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Epidemiology

Incidence of BRCA1 and BRCA2 mutations in 54 Chilean families with breast/ovarian cancer, genotype–phenotype correlations

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Summary

Our aim was to analyze the incidence of mutations in BRCA1 and BRCA2 genes in 54 families with breast/ovarian cancer. Families were selected from three Institutions following the standard criteria for hereditary breast/ovarian cancer. PCR amplification of all exons was performed, followed by SSCP, heteroduplex, PTT and sequencing analysis. We identified eight truncation mutations, three in the BRCA1 gene and five in the BRCA2 gene. Three of these mutations have not been reported previously by other groups: 308insA in one family, 3936 C > T in two families, for BRCA1, and 4970insTG in one family for BRCA2. In addition two families having Ashkenazi Jewish ancestors present the well known mutations 185delAG and 6174delT. Interestingly, 5 out of 11 families have mutations recurrent in Spanish families. Among the 54 families selected, seven have breast and ovary cancer cases, and only two presented a mutation in BRCA1 or BRCA2 genes. Other cancers as prostate and stomach are frequent among relatives carrying the mutation. Five cases of very early onset (< 31 years old) breast cancer were detected. The frequencies of BRCA1 (0.074) and BRCA2 (0.13) mutations in our families is low but similar to the incidence found in other populations, like in Spain. Since is widely known that risk factors that modulate the development of breast cancer such as lifestyle risk factors, geographic location, country of origin and socioeconomic status, besides a familial history of breast cancer our findings suggest that the history of colonization and immigrations is very relevant when studying hereditary factors associated to breast cancer.

Introduction

In Chile, Breast cancer is the third cause of death by cancer among the general population with annual incidence rates of 13 per 100,000 women (National Statistics Institute Chilean Health Ministry, 2001). Two genes, BRCA1 [1] and BRCA2 [2] have been identified for familial breast cancer. Since the identification of these genes more than 1647 germ-line mutations, polymorphisms, and unclassified variants have been reported for BRCA1 and 1406 for BRCA2 in breast and/or ovarian cancer families. The proportion of high-risk families with breast or ovarian cancer attributable to BRCA1 or BRCA2 mutations varies widely among populations [3,4]. To date there are no extensive screening studies reported on the genetic susceptibility of familial breast cancer in Latin American populations, other than Mexico [5] Due to the extended Spanish colonization of Latin America, since the XVI century, we could assume an important genetic influence in families with mutations in these BRCA1 and BRCA2 genes, without dismissing the relevant Amerindian component as well.

In the present study we describe a screening of mutations on the BRCA1 and BRCA2 genes in 54 Chilean families with two or more members affected with breast cancer and/or ovarian cancer.

Methods

Families and patients

Seventy-seven patients from to 54 families with breast or, breast and ovarian cancer were screened for mutations in the BRCA1 and BRCA2 genes. Families were selected by any of the following criteria: three cases of breast cancer in first degree relatives; or two cases of breast cancer in first degree relatives, one diagnosed before age 40; or one breast cancer and one ovarian cancer in first degree relatives. The selected individuals are patients of three Cancer Centers in Santiago, Chile. After informed consent, blood samples were obtained from affected individuals and relatives. DNA was isolated from peripheral blood lymphocytes of each patient, as previously described [6].

Mutation screening

PCR amplification covering coding sequences and intron-exon boundaries, of exons 2 to 24 was performed by standard methods. The coding region of exon 11 for BRCA1, and exons 10 and 11 for BRCA2, were screened through heteroduplex analysis [7] and Protein Truncation Test (PTT) [8] for some families. The remaining exons were analyzed by single strand conformational polymorphism (SSCP). Briefly, PCR products were denatured 95° for 5 min, cool on ice for 5 min, and submitted to electrophoresis on MDE 0.5× (FMC Bioproducts) at 18°, 3W for 13 h. The gels were silver stained after electrophoresis. PCR products or peptides showing an abnormal migration on heteroduplex, SSCP or PTT, were subjected to direct sequencing, using the dsDNA Cycle Sequencing System (Life Technologies), and γ-^{32}P-ATP. Electrophoresis of sequencing gels was carried out onto 6% acrylamide/bisacrylamide (19:1) gels in the presence of 8 M urea. Gels were dried and subjected to autoradiography for 24–48 h.

