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Prevalence of BRCA1 and BRCA2 gene mutations in families with medium and high risk of breast and ovarian cancer in Brazil

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Of all malignant neoplasias affecting women, breast cancer has the highest incidence rate in Brazil. The objective of the present study was to determine the frequency of genetic modifications in families with medium and high risk for breast and ovarian cancer from different regions of Brazil. An exploratory, descriptive study was carried out on the prevalence of the BRCA1 and BRCA2 mutations in case series of high-risk families for breast and/or ovarian cancer. After heredogram construction, a blood sample was taken and DNA extraction was performed in all index cases. The protein truncation test was used to screen for truncated mutations in exon 11 of the BRCA1 gene and in exons 10 and 11 of the BRCA2 gene. Of the 612 individuals submitted to genetic testing, 21 (3.4%), 19 women and 2 men, had mutations in the BRCA1 or BRCA2 genes. Of the 19 BRCA1 mutations found in the 18 participants, 7 consisted of ins6kb mutations, 4 were 5382insC, 3 were 2156delGinsCC, 2 were 185delAG, 1 was C1201G, 1 was C3522T, and 1 was 3450del4. With respect to the BRCA2 gene, 3 mutations were found: 5878del10, 5036delA and 4232insA (one case each). The prevalence of germline mutations in the BRCA1 and BRCA2 genes found in the present study was lower than reported by other studies on high-risk Brazilian populations. The inclusion of individuals with medium risk may have contributed to the lower prevalence observed.

Key words: Breast cancer; BRCA1; BRCA2; Mutations; Brazil

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Introduction

Breast cancer has the highest incidence rate of all malignant neoplasias affecting women in Brazil. According to estimates by the Brazilian Ministry of Health, about 49,400 new cases occurred in the country in 2008 and more than 10,000 deaths from breast cancer are recorded annually (1).

Despite the studies on the association of several risk factors, such as exposure to endogenous and exogenous steroid hormones, diet, and physical activity, among others, a comprehensive understanding of all factors involved in breast carcinogenesis is not available (2).

The advances in the study of the molecular genetics of breast cancer have gradually contributed to a better understanding of the biological process that leads a normal breast cell to be transformed into a malignant one with invasive and metastatic properties. Carriers of germline mutations in the susceptibility genes for breast cancer, BRCA1 and BRCA2, are particularly at risk of developing breast cancer (3), and the same has been observed in Brazil (4).

Familial antecedents of breast or ovarian cancer can usually be traced in about 5-8% of all cases of breast cancer. BRCA1 and BRCA2 are tumor suppressor genes described as causing hereditary breast-ovarian cancer

syndrome. In addition to these, other genes are also believed to be involved in this syndrome, since a certain percentage of families with a presumed clinical diagnosis of the syndrome do not show either BRCA1 or BRCA2 mutations (5).

BRCA1 is located on chromosome 17 and is a large gene containing a coding region with 5592 bp. The BRCA2 gene is located on chromosome 12 and is even larger than the BRCA1 gene, with a coding region containing 10,254 bp (6).

The BRCA1 gene is known to participate in the process of epithelial cell proliferation in mammals in response to hormone stimulation, in the process of apoptosis, recombination control, and in maintaining genome integrity after binding to the proteins related to these activities. There is evidence of the interaction of the BRCA1 protein with Rad51, which would be related to the function of maintaining cell genome integrity (7).

The BRCA2 gene acts as a tumor suppressor and has activity related to transcription activation, as well as involvement in the DNA repair system. The BRCA2 protein also interacts with the Rad51 and Dss1 proteins. The BRCA2 and Dss1 complex is bound by a single DNA helix and this may be the means of transporting Rad51 to the sites at which there is a defect in the DNA (8).

Mutations in the BRCA1 and BRCA2 genes have diverse clinical characteristics. Women carrying the BRCA1 mutation have an 85% risk of developing breast cancer before 70 years of age and a 63% risk of developing ovarian cancer (9). The tumors resulting from mutations have a higher incidence of estrogen- and progesterone-negative receptors and p53-positive alterations, indications of more aggressive tumors (10). Women with the BRCA2 gene mutation have an 85% risk of developing breast cancer before 70 years of age and a 27% risk of developing ovarian cancer (9). Men with the BRCA2 gene mutation are at an increased risk for breast cancer, and studies suggest that there is also an increase in the incidence of prostate cancer in these individuals (11). Mutations in the BRCA2 gene are present in about 10% of families with hereditary pancreatic cancer (12). Primary peritoneal carcinomatosis and cancer of the fallopian tubes are also typically related to BRCA1 and BRCA2 (13). In addition, BRCA1 is associated with bowel tumors, melanoma, leukemia and lymphoma, and BRCA2 with tumors of the pancreas, colorectum, prostate, stomach, head and neck, lymphoma, and melanoma (14).

