Short Report

BRCA1 and BRCA2 mutations among familial breast cancer patients from Costa Rica


The contribution of mutations in BRCA1 and BRCA2 genes to the burden of breast cancer in Costa Rica has not been studied. We estimated the frequency of BRCA mutations among 111 Costa Rican women with breast cancer and a family history of breast cancer. These women were mainly from the metropolitan area of San José. A detailed family history was obtained from each patient and a blood sample was processed for DNA extraction. Mutations in BRCA1 and BRCA2 were sought using a combination of techniques and all mutations were confirmed by direct sequencing. Four different mutations were identified in five patients (four in BRCA2 and one in BRCA1) representing 4.5% of the total. Two unrelated patients were found to have a BRCA2 5531delTT mutation. Other BRCA2 mutations included C5507G and 6174delT. Only one BRCA1 mutation was found (C3522T). The family with the BRCA1 mutation had five cases of gastric cancer. Families with BRCA2 mutations were also reported to have cases of gastric and prostate cancers; however, the full range of cancers associated with BRCA1 and BRCA2 mutations in Costa Rica has not yet been established.

Conflict of interest
Nothing to declare.

Costa Rica is a small Central American country with approximately 4.5 million inhabitants. In Costa Rica, breast cancer is the cancer with the highest incidence and mortality in women. In 2008, there were 931 new cases of breast cancer in Costa Rica and 274 women died of breast cancer (1). The annual age-standardized incidence rate for breast cancer in Costa Rica is estimated to be 42.9 per 100,000 (1) compared with 83.2 per 100,000 in Canada (1) and 92 per 100,000 (whites) in the United States (SEER Registry).

Both BRCA1 and BRCA2 mutations confer susceptibility to breast cancer, with the cumulative risk of breast cancer in women up to 70 years of age estimated to be from 50% to 80% (2–4). However, the risk of breast cancer may vary according to the gene, specific mutation, country of residence, and family history (2,5–7).

Because breast cancer is a major public health problem in Costa Rica, it is important to establish the profile of mutations of BRCA1 and BRCA2 genes in this country. Knowledge about the presence of founder mutations in Costa Rica and other Latin American countries will enable the development of novel prevention strategies and may impact on treatment. It is hoped that genetic testing in developing countries such as Costa Rica can be made affordable to most women. Ultimately, affordable genetic testing can be made possible through the detection of founder mutations because the cost of testing for these is low compared to the cost of complete sequencing of both BRCA1 and BRCA2 genes (8). Founder effects have been seen in specific ethnic groups such as the Ashkenazi Jewish (9, 10), Polish (11), and French-Canadians (12).
We sought to identify the common BRCA mutations by performing genetic testing on women who were at high risk of carrying a mutation. Given that families with multiple cases of breast or ovarian cancers are the most likely to carry a mutation in BRCA1 or BRCA2 (13), an efficient way to identify the genetic profile of BRCA mutations in a country is to select families with multiple cases of breast or ovarian cancer. Similar studies have been conducted in Colombia, Chile, and Mexico (14–18). We conducted a mutation analysis of BRCA1 and BRCA2 on 111 Costa Rican families with breast cancer and a family history of breast cancer in order to identify germline mutations in these genes.

**Material and methods**

**Patient population**

Study subjects were from Costa Rican breast cancer families, diagnosed at age 18 or older from April 2007 to February 2009. Families were selected on the basis of having had at least three individuals diagnosed with breast cancer at any age or ovarian cancer on one side of the family or at least two individuals diagnosed with breast cancer under the age of 50 on one side of the family. Patients were recruited from both public and private settings in various regions of Costa Rica. The majority of the subjects (56.9%) resided in the metropolitan area of San José, which has a population of approximately 1.2 million inhabitants.

The patients were identified and referred by physicians, oncologists and gynecologists. Patients were also referred by the Fundación Nacional de Solidaridad contra el Cáncer de Mama (FUNDESO) and Fundación Esperanza, Movimiento Elige Vivir. The institutional review boards at the participating centers approved the protocol and all the women in the study provided written informed consent. Once patients were identified, they were asked to go to the Universidad de Ciencias Médicas (UCIMED) to participate in the study. At the clinic appointment, a research coordinator interviewed the patient and recorded the family history.

In total, 111 women were interviewed and agreed to participate. On average, 2 years had elapsed between the date of breast cancer diagnosis and the date of interview. Tumor histology, tumor size, lymph node involvement and grade were abstracted from the Registro Nacional de Tumores, RNT (National Cancer Registry).

