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Prevalence of BRCA1 and BRCA2 mutations in breast cancer patients from Brazil

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Abstract The contribution of BRCA1 and BRCA2 to breast cancer incidence in Brazil has not yet been explored. In order to estimate the proportion of breast cancers due to BRCA1 and BRCA2 mutations in Brazil, we conducted a study of unselected breast cancer patients from Rio de Janeiro, Brazil. We enrolled 402 women with breast cancer from a large public hospital and two private medical clinics in the city. A detailed family history was obtained from each patient and a blood sample was obtained for DNA analysis. Mutations in BRCA1 and BRCA2 were sought using a combination of techniques, but all mutations were confirmed by direct sequencing. Overall, nine mutations were identified (six in BRCA1 and three in BRCA2) representing 2.3% of the total. The most common mutation, 5382insC in BRCA1, was seen five times and accounted for 56% of all identified mutations. A second mutation, in BRCA2 (6633del5) was seen in two unrelated women. In summary, BRCA1 and BRCA2 mutations are not uncommon in Brazilian women with breast cancer. It appears that a small number of founder mutations may be predominant. Moreover, a small

All studies listed in this paper are in accordance with the ethical standards of the regional hospitals in the listed study area.

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number of founder mutations may be prevalent in Brazil, raising the possibility that a rapid and inexpensive genetic test may be developed to screen for inherited susceptibility to breast cancer in Brazil.

Keywords Brazil · BRCA1 · BRCA2 · Breast cancer · Hereditary

Introduction

Breast cancer is the most common cause of death in women between the ages of 40 and 69 years in Brazil. Brazil is the fifth largest country in the world, with 186,000,000 inhabitants. Approximately 50,000 new cases of breast cancer are diagnosed annually in Brazil. The relative contributions of environmental and genetic factors to the breast cancer burden have not yet been studied.

Both BRCA1 and BRCA2 confer susceptibility to breast and ovarian cancer, and a high proportion of familial breast cancers are associated with mutations in BRCA1 and BRCA2. Approximately 10% of women who are diagnosed with breast cancer report a family history of the condition, but the minority of these will be found to carry a germ-line mutation in one of these genes. Because one of the characteristics of hereditary breast cancer is a tendency towards young age at onset, families with multiple cases of early-onset breast cancer or ovarian cancer are the most likely to carry a mutation in BRCA1 or BRCA2 [1]. An 8–10 year decline in the mean age of onset of breast/ovarian cancer from one generation to another was reported in 260 families in Brazil, showing a marked familial aggregation of these tumors [2]. The lifetime risk of breast cancer in women who carry BRCA1 or BRCA2 mutations is as high as 80%, but the absolute risk may vary according to the specific mutation and the country of residence [3–7].

At present, genetic testing is offered in many centers of North America, Europe, Australia and Israel, but is not commonly available in South America. Genetic testing is gaining acceptance worldwide because of the increasing numbers of preventive options available to women with a mutation, and because of the development of novel, individualized, cancer therapies [8].

The prevalence of BRCA-associated breast carcinoma in the Brazilian population has not yet been evaluated. To determine the prevalence of BRCA germline mutations in Brazilian breast cancer patients, we performed mutation analysis of BRCA1 and BRCA2 on 400 unselected patients with breast cancer from Rio de Janeiro.

Materials and methods

Patient population

We conducted a study of unselected breast cancer patients in Rio de Janeiro, Brazil, from February 2003 to September 2005. Patients are treated for breast cancer in both public and private settings in Brazil. To ensure that we included patients from a range of ethnic and social backgrounds, patients were recruited both from a large public hospital (Hospital of the Federal University of Rio de Janeiro) and from two private medical clinics which provide oncology services (The Centre of Tumor Treatment, Botafogo, Rio de Janeiro, and the Clinic of Roberto Vieira in Copacabana, Rio de Janeiro).

The study included components evaluating diet, reproductive and lifestyle factors, and hereditary factors. The results of the hereditary factors are reported here and the results of other analyses will be reported elsewhere. The institutional review boards at the participating centers approved the protocol and all of the women in the study gave written, informed consent. Patients were approached to participate in the study during an out-patient visit to the medical oncology clinic, or during hospital admission.