Recognition of risk groups is fundamental in ensuring the development and implementation of screening strategies, early diagnosis and preventive measures for these families. Women with BRCA1 and BRCA2 mutations have an extremely high risk of developing breast and ovarian cancer. Men with BRCA2 mutations also have an increased risk of developing breast and prostate cancer at a young age. Therefore, high-risk families need specific screening and prevention programs. In addition, identification of genetic modifications may be used as a prognostic method and to define therapeutic strategies (15).

The objective of the present study was to determine the frequency of genetic modifications in a medium- and high-risk population for breast and ovarian cancer in different Brazilian states.

Patients and Methods

In 1995, the Fernandes Figueira Institute, Oswaldo Cruz Foundation, implemented a study on breast cancer and genetics involving the following Brazilian institutes: Breast Institute, Porto Alegre, Rio Grande do Sul; São Marcos University, São Paulo; Association for the Combat of Cancer, Goiás, Goiânia; Santa Rita Hospital, Dourados, Mato Grosso do Sul; State Department of Health, Campo Grande, Mato Grosso do Sul; Women's Association for Education and Combat of Cancer, Vitória, Espírito Santo; School of Medical Sciences of the Foundation University of Pernambuco, Recife, Pernambuco; Dr. Amaral de Carvalho Foundation, Jaú, São Paulo; Foundation Federal University of Piauí, Teresina, Piauí; Federal University of Ceará, Fortaleza, Ceará; Januário Cicco Teaching Maternity Hospital, Natal, Rio Grande do Norte; Napoleão Laureano Hospital, João Pessoa, Paraíba.

An exploratory, descriptive study was carried out on the prevalence of BRCA1 and BRCA2 mutations in a case series of families at medium or high risk for breast and/or ovarian cancer. Participants were enrolled by specialists in mastology, nurses, psychologists, and clinical oncologists according to one of the following criteria: women with a history of two or more relatives with breast and/or ovarian cancer along three or more generations; two or more cases of breast and/or ovarian cancer in first-degree relatives; male relatives with breast cancer diagnosed under 35 years of age, or cases of bilateral breast cancer.

A blood sample was taken and sent to the Laboratory of Applied Molecular Biology, the Fernandes Figueira Institute, FIOCRUZ, Rio de Janeiro, where DNA extraction was performed.

Personal data on the following variables were collected: gender, age, city of residence, family history of cancer, number of cases of cancer in the family, number of cases of breast, ovarian, prostate, bowel, and other types of cancer among relatives, familial relationships between family members with cancer, presence of cancer and

topography, and the presence of the BRCA1 and BRCA2 gene mutations. To analyze family history of cancer, information on family antecedents was collected using specific questionnaires, and the respective familial cancer heredograms were constructed. The analysis and sequencing of DNA extracted from blood samples were carried out at the following institutions: Laboratory of Applied Molecular Biology, Fernandes Figueira Institute, FIOCRUZ, Rio de Janeiro, Brazil; Myriad Diagnostic, Salt Lake City, Utah, USA; Cambridge University, UK; International Agency of Research in Cancer (IARC); World Health Organization, Lyon, France; Narod Laboratory, Toronto, Canada.

The protein truncation test was used to screen for truncated mutations in exon 11 of the BRCA1 gene, and in exons 10 and 11 of the BRCA2 gene. This screening covers approximately 58% of the coding region of BRCA1 and 50% of the coding region of BRCA2. All the mutations detected by the screening protocols were confirmed by DNA sequencing.

A database was created to store the collected data, which were later analyzed using the Epi-Info software. The frequency distribution of the variables studied is presented with their respective 95% confidence intervals.

All participants signed an informed consent form giving their permission for genetic testing. The study protocol was approved by the Research and Ethics Committee of the Fernandes Figueira Institute, Oswaldo Cruz Foundation.

Results

A family history of cancer and the prevalence of germline mutations in BRCA1 and BRCA2 were investigated in 612 individuals. Of the participants submitted to genetic testing, only 21 (3.4%), 19 women and 2 men, had mutations in the BRCA1 or BRCA2 genes consisting of 10 different alterations distributed throughout the coding region of both genes (Table 1). Mutations in BRCA1 were found in 18 participants (2.9%; 95%CI: 1.8-4.6) and mutations in BRCA2 were found in 3 (0.5%; 95%CI: 0.1-1.4). In one case, two mutations were found in BRCA1.

Of the 19 BRCA1 mutations found in the 18 participants, 7 consisted of ins6kb mutations, 4 were 5382insC, 3 were 2156delGinsCC, 2 were 185delAG, 1 was C1201G, 1 was C3522T, and 1 was 3450del4.

With respect to the BRCA2 gene, 3 mutations were found: 5878del10, 5036delA and 4232insA (one case each).