**Laboratory methods**

A blood sample was obtained for 111 patients. The samples were stored at the UCIMED, San José at −70°C until they were shipped to Dr Narod’s laboratory in Toronto. DNA was extracted from blood samples using Puregene DNA extraction kits in Toronto. Exon 10 of BRCA1 and exons 10 and 11 of BRCA2 were screened by the protein truncation test (PTT). Primer sequences used to amplify overlapping fragments for the PTT were obtained from the Breast Cancer Information Core.

PTT was performed using the TNT™ rabbit reticulocyte lysate system (Promega Corporation, Madison, WI), involving [35S] methionine/cysteine (New England Nuclear, Boston, MA) for protein detection. All samples were then screened for the presence of the BRCA1 exon-13 6-kb duplication (19). We also tested for the three common mutations, BRCA1 185delAG, 5382insC and BRCA2 6174delT (20, 21) that are most commonly seen in Ashkenazi Jews and others of eastern European ancestry. These founder mutations were assayed using a rapid multiplex method (11). All deleterious mutations were confirmed by direct DNA sequencing. Bidirectional Sanger sequencing was used for the confirmation of sequence variants. Mutations were aligned with the BRCA1 reference sequence (accession no. U14680) and BRCA2 reference sequence (accession no. U43746).

**Results**

A total of 111 patients were tested for BRCA1 and BRCA2 mutations using a combination of laboratory techniques. All of the patients had a family history of breast cancer in first- or second-degree relatives. The mean age of breast cancer diagnosis was 43 years (range 27–75 years); 35.1% of the patients were diagnosed before the age of 40 and 75% were diagnosed before the age of 50.

A mutation was found in five patients (4.5%); four in BRCA2 (3.6%) and one in BRCA1 (0.9%) (Table 1). All mutations resulted in premature protein truncation and were predicted to be deleterious. The BRCA2 5531delTT mutation was detected in two different families. A mutation was seen in 3 of 39 patients diagnosed with breast cancer at age 40 or below (7.7%), 1 of 44 patients diagnosed between ages of 41 and 50 (2.3%), and 1 of 28 patients diagnosed above age 50 (3.6%).

The age of diagnosis of breast cancer in the women with a BRCA mutation was lower (mean 39 years) than that of the corresponding non-mutation carriers (mean 46 years). There were 12 patients with a family history of ovarian cancer in a first- or second-degree relative; among these, no mutations were seen. A mutation was found in 3 of 34 patients (8.8%) with a first- or second-degree relative with gastric cancer and in 2 of 26 patients (7.7%) with a first- or second-degree relative affected with prostate cancer. The pedigrees of the families with mutations are shown in Fig. 1. In the family with a BRCA1 mutation, five cases of gastric cancer were reported. We did not have pathologic confirmation of the gastric cancer and these individuals were not available for genetic testing.

**Discussion**

We identified a deleterious BRCA1 or BRCA2 mutation in 4.5% of Costa Rican breast cancer patients selected for a family history of breast cancer. This frequency of BRCA mutations is similar to that reported for familial breast cancer cases from Mexico (3.9%) (14, 15); but lower than the frequency found in Chile (20.4%) (14, 15); however, ascertainment of families.
Table 1. BRCA1 and BRCA2 mutations identified in Costa Rican familial breast cancer patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gene</th>
<th>Exon</th>
<th>Mutation</th>
<th>HGVS nomenclature</th>
<th>Codon change</th>
<th>Age of diagnosis</th>
</tr>
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<tbody>
<tr>
<td>CM24</td>
<td>BRCA1</td>
<td>11</td>
<td>C3522T</td>
<td>c.3403C&gt;T</td>
<td>Q1135X</td>
<td>49</td>
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<tr>
<td>CM2</td>
<td>BRCA2</td>
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<tr>
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<td>BRCA2</td>
<td>11</td>
<td>5531delTT</td>
<td>c.5303_5304delTT</td>
<td>Stop 1772</td>
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<tr>
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<td>11</td>
<td>5531delTT</td>
<td>c.5303_5304delTT</td>
<td>Stop 1772</td>
<td>3</td>
</tr>
</tbody>
</table>

Family with BRCA1 mutation:
CM24 (BRCA1 C3522T)

Family with BRCA2 mutations:
CM2 (BRCA2 C5507G)

