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Short Communication

BRCA1 mutations in Brazilian patients

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Abstract

BRCA1 mutations are known to be responsible for the majority of hereditary breast and ovarian cancers in women with early onset and a family history of the disease. In this paper we present a mutational survey conducted in 47 Brazilian patients with breast/ovarian cancer, selected based on age at diagnosis, family history, tumor laterality, and presence of breast cancer in male patients. All 22 coding exons and intron-exon junctions were sequenced. Constitutional mutations were found in seven families, consisting of one insertion (insC5382) in exon 20 (four patients), one four base-pair deletion (3450-3453delCAAG) in exon 11 resulting in a premature stop codon (one patient), one transition (IVS17+2T > C) in intron 17 affecting a mRNA splicing site (one patient), and a C > T transition resulting in a stop-codon (Q1135X) in exon 11 (one patient). The identification of these mutations which are associated to hereditary breast and ovarian cancers will contribute to the characterization of the mutational spectrum of *BRCA1* and to the improvement of genetic counseling for familial breast/ovarian cancer patients in Brazil.

Key words: BRCA1, breast cancer, ovarian cancer.

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The Breast Cancer Linkage Consortium dataset indicates that the proportion of familial breast and ovarian cancers associated to *BRCA1* or *BRCA2* may be as high as 98% (Martin *et al.*, 2001). Mutations in *BRCA1* account for 45% of families with multiple cases of breast cancer and for at least 80% of families with early-onset breast and/or ovarian cancer (Miki *et al.*, 1994). Moreover, 88% of families with at least four cases of early-onset breast cancer and one case of ovarian cancer are related to mutations in *BRCA1* and *BRCA2* (Unger *et al.*, 2000). Furthermore, germ line *BRCA1* or *BRCA2* mutation carriers also have an increased risk of developing other cancers in prostate, stomach, colon, pancreas, fallopian tubes and uterus when compared to the general population (Offit, 1998; Brose *et al.*, 2002). However, the alternative possibility of mutations in other, still unidentified susceptibility genes with low penetrance or chance clustering in families with multiple cases of breast/ovarian cancer without mutations in *BRCA1* or *BRCA2* remains to be determined.

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More than 800 different mutations were reported in *BRCA1*, and most of them are listed in the Breast Cancer Information Core (BIC - http://research.nhgri.nih.gov/bic/). Mutation carriers have an increased risk of developing breast cancer at different ages, with estimates of approximately 37% up to 40 years, 66% up to 55, 73% up to 70, and 82% over their entire lifetime (Hall *et al.*, 1990; Brose *et al.*, 2002). As for ovarian cancer, the risk was estimated to be 29% up to 50 years and 40% up to 70 years (Ford *et al.*, 1998; Kasprazak *et al.*, 1999; Brose *et al.*, 2002).

Mutation detection and estimates of prevalence, however, are restricted to North America and Europe (Los Rios *et al.*, 2001, Loman *et al.*, 2001, Brose *et al.*, 2002), while data from other populations are scarce. For instance, most studies carried out in individuals of African origin are restricted to African Americans (Olopade *et al.* 2003; Kinney *et al.*, 2001; Shen *et al.*, 2000; Gao *et al.*, 2000). Altogether, studies in Latin American populations are so far restricted to a few reports: (i) mutational data and description of rare

variants of unknown significance in *BRCA1* and *BRCA2* in Mexico (Ruiz-Flores *et al.*, 2002); (ii) a study of an Uruguayan population describing six different mutations, five in *BRCA2* and one in *BRCA1* (Delgado *et al.*, 2003); (iii) a study of 64 Brazilian patients (Simon *et al.*, 2003) with a family history of breast and/or ovarian cancer, with 17 detected variants including the classical Askhenazi Jewish mutations (185-186delAG and 5382insC in *BRCA1*, and 6174delT in *BRCA2*); (iv) another Brazilian study describing a nonsense mutation and a missense mutation of unknown value (Corvello *et al.*, 1998); and (v) two Chilean studies (Trincado *et al.*, 1999; Jara *et al.,* 2002) testing women (with a family history of breast cancer) for the mutations 185-186delAG, 5382incC and 6174delT (in *BRCA2*), which did not find these mutations in the sampled individuals.

