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Frequency of Mutations in Brca1 and Brca2 Genes in Women with Breast Cancer in Córdoba, Argentina

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Abstract: *The discovery of BRCA1 and BRCA2 genes has led to the introduction of increasingly sophisticated genetic tests to measure the risk of hereditary breast cancer, among other things. The aim of this study was to determine the prevalence of the mutation in 24 women from Córdoba with breast / ovarian cancer (BC / OC) and with at least two relatives with breast cancer. Although there are recurrent mutations, which appear repeatedly in unrelated families, with highest prevalence as in the Jewish ethnicity, this is the first study on the population of the province of Córdoba, Argentina.*

Keywords: *BRCA 1 y 2, mutation,hereditary cancer,breast cancer, ovarian cancer*

1. INTRODUCTION

Breast cancer ranks third in frequency of all patients with cancer in the world and more than six hundred thousand new cases are reported each year. In developing countries it is the second most common cancer (1). In Argentina it is the leading cause of tumor deaths in women. Each year approximately 5,400 women die (1) and it is estimated that about 17,000 new cases (2) are diagnosed. It is estimated that 18,000 new cases will occur per year, which represents 17.8% of total cancer incidence in Argentina. Argentina, after Uruguay, is the American country with the highest death rate from breast cancer (with 20.1 and 24.3 deaths per 100,000 women respectively). While Bolivia, Ecuador and Mexico have the lowest rates (with 7.6, 10 and 10.5 deaths per 100,000 women respectively) .The highest breast cancer mortality rates are between 50 (41.6 per 100,000 women) and 80 years old (215.8 per 100,000 women). Breast cancer is the most prevalent cancer in women, with a rate of 74 cases per 100,000 women. Over 75% of women with breast cancer have no family history of the disease. Breast carcinoma is a heterogeneous neoplasm from clinical, morphological and molecular point of view due to the variability in the expression of multiple genes, where each type of carcinoma presents its own histopathologic features and biological behavior. Regarding the different phenotypical changes a molecular classification in five subtypes has been made, four subtypes of clinical relevance (3):1) subtype overexpressing Her2 neu, Her 2 positive 2) Luminal A-like subtype 3) Luminal B-like subtype 4) Triple negative subtype. The practical importance of the molecular classification of breast carcinoma lies in the prognosis of patients. Worse prognosis subtypes are those that have an over expression of Her2 neu triple negative, presenting a shorter survival and disease-free time. In addition, the triple negative basal subtype has the highest mutation frequency in BRCA1 gene and P53 (4).

For breast cancer early detection is critical because tumors of less than 1 centimeter have up to 90% chance of cure. 1% of breast cancers occur in men (5).

Since the discovery of susceptibility genes for breast cancer (BC) and ovarian cancer (OC), BRCA1 (17q 21, MIM 113705) and BRCA2 (13q 14, MIM 600185) (6), it is known that 5% BC cases can be explained by the existence of pathogenic mutations in these genes. The pattern of genetic predisposition that BRCA1 and BRCA2 genes follow is of high penetrance autosomal dominant type, in which the inheritance of a single mutation in one of these 2 genes confers a high risk of developing the disease throughout life (a 45-85% risk of BC and a 11-63% OC) (7.8). The spectrum of mutations

in the BRCA1 and BRCA2 genes can have large differences in different communities due to their ethnic diversity (9). Some populations or ethnic groups have founder mutations, from a common ancestor, which appear recurrently in most families with BC / OC hereditary of those populations (10, 11).