Results

Seventy seven Chilean women from 54 selected families, with breast, or breast and ovarian cancer (Table 1) were screened for germline mutations in the BRCA1 and BRCA2 genes. Twelve families had only 2–5 breast cancer cases, six families presented breast and ovary cancer cases simultaneously, and the remaining families showed other cancer types being stomach and prostate cancers the most frequent ones (Table 1).

Molecular screening of BRCA1 gene

Molecular screening of the BRCA1 gene revealed the presence of two truncating mutations, c.3936C > T previously reported by our group [9] and one novel mutation c.308insA. The first mutation was found in families 3 and 14, and the second one in Family 5 (Table 2). A third mutation c.185_186delAG was found in Family 19, which has Ashkenazi Jewish ancestry. Family 5 includes mother, daughter, and maternal cousin diagnosed with breast cancer at ages of 68, 43 and 30 respectively. In this family the maternal cousin died at 31 years of age. A frame shift mutation was found in exon 5 consisting of an A insertion at position 308 in the cDNA, which leads into a stop at codon 65 (Table 2). We screened for this mutation in four nonaffected relatives. The mutation was present in three women, aged 38, 28, and 80 suggesting a case of incomplete penetrance in the latter case. The mutation c.3936C > T in Family 14, which shows several cases of cancer, including six cases of breast cancer (at least five detected between 28 and 41 years old), three cases of ovarian cancer, two cases of prostate cancer, and two cases of stomach cancer. This truncating mutation causing a stop at codon 1273, in exon 11, was found through PTT and sequence analysis (Table 2). It is interesting to note that there are three individuals in this family which are obligate carriers of the same mutation and were affected by prostate cancer (2), ovarian cancer (1) and stomach cancer (2). The c.3936 C > T mutation was analyzed in 17 healthy relatives who agreed to be tested. Eight women between 24 and 51 years old were carriers of the mutation, and nine women between 29 and 72 years old were not [9] The same mutation was found in Family 3. This is a small family, presenting two sisters with breast cancer, one bilateral, and two maternal uncles with stomach (age at diagnosis 40 years old) and prostate cancer (50 years old) respectively. The mother of the proband, who is not affected, and is suspected to have been the carrier of the mutation died at 80 years old, before starting this study. Family 19, has Ashkenazi Jews ancestors, and shows one of the most frequent mutations found in this population, c.185_186delAG, detected in the only affected woman who is still alive. The index case has two breast cancer relatives from the maternal side and one from the paternal side.

The screening of all families also revealed the presence of several polymorphisms and allelic variants as shown in Table 3. Two of these polymorphisms have not been previously described, c.560 + 18 to c.561–4206del(CTT)6, and, c.560 + 41 to c.561–4190 delC(T)10,. Interestingly, five polymorphisms: c.560 + 18 to c.561−4206del(CTT)6, c.560 + 41 to c.561−4190delC(T)10, c.660 + 2421 to 661−58del(T)7, c.4956 A > G and c.5106−68 A > G form a haplotype, found in 31 out of 68 heterozygous and homozygous patients. The haplotype frequency in this group of patients is 0.316. This haplotype has also been detected in a frequency of 0.402 in 92 chromosomes from 46 Chilean controls individuals. Due to the important aboriginal admixture, 30%, of the Chilean population [10], we tested the presence of the same haplotype in a sample of 40 Chilean aborigines (80 chromosomes), and found a frequency of 0.275 for the haplotype. No association of this haplotype to breast cancer was found. It is also interesting to note that this haplotype is present in chromosomes with diverse ethnic