In Brazil, mutational surveys of *BRCA1/BRCA2* are needed for determining which mutations are relevant for the prevalence of hereditary breast/ovarian cancer, in view of the multi-ethnic composition of the population (Alves-Silva *et al.*, 2000, Carvalho-Silva *et al.*, 2001). The investigation of this multi-ethnic population might lead to the discovery of new pathologic variants, resulting in a different mutational profile from those of European and North American populations.

Here we present the results of a mutational survey carried out in 47 Brazilian patients with breast/ovarian cancer, selected according to several inclusion criteria for high-risk inherited susceptibility. This survey, restricted to *BRCA1*, was based on DNA sequencing of coding and intron/exon flanking regions.

Patients

Forty-seven unrelated patients were selected according to criteria based on a number of previous reports (Friedman *et al.*, 1994; Langston *et al.*, 1996; Hamann *et al.*, 1997; Ford *et al.*, 1998), listed in Table 1. All selected patients and their tested relatives signed informed consent forms guaranteeing privacy. Five milliliters of peripheral blood were collected for genomic DNA isolation, as described by Miller *et al.* (1988). All patients were unrelated. The mean age for first breast tumor diagnosis was 46 years, ranging from 23 to 76, and for the second breast tumor it was 47.1 years, ranging from 38 to 61. For ovarian cancer, the mean age of diagnosis was 36 years, ranging from 20 to 43. Two patients were of Ashkenazi Jewish origin, four patients were of European origin, one of them with a Ukrainian and an Italian parent, two patients of Italian origin (one of them being Italian), and one patient with a German and a Portuguese parent. The other 38 patients were from the local Brazilian population of multi-ethnic origin.

Exon amplification and DNA sequencing

The 22 *BRCA1* coding exons and flanking intron-exon regions were PCR-amplified with 27 primer pairs.

Table 1 - Number of patients included in each selection criterion. A total of 47 unrelated patients were selected, with some of them fitting more than one criterion[1].

Criterion of inclusion	Patients
I. Breast cancer patient with family history of breast cancer and one or more cases of ovarian cancer at any age	2
II. Breast cancer patient diagnosed before age 50, with family having more than three cases of breast cancer diagnosed before age 50	8
III. Breast cancer patient and a first-degree relative with breast and/or ovarian cancer diagnosed before age 50	27
IV. Male patient with breast cancer diagnosed at any age	4
V. Patient with bilateral breast or ovarian cancer diagnosed before age 50	13
VI. Ashkenazi Jewish patient with breast or ovarian cancer at any age	2

[1]Some patients fitted more than one criterion: one patient fitted I and V; one fitted I, II and III; one 5382insC mutation carrier fitted III and V; two patients fitted II and III; and two carriers of different mutations (5382insC and 3450-3453delCAAG) fitted II, III, and V.

Most primer sequences were described by Miki *et al.*, (1994). In view of its large size, exon 11 was amplified in seven overlapping fragments (named 11A to 11G), using the following primer pairs:

11A-f GGAATTAAATGAAAGAGTATGAGC/11A-r TGTGAGGGGACGCTCTTG; 11B-f TTGGGAAAACCTATCGGAA/ 11B-r ATCTTTGGGGTCTTCAGCA; 11C-f GTGTTCAAATACCAGTGAACTTA/ 11C-r GGAGCCCACTTCATTAGTAC; 11D-f CCAAGTACAGTGAGCACAATTA/ 11D-r GTGCTCCCAAAAGCATAAA; 11E-f CAGGAAATGCAGAAGAGGAATGTG/ 11E-r GAGCCCACTTCATTAGTAC; 11F-f CCAAGTACAGTGAGCACAATTA/ 11F-r GTGTTGGAAGCAGGGAAGCTCTTC; and 11G-f GAAGAGCTTCCCTGCTTCCAACAC/ 11G-r GTGCTCCCAAAAGCAT AAA (f = forward primer; r = reverse primer). PCR amplifications were carried out in a final volume of 50 µL containing: 50 mM Tris pH 9.0, 1.5 mM $MgCl_2$, 40 mM KCl, 250 µM of each dNTP, 50 pmol of each primer and 1 U of *Taq* DNA polymerase and 100-200 ng of genomic DNA. Amplification cycles were: 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, except for exon 2 and 20 (94 °C for 4 min followed by 35 cycles of 94 °C for 45 s, 60 °C for 30 s, and 72 °C for 30 s), exon 8 (94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, 47 °C for 30 s, and 72 °C for 30 s), and the fragment 11G of exon 11 (94 °C for 5 min, followed by 40 cycles of 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 1 min). Amplification products were purified with GFX^{TM}PCR DNA and Gel Band Purification Kit (Amersham Biosciences) or Wizard PCR Preps DNA Purification System Kit (Promega), according to the manufacturer's guidelines. Subsequently, purified PCR products were sequenced in both forward and reverse directions, using the DYEnamyc^{TM} ET Terminator

Cycle Sequencing Premix Kit or the DYEnamic ET Dye Terminator Cycle Sequencing Kit for Megabace (both from Amersham Biosciences). Sequencing reactions were run in an ABI Prism 377 DNA Sequencer (Applied Biosystems) or in a MegaBACE 1000 DNA Sequencer (Amersham Biosciences). Sequence analyses were performed with the software Sequence Analysis version 3.3 (for ABI-Prism 377) or Sequence Analysis version 2.1 (for Megabace 1000). All mutations were observed in both strands and confirmed by another PCR reaction with material from the same patient.

Twenty seven patients (48%) fulfilled criterion III (proband with one first-degree relative with breast and/or ovarian cancer diagnosed before age 50), 13 patients (23%), criterion V (proband with bilateral breast or ovarian cancer diagnosed before age 50), eight patients (14%), criterion II, four patients (7%), criterion IV (one of them with two relatives with breast cancer, and another with a relative with bilateral breast cancer diagnosed at 36 and 50 years, respectively), two Ashkenazi Jewish patients (4%) followed criterion VI, and two other patients (4%), criterion I. Some patients fitted more than one criterion: one patient met criteria I and V; one, criteria I, II and III; one mutation carrier of 5382insC fulfilled criteria III and V; two patients, criteria II and III; and two carriers of different mutations (5382insC and 3450-3453delCAAG) fitted criteria II, III, and V (Table 1).

The average age at diagnosis was 41.6 years, ranging from 25 to 48 for first tumor diagnosis. Only one patient with bilateral tumors had the second tumor diagnosed after age 50, while the other two patients with bilateral tumors had their first tumor diagnosed at 33 and 47 years, and the second tumor at 38 and 50 years, respectively.

Tumor-associated mutations were found in seven (15%) of the 47 patients (Table 2). They comprised: (i) an identical insertion (5382insC) in exon 20 in four patients; (ii) a four base-pair deletion (3450-3453delCAAG) in exon 11 resulting in an early stop codon in one patient; (iii) a transition (IVS17+2T > C) in intron 17 affecting a mRNA splicing site in one patient; and (iv) a C > T transition (Q1135X) in exon 11 resulting in a stop-codon in another patient. All these mutations were present in heterozygosis and, to our knowledge, the carriers came from unrelated families.

The frequency of *BRCA1* mutations detected in our sample (15%) was similar to those reported previously using similar inclusion criteria and mutation detection methodologies (Frank *et al.*, 1998; Durocher *et al.* 1996). However, in studies using more stringent inclusion criteria for a hereditary profile (like families with three cases of breast cancer at a young age or two cases of breast cancer and one case of ovarian cancer, both diagnosed at a young age), mutations were detected in 40% of patients (Simard *et al*., 1994). Conversely, the proportion of detected tumor-associated mutations was lower (12.8%) with less stringent criteria (Shattuck-Eidens *et al.*, 1997).