The ins6kb mutation comprised 33.3% of all mutations found in this study. Of the 7 participants with this mutation, two were from the State of Rio de Janeiro and 5 from the State of Rio Grande do Sul. The participant with both mutations was a male who, in addition to the ins6kb mutation, also had the 2156delGinsCC mutation.

Discussion

Sequencing of the BRCA1 and BRCA2 genes, the principal genes related to hereditary breast and ovarian cancer, resulted in the classification of a large number of mutations in both genes. With the exception of specific ethnic groups, there is no predominant mutation responsible for the majority of cases of hereditary breast cancer; however, in some locations, recurrent mutations have been described, facilitating diagnosis (16). Among the 18 mutations observed in our medium- and high-risk families series, 6 (33.3%) were founder mutations commonly detected among Ashkenazi Jews.

Despite the high prevalence of breast cancer in the Brazilian population, no systematic study had been previously carried out on BRCA1 and BRCA2 mutations among patients with either breast cancer or a high-risk family history. To our knowledge, this study is the first comprehensive investigation describing the prevalence of such mutations in the Brazilian population, and included indi-

Table 1. Germline BRCA1 and BRCA2 mutations in 612 subjects.

Patient	State	Gender	Gene	Exon	Mutation
24637	SP	female	BRCA1	11	C1201G
24751	PI	female	BRCA1	11	3450del4
24755	PI	female	BRCA2	11	5878del10
24774	PI	female	BRCA2	11	5036delA
24863	RJ	female	BRCA2	11	4232insA
25214	RS	female	BRCA1	13	ins6kb
25215	RS	female	BRCA1	13	ins6kb
25243	RS	female	BRCA1	20	5382insC
25245	RS	female	BRCA1	20	5382insC
25257	RS	female	BRCA1	20	5382insC
25265	RS	female	BRCA1	13	ins6kb
25266	RS	female	BRCA1	13	ins6kb
25275	RS	female	BRCA1	20	5382insC
25336	RS	female	BRCA1	13	ins6kb
25350	RJ	female	BRCA1	2	185delAG
25359	RJ	female	BRCA1	13	ins6kb
25360	RJ	male	BRCA1	13	ins6kb
25360	RJ	male	BRCA1	11	2156delGinsCC
25364	RJ	female	BRCA1	11	2156delGinsCC
25367	RJ	male	BRCA1	11	2156delGinsCC
25383	RJ	female	BRCA1	11	C3522T
25404	RJ	female	BRCA1	2	185delAG

SP = State of São Paulo; RJ = State of Rio de Janeiro; PI = State of Piauí; RS = State of Rio Grande do Sul.

viduals from all five geographic regions of the country.

Gomes et al. (17) carried out a pioneering, case-control study on the prevalence of BRCA1 and BRCA2 mutations in patients with breast cancer in the State of Rio de Janeiro. These investigators reported a mutation prevalence of 2.3%, suggesting that in every 50,000 cases of breast cancer diagnosed in Rio de Janeiro, 1000 are related to germline mutations in these genes. In that study, most of the mutations (50%) found were in 5382insC in exon 20 of the BRCA1 gene. In the present study, however, this mutation was found in only 4 (19.1%) cases. Gomes et al. (17) failed to find the ins6kb mutation in any of their cases, probably due to the differences in the characteristics of the studied populations.

In another study carried out in Brazil, Dufloth et al. (18) reported the prevalence of BRCA1 and BRCA2 mutations in 31 patients with breast cancer and a positive family history in the State of São Paulo. Four mutations were identified (12.9%), 1 in the BRCA1 gene and 3 in the BRCA2 gene. The mutation in the BRCA1 gene was the founder mutation, 5382insC, also found in 4 participants in the present study.

In an additional Brazilian study including patients recruited from high-risk genetic clinics in the cities of Rio de Janeiro and Sao Paulo, Ewald et al. (19) described a prevalence of 3.4% (5/145) for BRCA1 mutation 5382insC.

A recent large-scale analysis of the mutation of these two genes in medium- and high-risk families indicated that, in many populations, only 30-60% of cases of hereditary breast cancer were attributed to mutations in the BRCA1 and BRCA2 genes. Incidence rates of 21 and 9%, respectively, were found in the UK, 24 and 18% in France, 40 and 16% in Canada, and 39 and 25% in the US. In Switzerland and Hungary, approximately 35% of these families had mutations in both genes. In isolated populations, this situation may be different. In Iceland, a single mutation found in the BRCA2 gene is responsible for the majority of cases of hereditary breast cancer. In the Ashkenazi Jewish population, however, a very high rate has been reported for the following three mutations: 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 (16,20).