Table 1. Ethnic origin of families and type of tumors included in this study

Family	Ethnicity	Type and number of cancer cases per family
1	Jewish/German	Breast (4); bone (1)
2	Chilean	Breast (2); ovary (1)
3	Chilean	Breast (2); breast bilateral (1); cervix (1); prostate (1); stomach (1); brain (1); Osteosarcoma (1)
4	Chilean	Breast (4); breast bilateral (1); stomach (1); bladder (1); internal (1)
5	Chilean	Breast (3)
6	Chilean	Breast (4); internal (1)
7	Chilean	Breast (2); Fibroadenoma (2); liver (1); other (1)
8	Italian	Breast (4); breast bilateral (1); stomach (1); other (1)
9	Chilean	Breast (2)
10	French/German	Breast (2); ovary (1); cervix (1); uterus (1); stomach (1); Oesophagus (1); lung (1); internal (1)
11	Chilean/Italian	Breast (2); breast bilateral (1); ovary (1); stomach (1); Oesophagus (1); testis (1); leukemia (1); sarcoma (1); lymphoma (1)
12	Italian	Breast (3)
13	Chilean	Breast (3); stomach (1)
14	Chilean	Breast (6); ovary (2), uterus (1); prostate (2); stomach (2)
15	Chilean	Breast (2); bladder (1); Oesophagus (1); uterus (1); mieloma (1)
16	Chilean	Breast (2)
17	Chilean	Breast (3)
18	Chilean/German	Breast (4)
19	Russian/Jewish	Breast (4); internal (1)
20	Chilean	Breast (3)
21	Italian/Chilean	Breast (2); stomach (1)
22	Chilean	Breast (3); uterus (1); liver (1)
23	Chilean	Breast (4)
24	Chilean/Spanish	Breast (3), stomach (1), larynx (1), skin(1), leukemia(1)
25	Chilean	Breast (2); stomach (1)
26	Chilean	Breast (4); testis (1); Oesophagus (1); lung (1)
27	Spanish	Breast (6); breast bilateral (1); prostate (4); colon (2); stomach (1)
28	French/Spanish	Breast (5); ovary (1); colon (3)
29	Chilean	Breast (4); breast bilateral (1);
30	Chilean/Spanish	Breast (4); Oesophagus (1)
31	Chilean/French	Breast (3); lymph node (1)
32	Chilean/Spanish	Breast (3); other (1)
33	Chilean	Breast bilateral (1)
34	Croatian/Spanish	Breast (2)
35	Chilean/Spanish	Breast (6); breast bilateral (1); lung (1); brain (1)
36	Chilean/Spanish	Breast (3); prostate (1); pancreas (1); stomach (1)
38	Italian	Breast (3)
39	Chilean/Spanish	Breast (2); prostate (1)
40	Chilean	Breast (5)
41	Chilean	Breast (2)
42	Chilean	Breast bilateral (1); ovary (1)
43	Chilean	Breast (1); stomach (1); ovary (1)
44	Chilean	Breast (4); other (1)
45	Chilean	Breast (3); uterus (1); stomach (1); other (1)
46	Chilean/Danish	Breast (6); stomach (1); other (1)
47	Chilean	Breast (2); internal (1)
48	Arabian/Spanish	Breast (3); one in man
49	German	Breast (2); cevix (1); pancreas (1); skin (1)
50	Spanish	Breast (2); breast bilateral (1); pancreas (2)
51	Chilean/Peruvian	Breast (5); breast bilateral (1); Oesophagus (1)
52	Chilean/Italian	Breast (3); testis (2)
53	Chilean	Breast (2); uterus (1); stomach (1)
54	Chilean	Breast (2); uterus (1); colon (1); lung (2)

Table 2. BRCA1 germline mutations in Chilean breast/ovarian cancer families

Family	Exon	Mutation	Change (codon)	Reference
3	11	c.3936 C > T	Stop at codon 1273	BIC no. 5152
5	5	c.308_309 ins A	Stop at codon 65	*Novel*
14	11	c.3936 C > T	Stop at codon 1273	BIC no. 5152
19	2	c.185_186 del AG	Stop at codon 39	Struewing et al. (1995)

Table 3. BRCA1 polymorphisms and variants in Chilean breast/ovarian cancer

Exon(E)/intron(I)	Sequence variant or alteration	Predicted effect	Allelic frequency	Reference
I7	c.560 + 18 to c.561−4221 del CTT	Unknown	0.316	*Novel*
I7	c.560 + 41 del C	Unknown	0.316	*Novel*
I7	c.560 + 42 to c.561−4190 del (T)10	Unknown	0.316	*Novel*
I8	c. 661−58 del T	Unknown	0.316	BIC no. 4687
E11	c.1186A > G	p.Q 356 R	0.007	BIC no. 2558
E11	c.3232 A > G	p.E 1038 G	0.0147	BIC no. 1087
E11	c.3238 G > A	p.S 1040 N	0.0147	BIC no. 1089
I16	c.5106−68 A > G	Unknown	0.316	BIC no. 4770
E16	c.4956 A > G	p.S 1613 G	0.316	BIC no. 1140
E11	c.3667 A > G	p.K 1183 N	0.0147	BIC no. 1099
I18	c.5271 + 66 G > A	Unknown	0.007	BIC no. 2896
E16	c.5075 G > A	p. M1652 I	0.007	BIC no. 1143

origins (Amerindians, Ashkenazim and Europeans) suggesting a very old ancestry.