In total, 403 women were approached to participate, of who 402 agreed (one woman did not wish to receive a genetic test result). The 402 women were diagnosed with invasive breast cancer between the ages of 25 and 67, between the years 1978 and 2005. On average, 2.7 years had elapsed between the date of diagnosis and the date of interview. Fifty-two percent of the patients were recruited from public hospitals and 48% were recruited from private clinics.

All participants were interviewed in person for their family history of cancer, with specific reference to a history of breast or ovarian cancer. Tumor histology, tumor size, lymph node involvement and grade were abstracted from the medical records. Questionnaires on family histories and lifestyle factors were completed for 381 of the 402 cases. For the other 21 cases, only limited information, including age of diagnosis, was available.

Laboratory methods

Lymphocyte deoxyribonucleic acid (DNA) was prepared from whole blood by standard procedures. All samples were screened for four common alterations. These were the BRCA1 185delAG, 5382insC and the BRCA2 6174delT mutations common to Ashkenazi Jews and others of eastern European ancestry. These founder mutations were assayed using a rapid multi-

plex method [9]. We tested separately for the presence of the BRCA1 exon-13 6-kb duplication [10]. Exon 11 of BRCA1 and exons 10 and 11 of BRCA2 were then screened by the protein truncation test (PTT). Primer sequences used to amplify overlapping fragments were obtained from the Breast Cancer Information Core (BIC). PTT was performed using the TNT^{TM} rabbit reticulocyte lysate system (Promega), involving [^{35}S]methionine/cysteine (New England Nuclear) for protein detection.

Cases not found to carry a mutation by the preceding methods, but with a strong family history of breast and ovarian cancer, were then screened for additional BRCA1 and BRCA2 mutations throughout the entire coding regions of the two genes. For BRCA1, fluorescent multiplex denaturing gradient gel electrophoresis (DGGE) was used [11] (all of the remaining coding exons, the exon–intron boundaries, and the beginning and end of exon 11 were included; the noncoding exons 1a and 1b, and the noncoding part of exon 24 were excluded). For BRCA2, denaturing high performance liquid chromatography (DHPLC) was employed to screen the remaining coding exons and exon–intron boundaries in that gene [12].

All variants identified by PTT, DGGE and DHPLC were confirmed by direct DNA sequencing. All of the observed mutations included in this report are considered to be deleterious.

Results

A total of 402 patients were tested for BRCA1 and BRCA2 mutations using a combination of laboratory techniques. All 402 patients were tested for four of the most commonly reported mutations and for protein-truncating mutations within exons 11 of BRCA1 and exons 10 and 11 of BRCA2 (using the PTT test). In addition, 12 patients with a strong family of breast and/ovarian cancer had comprehensive testing, which included all coding regions of BRCA1 and BRCA2.

The mean age of the patients at diagnosis was 46.4 years (range 25–67 years) and the average age at interview was 49.1 years (range 25–82 years). Twenty-three percent of the patients were diagnosed before the age of 40 and 66% were diagnosed before the age of 50. Family histories were available for 381 patients (95%), of which 73 (19%) had a first-degree relative diagnosed with breast or ovarian cancer.

Overall, there were nine mutations identified (2.3%), including six in BRCA1 (1.5%) and three in BRCA2 (0.75%) (Table 1). Five of the six BRCA1 mutations were the 5382insC founder mutation and two of the three BRCA2 mutations were 6633del5. In total, seven of the nine mutations (78%) were founder mutations; i.e. were seen in more than one family. The 5382insC mutation alone represented 56% of all mutations in the study. This mutation appears to be present in 1.3% of all Brazilian breast cancer patients.

A mutation was seen in five of 87 patients diagnosed with breast cancer at age 40 or below (5.7%), in three of 167 patients diagnosed between ages of 41 and 50 (1.8%) and in one of 127 cases diagnosed above age 50 (0.8%).

A mutation was seen in five of 73 patients (6.8%) with a family history of breast or ovarian cancer in a first-degree relative and in three of 308 (1.0%) of patients with no family history in a first-degree relative. Overall, eight of nine women with a mutation had either early-onset breast cancer (diagnosed at age 50 or below) or had a first-degree relative affected with breast or ovarian cancer.

Because of the high prevalence of the 5382insC mutation in the Brazilian breast cancer population, we tested this mutation in 319 healthy controls in order to attempt to estimate the population allele frequency. No mutant allele was found in the controls.

