect the splicing process by altering important sequences. The variants c.7697T>C (p.I2496T) and c.8237C>T (p.S2670L) in *BRCA2* lay in a region of the gene through which it has been shown that BRCA2 interacts with DSS1 (amino acids 2378 to 3115) (Yang et al., 2002), a protein that stabilizes BRCA2 and regulates its function during DNA repair, acting as a co-factor (Kojic and Holloman, 2004). Both of these mutations change polar for nonpolar amino acids and therefore may affect the interaction between these two proteins. Finally, three novel variants, g.6002C>T in *BRCA1* and c.10594G>T and c.11323T>C in *BRCA2*, were found in the 3′-UTR region. These variants could affect the stability, localization or transportation of the mRNA given the important participation of the 3′-UTR in the fate of this molecule.

In conclusion, the prevalence of mutations of the *BRCA1* and *BRCA2* genes found in this study among patients with breast cancer was 17.2% (10/58), which is similar to what has been reported in other countries for breast cancer families. This study represents the first analysis of *BRCA* disease-associated mutations in Venezuela. The ethnicity of our population, as well as the heterogeneous and broad spectrum of *BRCA* genes mutations, must be considered to optimize genetic counseling and disease prevention in affected families.

ACKNOWLEDGEMENTS

We thank J. Bubis and D. Ajami for their critical reading of the manuscript. This study was supported by a research grant from LOCTI Nº 5662-08

ELECTRONIC-DATABASE INFORMATION

The URLs for data presented in this article are as follows:

BIC database, (http://research.nhgri.nih.gov/bic/) last viewed April, 2011

ChartsBin statistics collector team 2010, *Current Worldwide Breast Cancer Incidence Rate*, ChartsBin.com, (http://globocan.iarc.fr/factsheets/cancers/breast.asp) last viewed September, 2011

REFERENCES

ALBÁNEZ S, RUIZ-SAEZ A, BOADAS A, DE BOSCH N, PORCO A (2011) Identification of factor VIII gene mutations in patients with severe haemophilia A in Venezuela: identification of seven novel mutations. Haemophilia 17: e913–e918

BOWEN DJ, KEENEY S (2003) Unleashing the long-distance PCR for detection of the intron 22 inversion of the factor VIII gene in severe haemophilia A. Thromb Haemost 89: 201-2.

BROSE MS, VOLPE P, PAUL K, STOPFER JE, COLLIGON TA, CALZONE KA, WEBER BL (2004) Characterization of two novel BRCA1 germ-line mutations involving splice donor sites. Genet Test 8: 133-8.

CAPOTE L (2006) Aspectos epidemiológicos del cáncer en Venezuela. Rev venez oncol 18: 269-281.

CHAPPUIS PO, NETHERCOT V, FOULKES WD (2000) Clinico-pathological characteristics of BRCA1- and RCA2-related breast cancer. Semin Surg Oncol 18:287-95.

DEN DUNNEN JT, ANTONARAKIS SE (2001) Nomenclature for the description of human sequence variations. Hum Genet 109: 121-4.

DUFLOTH MR, CARVALHO S, HEINRICH JK, SHINZATO JY, CABELLO DOS SANTOS C, SCHMITT F, ZEFERINO LC (2005) Analysis of *BRCA*1 and *BRCA*2 mutations in Brazilian breast cancer patients with positive family history. Sao Paulo Med J 123:192-197.

FORD D, EASTON DF, STRATTON M, NAROD S, GOLDGAR D, DEVILEE P, BISHOP DT, WEBER B, LENOIR G, CHANG-CLAUDE J, SOBOL H, TEARE MD, STRUEWING J, ARASON A, SCHERNECK S, PETO J, REBBECK TR, TONIN P, NEUHAUSEN S, BARKARDOTTIR R, EYFJORD J, LYNCH H, PONDER BA, GAYTHER SA, ZELADA-HEDMAN M AND THE BREAST CANCER LINKAGE CONSORTIUM (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 62: 676-89.

FRIEDMAN LS, OSTERMEYER EA, SZABO CI, DOWD P, LYNCH ED, ROWELL SE, KING MC (1994) Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nat Genet 8: 399-404.

GALLARDO M, SILVA A, RUBIO L, ALVAREZ C, TORREALBA C, SALINAS M, TAPIA T, FAUNDEZ P, PALMA L, RICCIO ME, PAREDES H, RODRIGUEZ M, CRUZ A, ROUSSEAU C, KING MC, CAMUS M, ALVAREZ M, CARVALLO P (2006) Incidence of BRCA1 and BRCA2 mutations in 54 Chilean families with breast/ovarian cancer, genotype–phenotype correlations. Breast Cancer Res and Treat 95:81-87.

GANGULY A, ROCK MJ, PROCKOP DJ (1993) Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. Proc Natl Acad Sci U S A 90: 10325-9.

GANGULY A, LEAHY K, MARSHALL AM, DHULIPALA R, GODMILOW L, GANGULY T (1997) Genetic testing for breast cancer susceptibility: frequency of BRCA1 and BRCA2 mutations. Genet Test 1:85-90.

GOMES M, COSTA M, BOROJEVIC R, MONTEIRO A, VIEIRA R, KOIFMAN S, JORGE KOIFMAN R, LI S, ROYER R, ZHANG S, NAROD S (2007) Prevalence of BRCA1 and BRCA2 mutations in breast cancer patients from Brazil. Breast Cancer Res Treat 103:349-353.

HONRADO E, BENÍTEZ J, PALACIOS J (2005) The molecular pathology of hereditary breast cancer: genetic testing and therapeutic implications. Mod Pathol 18: 1305-20.