Fig. 1. Pedigrees of subjects with BRCA1 or BRCA2 mutations. Circles indicate women and squares indicate men. Patterned circles indicate women affected with cancer. Patterned squares indicate men affected with cancer. Diagonal slash indicate deceased. OV, ovarian cancer, BR, breast cancer, PROS, prostate cancer, Ut, uterine cancer, Mel, melanoma, Cerv, cervical cancer. The numbers following the abbreviations indicate age of diagnosis. The plus sign indicates the presence of a germline BRCA1 or BRCA2 mutation.

and testing criteria vary between studies. Studies such as these, on a small number of familial breast cancer cases are inexpensive and have the potential to identify founder mutations. However, studies on unselected breast cancer cases are better suited to estimate the genetic burden of breast cancer in a particular country. The population of Costa Rica is young and the majority of women with breast cancer are diagnosed under the age of 50. Given the strong relationship between age of onset and the presence of a BRCA mutation, we might expect to see a higher proportion of genetic cases in Costa Rica than in countries such as Canada where the age of onset is typically later.

The BRCA2 5531delTT mutation, which was found twice in this study, has been reported four times in the Breast Information Core (BIC) database; three patients were of Western European descent. The two families with this mutation were from the San Jose area and were not known to be related. This suggests that this may be a founder mutation in Costa Rica. The BRCA2 C5507G mutation has only been reported once in the BIC database and the ethnicity of this individual was not specified. The BRCA1 Q1135X (3522 C>T) mutation has been reported in four individuals in the BIC database, two of whom were of Western European ethnicity, and one of whom was of Caucasian ancestry. The BRCA2 6174delT mutation is relatively rare outside of the Jewish population. The Costa Rican family with this mutation was of German–Jewish background.
The three founder mutations that are common among Ashkenazi Jews are also represented in Latin America. The \textit{BRCA1} 185delAG mutation, which is the most common mutation in Ashkenazi Jews, has been reported in Spain and in Mexico \citep{22, 23}. The 5382insC mutation is common among Jews and in Slavic populations. The \textit{BRCA1} 5382insC mutation accounted for 56\% of all identified mutations in a study of unselected Brazilian women with breast cancer, who were not known to be of Jewish or Slavic origin \citep{24}. The \textit{BRCA1} 5382insC mutation has also been observed in a Costa Rican family in our clinic in Toronto (unpublished data). The third mutation, \textit{BRCA2} 6174delT, was observed in one family in this study. It will be of interest to explore the possibility of these Jewish founder mutations being present in other Hispanic populations.

The observation of \textit{BRCA2} mutations in families with multiple cases of gastric cancer is consistent with the findings of Jakubowska et al., which suggest that clustering of ovarian and stomach cancers in Polish families predicts the presence of a \textit{BRCA2} mutation \citep{25}. This is of particular importance, because there is a very high incidence of gastric cancer in Costa Rica. One family (CM24) with five relatives affected with gastric cancer was detected to have a \textit{BRCA1} mutation (C3522T). The presence of ovarian cancer in a family is predictive of the presence of a \textit{BRCA1} mutation \citep{26}, but in this study, none of 12 families with ovarian cancer had a mutation.

A limitation of this study was the fact that the entire gene coding sequence of \textit{BRCA1} and \textit{BRCA2} was not screened for families without mutations. The protein

\textit{Fig. 1. Continued}
truncation test, performed for exon 11 of BRCA1 and exons 10 and 11 of BRCA2, covers approximately 65% of the coding region of both genes (27). Furthermore, up to 10% of mutations are due to large genomic rearrangements and deletions (28, 29) and these would not have been identified using the methods employed in this study.

Efforts to determine the frequency and spectrum of BRCA1 and BRCA2 mutations in several Central and South American countries are underway. Mutation analyses have been performed in Brazil, Cuba, Colombia, Mexico, and Chile (8, 14–18, 30–32). Founder mutations have been reported to account for a larger proportion of mutations in Colombia (16, 33). Many Hispanics are of mixed ancestry (mestizo population), or have common Spanish/Indigenous ancestors. It is of interest to evaluate the presence of the Spanish founder mutations in Costa Rica; there are nine founder mutations identified in Spain and our mutation analysis techniques covers seven of these (22). None of these seven Spanish founder mutations were seen in our 111 families. It would be beneficial to develop a fast and affordable multiplex panel that tests for BRCA mutations identified in women of Hispanic ancestry. As a result, it is important to compare results from mutation studies from all Latin American countries so that most common mutations are identified and added to a registry of Hispanic mutations. We expect to see the same BRCA mutations in different regions of Latin America at different frequencies. Our goal is to ensure that Costa Rican and Latin American women who are found to have mutations would be in a position to benefit from major advances in the prevention and treatment of hereditary breast cancer.

Acknowledgements

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References