The state of BRCA1 / 2 mutation in subgroups of patients with breast cancer (BC) and ovarian cancer (OC) selected by age, family history and ethnicity has been little researched outside Europe and North America (12-14), with few reports from South America, where the effects of the founders Native American and the multiracial complex demographics of recent immigration, predict a high genetic variation (15). In fact, recent studies suggest a role of indigenous descent in patterns BRCA1 / 2 disease in Central and North America (16-21). Epidemiological data indicate that in Argentina BC incidence and mortality (22) is among the highest in the world. Historical records and epidemiological and molecular studies point to the variable mix of European, mainly Spanish and Italian, and the natives in more than 50% of Argentina's population (15, 23).Regarding the autosomal evidences of the mix, the European relation, the Native American and Western Africa in the genetic contribution to the Argentinians were estimated at 67.55%, 25.9% and 6.5%, respectively (24). Solano et al (25) conducted the first study that describes the genetic variants BRCA1 / BRCA2 in Argentinian patients with BC and OC, and highlights a significant impact of new mutations and genetic variants that may be regarded as supposedly South American. On the other hand, they confirmed the key role of Ashkenazi Jews BRCA1 and BRCA2 in Argentina.

The aim of this study was to determine the prevalence of the mutation in 24 women from Córdoba with BC / OC and with at least two relatives with breast cancer.

2. PATIENTS AND METHODS

2.1.Patients: The sample included 24 women , index case (first case study) of the city of Cordoba, Argentina with BC / OC. Age range of the sample ranged between 21 and 54 years (x = 29). Inclusion criteria were: a) women diagnosed with breast and / or ovarian cancer; b) age not less than 20 years; c) to accredit two or more relatives affected with breast cancer from the same lineage (same maternal or paternal family line), which was checked by medical and / or death certificate. All participants signed an informed consent, in which the study objectives and confidentiality of the results were explained. These were delivered to patients personally in a genetic counseling session.

2.2. DNA extraction from peripheral blood: Each woman incorporated to the research was extracted 2.5 ml peripheral blood using vacuum tubes with EDTA as anticoagulant. Genomic DNA from peripheral blood leukocytes was isolated using the purification kit (Qiagen, www.qiagen.com) after which it was measured with spectrophotometer Nanodrop-1000 (Thermofisher) establishing DNA concentration in 1 - 2 µl from samples, with a good purity degree. DNA was amplified through PCR (polymerase chain reaction) with specific primers previously described (26) for exons 2, 11 and 20 of the BRCA1 gene (RefSeq: NM 007294.3, NG 005905.2) and exons 11 and 12 of the BRCA2 gene (RefSeq: NM 00059.3; NG 012772.2). Then the photographic record of the 12 amplified fragments was performed by electrophoresis on agarose gel at 1.8% and visualization in UV transilluminator. The amplified products were purified with Wizard SV Gel kit and PCR Clean-Up System (Promega) and the fragments corresponding to the 3 exons of the gene BRCA 1 and 2 exons of the gene BRCA 2 were sequenced in both directions .Sequencing was performed with Big Dye Terminator v1.1 method Cycle Sequencing, technology (Applied Biosystems) with the sequencer ABI PRISM 310 Genetic Analyser (Applied Bio systems). The nomenclature used for the description of the identified variants follows the Human Genome Variation Society (HGVS) guidelines. Table 1 also includes the Cancer Information Core Website Internet (BIC) nomenclature. Unreported sense mutations (missense) or those without clinical significance in the BIC database were analyzed with the Aling-GVGD program (GVGC alignment analysis) (27). This program is a bioinformatics statistical model (predictive free access program) which takes into account the biophysical characteristics of proteins and amino acids (composition, polarity, and volume) that compares in multiple sequence alignments (multiple sequence alignment MSA) amino acids observed at each position, predicting some degree of clinical relevance of reversal substitutions (missense) in the genes of interest. The amino acid changes are grouped in a spectrum ranging from a probably pathogenic to a probably neutral (without clinical significance) position. For the BRCA1 gene, scores of Align-GVGD are classified as follows: C65- most likely to be pathogenic, C35 - C55 - less likely to be pathogenic, C15 - C25 - low probability of being pathogenic, C0 - least likely to be pathogenic.

3. RESULT AND DISCUSSION

After the analysis of the corresponding electropherograms of the 24 samples included in the study, 9 variants, detailed in Table, 1 were identified.