Molecular screening of BRCA2 gene

Molecular screening of BRCA2 revealed the presence of 5 mutations (Table 4). Family 21 presented a novel frame shift mutation c.4970insTG, leading into a stop at codon 1617. From the two women affected with breast in this family, one died at 50 years old, and her mother (diagnosed at 31 years old) was dead at age of 34, before starting this study. We identified three mutations already described for Spanish families: E49X [11] (Families 2 and 16), c.6857_6858delAA [12,13] (Family 27), and c.5373_5376delGTAT [11] (Families 24 and 25) (Table 4). In Family 2 the proband diagnosed at 35 years old of breast cancer, has a maternal aunt with ovarian cancer (diagnosed at 59, dead at 60 years old). In this case the mother of the proband, healthy at 70 years old is an obligated carrier of the mutation. Family 16, presenting the same mutation, has two affected women, the mother diagnosed at 55 years old and her daughter diagnosed at age of 35. There is no information of second degree relatives. In Family 27 the father, dead from prostate cancer, was the obligated carrier of the mutation. From the 11 siblings, 5 out of 6 women presented breast cancer (three died at ages of 43, 39 and 49), and 3 out of 5 men had prostate cancer (one dead at age of 61). This mutation has been associated to male breast cancer in Spanish families. In our family we tested three alive men for this mutation, two affected by prostate cancer (56 and 60 years old) and one not affected. Two of them were carriers of the c.6174delT, and one had prostate cancer. On the other hand one sibling affected by prostate cancer did not have the mutation. In this family we could not find a correlation of this mutation with prostate cancer, nor with breast cancer in men. Family 24 presents three cases of breast cancer, the proband diagnosed at 28 years old and dead at age of 33, her mother diagnosed at age of 45 and a maternal cousin diagnosed at 51, both alive. In Family 25 there are two cases of breast cancer, the proband with bilat-

Table 4. BRCA2 germline mutations in Chilean breast/ovarian cancer families

Family	Exon	Mutation	Change (codon)	Reference
1	11G	c.6174 del T	Stop at codon 2003	BIC no. 1042
27	11H	c.6857_6858 del AA	Stop at codon 2223	BIC no. 1152
21	11E	c.4970_4971 ins TG	Stop at codon 1617	*Novel*
24	11F	c.5373_5376 del GTAT	Stop at codon 1724	[11]
25	11F	c.5373_5376 del GTAT	Stop at codon 1724	[11]
2	3	c.373 G > T	Stop at codon 49	[11]
16	3	c.373 G > T	Stop at codon 49	[11]

eral breast cancer (52 and 54 years old) and her daughter diagnosed at 30 years old and dead at age of 32. In Family 1, an Ashkenazi Jewish, we detected the c.6174delT mutation (Table 4).

The screening revealed also the presence of several polymorphisms and allelic variants of BRCA2 as shown in Table 5. Two novel polymorphisms were found c.203insA in the 5′UTR in exon 2, and c.544 + 82_c.544 + 83 ins TTA, in intron 3. These sequence changes have been found in more than 1% of a sample from the control population from Chile. The other polymorphisms found, c.203G > A, c.1093A > C, c.3199A > G, c.7069 + 80_c.7070−3279delTTAA, and c.8034−14 T > C, have been described previously in BIC.

Discussion

Fifty four breast cancer families were screened for BRCA1 and BRCA2 mutations in Chile. In most of these families we obtained reliable information about causes of death for two generations. It is interesting to mention that ethnically the Chilean population is considered to be an admixture of 30% Amerindian origin and 70% European, mostly Spanish origin based on autosomal genetic markers [10]. In relation to mitochondrial DNA haplotypes, the Chilean population reveal a frequency of 0.84 on Amerindian matrilineal ancestors besides 0.16 of European [14,15], which is concordant with the history of colonization. All the families selected in this study lived in Chile for three or more generations, and 27 out of 53 had at least two surnames from Spanish origin (Table 1). Other families with different European origins were also included in this study and classified by the ethnic ancestry from the family branch carrying breast or ovarian cancer (Table 1). The determination of mitochondrial DNA haplotypes in the selected group of families showed a significant difference in the frequency of Amerindian matrilineal component (0.69) besides the general population (0.84) ($p = 0.0000428$).