The prevalence of germline mutations in the BRCA1 and BRCA2 genes found in the present study was 3.4% (21/612), lower than the rate reported by another study (18) in high-risk populations in Brazil (12.9%). The inclusion of individuals at medium risk may have contributed to the lower prevalence observed. Nevertheless, further studies should be carried out to establish with greater precision the significance of BRCA mutations in Brazilian women and to assess the prevalence of specific mutations.

References

1. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Secretaria de Atenção à Saúde. Instituto Nacional de Câncer. Coordenação de Prevenção e Vigilância (Brasil): Estimates 2008 - Câncer Incidence in Brazil. Rio de Janeiro: INCA. 2007. http://www.inca.gov.br. Accessed June 10, 2008.
2. Harris JR, Lippman ME, Morrow M. *Diseases of the breast*. Philadelphia: Lippincott Williams & Wilkins; 2004.
3. Nusbaum R, Isaacs C. Management updates for women with a BRCA1 or BRCA2 mutation. *Mol Diagn Ther* 2007; 11: 133-144.
4. Koifman RJ, Koifman S, Vieira RJ. Familial aggregation of breast/ovarian cancer: age of onset along subsequent generations in Brazil. *Cad Saúde Pública* 1998; 14 (Suppl 3): 181-185.
5. Nusbaum R, Vogel KJ, Ready K. Susceptibility to breast cancer: hereditary syndromes and low penetrance genes. *Breast Dis* 2006; 27: 21-50.
6. Welcsh PL, Owens KN, King MC. Insights into the functions of BRCA1 and BRCA2. *Trends Genet* 2000; 16: 69-74.
7. Jara L, Acevedo ML, Blanco R, Castro VG, Bravo T, Gomez F, et al. RAD51 135G>C polymorphism and risk of familial breast cancer in a South American population. *Cancer Genet Cytogenet* 2007; 178: 65-69.
8. Venkitaraman AR. A growing network of cancer-susceptibility genes. *N Engl J Med* 2003; 348: 1917-1919.
9. Llort G, Peris M, Blanco I. [Hereditary breast and ovarian cancer: primary and secondary prevention for BRCA1 and BRCA2 mutation carriers]. *Med Clin* 2007; 128: 468-476.
10. Musolino A, Bella MA, Bortesi B, Michiara M, Naldi N, Zanelli P, et al. BRCA mutations, molecular markers, and clinical variables in early-onset breast cancer: a population-based study. *Breast* 2007; 16: 280-292.
11. Kirchhoff T, Kauff ND, Mitra N, Nafa K, Huang H, Palmer C, et al. BRCA mutations and risk of prostate cancer in Ashkenazi Jews. *Clin Cancer Res* 2004; 10: 2918-2921.
12. Greer JB, Whitcomb DC. Role of BRCA1 and BRCA2 mutations in pancreatic cancer. *Gut* 2007; 56: 601-605.
13. Crum CP, Drapkin R, Kindelberger D, Medeiros F, Miron A, Lee Y. Lessons from BRCA: the tubal fimbria emerges as an origin for pelvic serous cancer. *Clin Med Res* 2007; 5: 35-44.
14. Foulkes WD. Clinically relevant biology of hereditary breast cancer. *Semin Oncol* 2007; 34: 379-383.
15. Berliner JL, Fay AM. Risk assessment and genetic counseling for hereditary breast and ovarian cancer: recommendations of the National Society of Genetic Counselors. *J Genet Couns* 2007; 16: 241-260.

16. Vehmanen P, Friedman LS, Eerola H, McClure M, Ward B, Sarantaus L, et al. Low proportion of BRCA1 and BRCA2 mutations in Finnish breast cancer families: evidence for additional susceptibility genes. *Hum Mol Genet* 1997; 6: 2309-2315.
17. Gomes MC, Costa MM, Borojevic R, Monteiro AN, Vieira R, Koifman S, et al. Prevalence of BRCA1 and BRCA2 mutations in breast cancer patients from Brazil. *Breast Cancer Res Treat* 2007; 103: 349-353.
18. Dufloth RM, Carvalho S, Heinrich JK, Shinzato JY, dos Santos CC, Zeferino LC, et al. Analysis of BRCA1 and BRCA2 mutations in Brazilian breast cancer patients with positive family history. *São Paulo Med J* 2005; 123: 192-197.
19. Ewald IP, Vargas FR, Moreira MA, Filho CM, Cunha DR, Ramos JP, et al. Prevalence of BRCA1 and BRCA2 founder mutations in Brazilian hereditary breast and ovarian cancer families. *J Clin Oncol* 2008; 26 (Suppl 15): 22108 (Abstract).
20. Beller U, Halle D, Catane R, Kaufman B, Hornreich G, Levy-Lahad E. High frequency of BRCA1 and BRCA2 germline mutations in Ashkenazi Jewish ovarian cancer patients, regardless of family history. *Gynecol Oncol* 1997; 67: 123-126.