Table 1 BRCA1 and BRCA2 mutations identified in Brazilian breast cancer patients

Patient	Gene	Exon	Mutation	Age of diagnosis	Family history
26257	BRCA1	11	3347delAG	Br32	Ov64
24579	BRCA1	20	5382insC	Br28	Br47
24122	BRCA1	20	5382insC	Br38	Br50
24535	BRCA1	20	5382insC	Br46	Br63
26288	BRCA1	20	5382insC	Br37, NHL35	Br42, Br35, Br30, Br32, Br36, Br38
28844	BRCA1	20	5382insC	Br41	Ut 56, Br60
26243	BRCA2	11	6174delT	Br48	Br37, MBr 70, Ov37
26260	BRCA2	11	6633del5	Br32	NA
24591	BRCA2	11	6633del5	Br53	Br49

NA Not available, *Br* breast cancer, *Ov* ovarian cancer, *NHL* non-Hodgkin lymphoma, *Ut* uterine cancer, *MBr* male breast cancer

Discussion

This is the first report of the prevalence of BRCA1 and BRCA2 mutations in a series of unselected breast cancer patients from Brazil. We identified a deleterious BRCA1 or BRCA2 mutation in 2.3% of unselected Brazilian women with breast cancer. In women who have inherited a mutation, the lifetime risk of breast cancer is between 65 and 85% by age 70 [7, 13–16]. The penetrance may vary according to the position of particular mutation, as the specific penetrance of the Brazilian mutations have not yet been estimated. In the only other study from Brazil published to date, Dufloth et al. [17] identified the 5382insC BRCA1 mutation in a breast cancer family from São Paulo and found a BRCA2 mutation (S2219X) in two families.

The most common mutation in the present study was the 5382insC mutation. This is the most common mutation identified worldwide and is found both among Ashkenazi Jews and women of Slavic origin [18, 19]. It is not clear how, or when, the mutation was introduced into Brazil, but a preliminary haplotype analysis confirms a common origin with the European mutation (Foulkes et al., manuscript in preparation). A possible explanation may reside in the fact that a large Jewish population used to live in Portugal until 1509, after which they were deported by the Inquisition. They relocated to other countries including Brazil, which was a Portuguese colony in the sixteenth century. In this sense, genetic studies carried out in the Brazilian population were able to identify some characteristics related to this Jewish ancestry [20]. The 5382insC mutation was found in five families in the current study and in one family in the previous study from São Paulo.

Elsewhere in South America, two mutation surveys have been completed in Chile. Jara et al. [21] found seven BRCA1 mutations and three BRCA2 mutations in 64 breast cancer families from Chile (all mutations were different). Gallardo et al. [22] identified three BRCA1 mutations and five BRCA2 mutations in 54 Chilean breast/ovarian cancer families. A single recurrent BRCA1 mutation (Q1273X) was seen (in two families). It is important to conduct similar mutation surveys in other South American countries so that the information can be compared and the most common mutations identified. This will facilitate genetic testing throughout the region.

Ours is the first study in South America which is based on unselected cases of breast cancer, and as such, permits the estimation of the proportion of cases due to BRCA mutations. We estimate this prevalence to be 2.3%, but this may be an underestimate. All families were screened for the most common mutations, and for mutations in the large exons; however, the entire gene coding sequences were screened only for the twelve cases with a strong family history. We estimate that the coding regions of the large exons contain approximately 75% of all mutations [23]. Furthermore, approximately 10% of mutations are due to large genomic rearrangements and deletions [24] and these would not have been identified using the strategy employed here. We included cases from different social strata in order for the population of Rio de Janeiro to be represented, but patients from other regions of Brazil were not included. Brazil is a large and populous country and the ethnic admixture varies from state to state.

This data supports the position that genetic testing for common founder mutations should be offered in Brazil to women with breast cancer before the age of 50 or with a family history of breast or ovarian caner in a first-degree relative. Brazil is the fifth largest country in the world, and we expect that approximately 1,000 of the 50,000 breast cancer cases diagnosed nationally in the region are attributable to BRCA founder mutations, including over 500 cases due to 5382insC alone. It is important that access to inexpensive genetic testing be made available to a large number of Brazilian women with breast cancer.

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