In this study we were able to identify 11 out of 54 families with BRCA1 or BRCA2 mutations. For BRCA1 three families (3, 5 and 19) had only breast cancer cases and one family (14) presented 6 breast and 2 ovarian cancer cases. Two truncating mutations not previously published were found, c.308insA and c.3936 C > T. The c.3936 C > T mutation has been reported twice in BIC, but there is no information on the ethnic origin or clinical characteristics of the patients. Family 14 carrying the c.3936 C > T mutation present other types of cancer such as prostate and stomach cancer, being obligated carriers of the BRCA1 mutation, strongly suggesting the involvement of the BRCA1 gene in other cancer types. It is interesting to note that these two types of cancer are the most frequent in our group of families. Family 3 sharing the same mutation also present two maternal uncles with stomach (age 40) and prostate (age 50) cancers. The c.308insA mutation in Family 5 is only associated to breast cancer cases with no history of other cancer types among the relatives of this family. These two mutations at BRCA1 gene seem to affect the three families in a very different manner. The c.308insA mutation creates a stop at codon 65, and the 3936 mutation creates a stop at codon 1273. The latter mutation seems to be more deleterious in the number and variability of tumors. In this sense, Risch et al [16] have described an increased breast cancer risk, for first degree relatives, when mutations are located in the 3′ fifth portion of the BRCA1 gene and Hohenstein and Fodde [17] reviewing information from other authors and their own experience, conclude that mutations in the middle portion of BRCA1 (nt 2401–4191) result in higher incidence of ovarian versus breast cancer, and mutations at the 5′ or 3′ ends of BRCA1 may result in unstable proteins with an attenuated effect. Perhaps the truncated protein formed from the c.3936C > T BRCA1 gene is somehow associated with other types of cancer in addition to breast and ovarian tumors. A functional analysis of these mutants could corroborate some of these hypotheses.

The BRCA2 mutation c.6857_6558delAA is associated with breast cancer in men, and ovary cancer for the Spanish families, but no association with these types of cancers has been found in Family 27 sharing the same mutation. Besides none of the Chilean families presenting mutations in BRCA2 gene have breast cancer cases in men.

We performed a haplotype analysis with 5 STR loci close to the BRCA2 gene, in the Spanish and the Chilean Family 27, and found a strong evidence of common ancestry [18]. The BRCA2 mutation c.5373_5376delGTAT, described in one Spanish family [11], is present in two Chilean families, Family 24 and Family 25. In both

Table 5. BRCA2 polymorphisms and rare variants in Chilean breast/ovarian cancer families

Exon (E)/intron (I)	Sequence variant or alteration	Predicted effect	Allelic frequency	Reference
E2	c.203 G > A	Unknown	0.013	BIC no. 1797
E2	c.203 ins A	Unknown	0.006	*Novel*
I3	c.544 + 82_c.544 + 83 ins TTA	Unknown	0.013	*Novel*
E10	c.1093 A > C	p.N 289 H	0.039	BIC no. 1129
E11	c.3199 A > G	p.N 991 D	0.039	BIC no. 1903
I11	c.7069 + 80_c.7070–3279 del TTAA	Unknown	0.104	BIC no. 2022
I16	c.8034−14 T > C	Unknown	0.006	BIC no. 2029

Chilean families cancer was detected at a very early age 28 and 30 years old, and both patients died 2 and 5 years, after diagnosis, respectively. The Spanish family presenting the same mutation, shows three cancer cases diagnosed at 40, 46 and 39 years old. The age of onset in the Spanish family is significantly different to the early ages at onset (28 and 30 years old) in the two Chilean families. In Families 27, 24 and 25, we found different expression of the mutations compared to the Spanish families [11–13], in relation to the type of cancer (c.6857_6558delAA, family 27) and the age of onset (c.5373_5376delGTAT, families 24, 25). These differences are in agreement with the belief that nongenetic factors as environment, lifestyle, and diet are directly implicated not only to the expression of cancer, but to the types of tissue affected, age at onset and degree of severity of cancer. Another important aspect is that only 2 out of 11 families with a mutation in BRCA1 or BRCA2 genes have an ovarian cancer, Family 14 and Family 2 (BRCA1 and BRCA2 respectively). This findings means that we did not come across with the higher incidence of ovarian cancers in families with mutations in BRCA1 as it has been described.