Despite being the best methodological procedures for mutation detection, PCR amplification and DNA sequencing failed to detect an important proportion of mutations (around 10-15%) like deletions, duplications and inversions encompassing large genome segments (Rohlfs *et al.*, 2000; Unger *et al.* 2000, Brown *et al.*, 2002, Montagna *et al.*, 2003). Therefore, patients with a strong familiar history but without detectable mutations in coding exons or intron-exon junction should not be excluded as potential mutation carriers. Detection must be based on other methodologies, like Southern blotting or long-distance PCR assays.

The relevance of a mutation can be evaluated by its frequency and penetrance in different human populations. Four of our patients, with bilateral ovarian or breast cancer (Table 1), carried a 5382insC, first described by Simard *et al.* (1994) and found to be very frequent in Ashkenazi Jews (0.13%; see Abeliovich *et al.*, 1997), East-European populations (Szabo and King, 1997), and nearly 10% of patients with *BRCA1* mutations (Dong *et al.*, 1998). Three of the four patients carrying this insertion in our sample have at least one European parent (Ukrainian/Italian, Italian, and Portuguese/German); the fourth patient did not provide any information on her ancestry. The fact that 5382insC occurred in unrelated patients suggests that larger-scale studies are necessary to evaluate the relevance of this mutation in Brazilian populations.

Table 2 - Mutations found in seven unrelated patients, inclusion criteria, type of cancer(s) (age at diagnosis), and number of previous reports at BIC (Breast Cancer Information Core).

Mutation	Inclusion criteria[1]	Type of mutation	Proband cancer (age at diagnosis)	Number of previous teports at BIC
5382 ins C	III and V	Frameshift	Bilateral Ovarian Cancer (45)	536
5382 ins C	II, III and V	Frameshift	Bilateral Breast Cancer (47 / 50)	536
5382 ins C	V	Frameshift	Bilateral Breast Cancer (47)	536
5382 ins C	V	Frameshift	Bilateral Breast Cancer (33 / 38)	536
Q1135X	III	Nonsense	Breast Cancer (46)	1
IVS17+2 T > G	III	Splicing site	Breast Cancer (25)	1
3450-3453delCAAG	II, III and V	Frameshift	Bilateral Breast Cancer (48 / 55)	24

[1]Some patients fitted more than one criterion.

Although 5382insC is the most frequently reported mutation in familial breast cancer (Foulkes *et al.*, 1999), we found it in two individuals with bilateral ovarian cancer: (i) a proband with a family history including one sister with breast cancer diagnosed at age 30 and her grandmother with a unilateral ovarian cancer diagnosed at age 60; and (ii) a woman with ovarian cancer diagnosed at the age of 39 years, who was related to a proband with bilateral breast cancer diagnosed at the age of 36/41 years, and with a strong family history of two other bilateral breast cancers (diagnosed at age 47/50 and 45/50, respectively,) and one unilateral breast cancer (diagnosed at age 50).

The four base-pair deletion (3450-3452delCAAG) in exon 11 was detected in a patient with bilateral breast cancer (diagnosed at age 48 and 55, respectively) and with the most impressive familial cancer history of all patients (5 cases of breast cancer diagnosed at ages 61, 46, 43, 42 and 36, respectively, and one case of ovarian cancer diagnosed at age 44). This deletion was first described by Durocher *et al.* (1996) in a patient diagnosed with breast cancer at 28 years and with ovarian cancer at 34 and with a strong family history (three breast cancers diagnosed at ages 26, 29 and 40 in different relatives). As this deletion was found in different ethnic groups like African Americans (Panguluri *et al.*, 1999) and West Europeans (Moller *et al.*, 2002), it is likely to be of ancient origin, although haplotype analysis might be required to confirm this hypothesis.

The two other mutations found (IVS17+2T > C and Q1135X) are rare variants, previously reported only once by BIC. The patient carrying IVS17+2T > C, which affects a splicing site, was 47 years old at the time of diagnosis and had a family history of breast cancer in first-degree relatives: her mother, diagnosed at age 69, and a sister at 44. The patient with Q1135X had breast cancer diagnosed at 46 years and a family history of breast cancer including her mother, diagnosed at 38 years, and her sister, diagnosed at 40 years.

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