JARA L, AMPUERO S, SANTIBÁÑEZ E, SECCIA L, RODRÍGUEZ J, BUSTAMANTE M, MARTÍNEZ V, CATENACCIO A, LAY-SON G, BLANCO R, REYES JM. (2006) BRCA1 and BRCA2 mutations in a South American population. Cancer Genet Cytogenet 166:36-45.

KING MC, MARKS JH, MANDELL JB (2003) Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 302: 643-6.

KOJIC M, HOLLOMAN WK (2004) BRCA2-RAD51-DSS1 interplay examined from a microbial perspective. Cell Cycle 3: 247-8.

KUPERSTEIN G, FOULKES WD, GHADIRIAN P, HAKIMI J, NAROD SA (2000) A rapid fluorescent multiplexed-PCR analysis (FMPA) for founder mutations in the BRCA1 and BRCA2 genes. Clin Genet 57: 213-20.

LAKHANI SR, VAN DE VIJVER MJ, JACQUEMIER J, ANDERSON TJ, OSIN PP, MCGUFFOG L, EASTON DF (2002) The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 20:2310–2318.

MCCARTHY EE, CELEBI JT, BAER R, LUDWIG T (2003) Loss of Bard1, the heterodimeric partner of the Brca1 tumor suppressor, results in early embryonic lethality and chromosomal instability. Mol Cell Biol 23: 5056-63.

MIKI Y, SWENSEN J, SHATTUCK-EIDENS D, FUTREAL PA, HARSHMAN K, TAVTIGIAN S, LIU Q, COCHRAN C, BENNETT LM, DING W ET AL. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66-71.

MITCHELL A (2002) DNA repair: molecular mimicry. Nat Rev Cancer 2 894: 1-5.

OZCELIK H, NEDELCU R, CHAN VW, SHI XH, MURPHY J, ROSEN B, ANDRULIS IL (1999) Mutation in the coding region of the BRCA1 gene leads to aberrant splicing of the transcript. Hum Mutat 14: 540-1.

Pathology of familial breast cancer: differences between breast cancers in carriers of BRCA1 or BRCA2 mutations and sporadic cases. (1997) Breast Cancer Linkage Consortium. Lancet 349: 1505-10.

REID LJ, SHAKYA R, MODI AP, LOKSHIN M, CHENG JT, JASIN M, BAER R, LUDWIG T (2008) E3 ligase activity of BRCA1 is not essential for mammalian cell viability or homology-directed repair of double-strand DNA breaks. Proc Natl Acad Sci U S A 105: 20876-81.

RODRÍGUEZ-LARRALDE A, CASTRO DE GUERRA D, GONZALEZ-COIRA M, MORALES J (2001) Frecuencia génica y porcentaje de mezcla en diferentes áreas geográficas de Venezuela, de acuerdo a los grupos RH y ABO. Interciencia 26: 8-12.

RUIZ-FLORES P, SINILNIKOVA O, BADZIOCH M, CALDERON -GARCIDUEÑAS AL, CHOPIN S, FABRICE O, GONZÁLEZ-GUERRERO JF, SZABO C, LENOIR G, GOLDGAR D, BARRERA-SALDAÑA H (2002) *BRCA1* and *BRCA2* Mutation Analysis of Early-Onset and Familial Breast Cancer Cases in Mexico. Hum Mutat 20:474-475.

SALGADO J, GIL C, ROBLES M, GUTIÉRREZ C, REYNA C, GARCÍA-FONCILLAS J (2008) A characterization of genetic haplotypes in BRCA1 identifies linkage disequilibrium with novel polymorphism in intron 7. Gene Ther Mol Biol 12: 175-180.

SARKAR G, YOON HS, SOMMER S (1992) Screening for mutations by RNA single-strand conformation polymorphism (rSSCP): comparison with DNA-SSCP. Nucleic Acids Res 20:871-878.

SHAPIRO MB, SENAPATHY P (1987) RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucleic Acids Res 15: 7155-74.

TESORIERO AA, WONG EM, JENKINS MA, HOPPER JL, BROWN MA, CHENEVIX-TRENCH G, SPURDLE AB, SOUTHEY MC (2005) Molecular characterization and cancer risk associated with BRCA1 and BRCA2 splice site variants identified in multiple-case breast cancer families. Hum Mutat 26: 495.

TORRES D, RASHID MU, GIL F, UMANA A, RAMELLI G, ROBLEDO JF, TAWIL M, TORREGROSA L, BRICENO I, HAMANN U. (2007). High proportion of BRCA1/2 founder mutations in Hispanic breast/ovarian cancer families from Colombia. Breast Cancer Res Treat 103:225-232.

WARNER E, FOULKES W, GOODWIN P, MESCHINO W, BLONDAL J, PATERSON C, OZCELIK H, GOSS P, ALLINGHAM-HAWKINS D, HAMEL N, DI PROSPERO L, CONTIGA V, SERRUYA C, KLEIN M, MOSLEHI R, HONEYFORD J, LIEDE A, GLENDON G, BRUNET JS, NAROD S (1999) Prevalence and penetrance of BRCA1 and BRCA2 gene mutations in unselected Ashkenazi Jewish women with breast cancer. J Natl Cancer Inst 91: 1241-7.

WOOSTER R, BIGNELL G, LANCASTER J, SWIFT S, SEAL S, MANGION J, COLLINS N, GREGORY S, GUMBS C, MICKLEM G (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378: 789-92.

WOOSTER R, NEUHAUSEN SL, MANGION J, QUIRK Y, FORD D, COLLINS N, NGUYEN K, SEAL S, TRAN T, AVERILL D ET AL. (1994) Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 265: 2088-90.

YANG H, JEFFREY PD, MILLER J, KINNUCAN E, SUN Y, THOMA NH, ZHENG N, CHEN PL, LEE WH, PAVLETICH NP (2002) BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. Science 297: 1837-48.