In this study we found five women diagnosed with breast cancer at very early ages, between 28 and 31 years of age, and two cases of incomplete penetrance. Prostate is the most frequent type of cancer related to mutation carriers, and stomach is the most frequent between relatives of the proband. Thompson and Easton [19] have described a high risk of developing prostate cancer in men carrying a mutation in BRCA1. In relation to BRCA2, the Breast Cancer Linkage Consortium [20] determined a relative risk of 4.6, and a cumulative risk of 20% for prostate cancer in men carrying a mutation. In relation to stomach cancer, a study from Jakubowska et al. [21] confirms that BRCA2 gene mutations, in families with breast cancer, are also associated with a higher than expected incidence of stomach cancer.

The low frequency of BRCA1 (7.4%) and BRCA2 (12.9%) mutations in the Chilean breast/ovarian cancer families correlates with three different studies made in Spanish families [22–25]. In two of these studies only the BRCA1 gene was screened, finding that 10 and 15% of families, respectively, have mutations. Osorio et al. [24] screened a total of 32 families, including 5 families with male breast cancer, and found a higher proportion of BRCA2 mutations (15.6%) than BRCA1 mutations (9.3%). It has been already pointed out [3,4] that the proportion of high-risk families having mutations in the BRCA1 and BRCA2 genes varies with the ethnicity of the population analyzed. Our results are in agreement with the history of colonization during the XVI and XVII centuries. The similarity in the mutation frequencies in both genes, with that reported for Spanish families, and the high percentage (45.4%) of Chilean families with Spanish mutations, found in our study is in part in agreement with the composition Chilean population, which is an admixture of Amerindian and European, mainly Spanish individuals. Interestingly the group of families selected in this study showed a significant low frequency of Amerindian mitochondrial DNA haplotypes. This finding is in agreement with a recent report by Joslyn et al. [26] describing a lower frequency of breast cancer in Hispanic versus non-Hispanic women in the US. Our results also show a high frequency (7 out of 11 families) of BRCA1/2 mutations of known Spanish or Ashkenazi Jewish origin, revealing a relevant non-Amerindian genetic component in familial breast cancer in relation to these genes.

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References

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, Sholnick MH: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66–71, 1994
2. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G, Barfoot R, Hamoudi R, Patel S, Rice C, Biggs P, Hashim Y, Smith A, Connor F, Arason A, Gudmundsson J, Ficenec D, Keisell D, Ford D, Tonin P, Biship DT, Spurr NK, Ponder BAJ, Eeles R, Peto J, Devilee P, Cornelisse C, Lynch H, Narod S, Lenoir G, Egilsson V, Barkadottir RB, Easton DF, Bentley DR, Futreal PA, Ashworth A, Stratton MR: Identification of the breast cancer susceptibility gene BRCA2. Nature 378: 789–792, 1995
3. Szabo CI, King M-C: Population genetics of BRCA1 and BRCA2. Am J Hum Genet 60: 1013–1020, 1997
4. Liede A, Narod SA: Hereditary breast and ovarian cancer in Asia: genetic epidemiology of BRCA1 and BRCA2. Hum Mutat 20: 413–424, 2002
5. Ruiz-Flores P, Sinilnikova OM, Badzioch M, Calderón-Garcidueñas AL, Chopin S, Fabrice JF, González-Guerrero JF, Szabo C, Lenoir G, Goldgar D, Barrera-Saldaña H: BRCA1 and BRCA2 mutation analysis of early-onset and familial breast cancer cases in Mexico. Hum Mutat 20: 474–475, 2002
6. Lahiri DK, Nurnberger J: A rapid non-enzymatic method for the preparation of the HMW DNA from blood for RFLP studies. Nucl Ac Res 19: 5444, 1991
7. Friedman LS, Ostermeyer EA, Szabo CI, Dowd P, Lynch ED, Rowell SE, King M-C: Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nat Genet 8: 399–404, 1994
8. Johannsson O, Ostermeyer EA, Hakansson S, Friedman LS, Johansson U, Sellberg G, Bronndum-Nielsen K, Sele V, Olsson H, King M-C, Borg A: Founding BRCA1 mutations in hereditary breast and ovarian cancer in Southern Sweden. Am J Hum Genet 58: 441–450, 1996
9. Gallardo M, Faúndez P, Cruz A, Rodriguez M, Alvarez M, Carvallo P: Screening for a BRCA1 mutation in a Chilean family with breast cancer. Rev Med Chile 132: 203–210, 2004

10. Cifuentes L, Valenzuela C, Cruz-Coke R, Armanet L, Lyng C, Harb Z: Genetic characterization of the hospital population of Santiago, Chile. Rev Med Chile 116: 28–33, 1988
11. Llort G, Munoz CY, Tuser MP, Guillermo IB, Lluch JR, Bale AE, Franco MA: Low frequency of recurrent BRCA1 and BRCA2 mutations in Spain. Hum Mutat 19: 307–313, 2002
12. Diez O, del Rio E, Domenech M, Sanz J, Cortes J, Brunet J, Alonso Mdel C, Baiget M: The identification of a new mutation in the BRCA2 gene by protein truncation analysis in a Spanish family with hereditary breast cancer. Med Clin (Barc) 112: 179–181, 1999
13. Diez O, Cortes J, Domenech M, Pericay C, Brunet J, Alonso C, Baiget M: BRCA2 germ-line mutations in Spanish male breast cancer patients. Ann Oncol 11: 81–84, 2000
14. Moraga M, Rocco P, Miquel JF, Nervi F, Llop E, Rothhammer F, Carvallo P: Mitochondrial DNA polymorphisms in southern Chilean aboriginal populations. Am J Phys Anthropol 113: 19–29, 2000
15. Rocco P, Morales C, Moraga M, Miquel JF, Nervi F, Llop E, Carvallo P, Rothhammer F: Genetic composition of the Chilean population. Analysis of mitochondrial DNA polymorphism. Rev Med Chile 130: 125–131, 2002
16. Risch H, McLaughlin J, Cole D, Rosen B, Bradley L, Kwan E, Jack E, Vesprini D, Kuperstein G, Abrahamson J, Fan I, Wong B, Narod S: Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 68: 700–710, 2001
17. Hohenstein P, Fodde R: Of mice and (wo)men: genotype–phenotype correlations in BRCA1. Hum Mol Genet 12 Spec No 2: R271–R277, 2003
18. Campos B, Díez O, Alvarez C, Palma L, Doménech M, Balmaña J, Sanz J, Ramirez A, Alonso C, Carvallo P, Baiget M: Haplotype of the BRCA2 6857delAA mutation in 4 families with breast/ovarian cancer. Med Clin (Barc) 123: 543–545, 2004
19. Thompson D, Easton D: Cancer incidence in BRCA1 mutation carriers. J Natl Cancer Inst 94: 1358–1365, 2002
20. The Breast Cancer Linkage Consortium, Cancer risks in BRCA2 mutation carriersJ Natl Cancer Inst 91: 1310–1316, 1999
21. Jakubowska A, Nej K, Huzarski T, Scott RJ, Lubinski J: BRCA2 gene mutations in families with aggregations of breast and Stomach cancers. Br J Cancer 87: 888–889, 2002
22. Diez O, Cortes J, Domenech M, Brunet J, Del Río E, Pericay C, Sanz J, Alonso C, Baiget M: BRCA1 mutation analysis in 83 Spanish in breast/ovarian cancer families. Int J Cancer 83: 465–469, 1999
23. Blesa JR, Garcia JA, Ochoa E: Frequency of germline BRCA1 mutations among Spanish families, from a Mediterranean area. Hum Mutat 15: 381–382, 2000
24. Osorio A, Barroso A, Martinez B, Cebrian A, San Roman JM, Lobo F, Robledo M, Benitez J: Molecular analysis of the BRCA1 and BRCA2 genes in 32 breast and/or ovarian cancer Spanish families. Br J Cancer 82: 1266–1270, 2000
25. Díez O, Osorio A, Durán M, Martinez-Ferrandis JI, de la Hoya M, Salazar R, Vega A, Campos B, Rodríguez-López R, Velasco E, Chaves J, Díaz-Rubio E, Cruz JJ, Torres M, Esteban E, Cervantes A, Alonso C, San Román JM, González-Sarmiento R, Miner C, Carracedo A, Armengod ME, Caldés T, Benítez J, Baiger M: Analysis of BRCA1 and BRCA2 genes in Spanish breast/ovarian cancer patiens: a high proportion of mutations unique to Spain and evidence of founder effects. Hum Mutat 22: 301–312, 2003
26. Joslyn SA, Foote ML, Nasseri K, Coughlin SS, Howe HL: Racial and ethnic disparities in breast cancer rates by age: NAACCR Breast Cancer Project. Breast Cancer Res Treat 92: 97–105, 2005

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