Table1.*Variants identified in 24 samples*

Exon	Variant (amino acid position)	Variant description	Nucleotide according to HGVS	Nucleotide according to BIC	GVGD analysis
11	D693N	p.Asp693Asn	c.2077G>A	2196G>A	CN
11	S694S	p.Ser694Ser	c.2082C>T	2201C>T	CN
11	N743Y	p.Asn743Try	c.2227A>T	2346A>T	CN
11	L771M	p.Leu771Met	c.2311T>A	2430T>A	CN
11	L771V	p.Leu771Val	c.2311T>G	2430T>G	CN
11	P871L	p.Pro871Leu	c.2612C>T	2731C>T	CN
11	E1038G	p.Glu1038Gly	c.3113G>A	3232G>A	CN
11	L1086X	pLeu1086Stop	c.3257T>G	nt3376T>G	MNS
11	K1183R	p.Lys1183Arg	c.3548A>G	3667A>G	CN

HGVS: Human Genome Variation Society. 'A' of the initiation codon (ATG) is numbered as nucleotide +1

BIC: Breast Information Core database. 'A' of the initiation codon(ATG) is numbered as nucleotide +120

GVGD analysis: See text description

CN: Without clinical importance according to BIC(*Clinically No important*)

MNS: Nonsense mutation (*Nonsense*)

In the sample of all cases studied, in 10/24 patients (40%) nucleotide alterations were not found in the analyzed exons and in 15/24 patients (60%) mutational variants were identified that consisted in a nucleotide substitution, no insertions or deletions were found. See Table 2.

From these nucleotide variants, in 2/15 samples (12%) nonsense mutation (nonsense) exon 11 was identified, L1086X c.3275T> G, which generates the appearance of a stop codon (stop) and the presumed abnormal protein synthesis (truncated protein). The remaining 7 variants correspond to nucleotide substitutions without clinical significance according to BIC. **See Table1**

Table2.*Summary of detected mutations - Gen BRCA 1 - exon 11*

Patie	No	Mutation								
nt Nº	Mutation	D693N	S694S	N743Y	L771V	L771M	P871L	E1038G	L1086X	K1183 R
1	+									
2									+	
3	+									
4	+									
5	+									
6								+		
7	+									
8								+		+
9						+				
10									+	
11			+		+			+		+
12								+		
13		+						+		+
14								+		+
15		+						+		+
16								+		+
17						+				

18								+		+
19	+									
20										+
21	+									
22	+									
23							+	+		+
24				+				+		+
Total	**10**	**2**	**1**	**1**	**1**	**2**	**1**	**12**	**2**	**10**
%	**40**	**8**	**4**	**4**	**4**	**8**	**4**	**48**	**12**	**40**

The extreme complexity and diligence of the study of these two genes in addition to the low prevalence of mutations in the population require the careful selection of women from families with chances of detecting a genetic alteration.

Although there is no unanimously established criterion, the main criteria for inherited predisposition risks are: a high number of BC and especially OC cases in the family, an early age at diagnosis and the presence of male BC, among others.

In our sample both cases with relevant mutations come from families with a family history of BC and OC (Table 3), with mutations only in the BRCA1 gene. The results could be biased due to the low number of families analyzed in this group and it would be necessary the study of a greater number of families, and to incorporate the full analysis of both genes with the complete sequencing system (Next Generation sequencing - NGS) because in both genes the mutations are distributed throughout the sequence and differ in each family.

Table3.*Family history of BC / OC and BRCA 1 and BRCA 2*

Patient Nº	**BRCA1**	**BRCA 2**	**Tumor**	**Family History**
1	Negative	Negative	Ovarian papillary serous adenocarcinoma	No
2	**Positive**	Negative	No	Yes
3	Negative	Negative	No	Yes
4	Negative	Negative	No	Yes
5	Negative	Negative	Triple negative breast cancer	No
6	Negative	Negative	Triple negative breast cancer	No
7	Negative	Negative	No	Yes
8	Negative	Negative	No	Yes
9	Negative	Negative	No	Yes
10	**Positive**	Negative	Triple negative breast cancer	Yes
11	Negative	Negative	Luminal B breast cancer	Yes
12	Negative	Negative	Triple negative breast cancer	Yes
13	Negative	Negative	Triple negative breast cancer	Yes
14	Negative	Negative	Triple negative breast cancer	No
15	Negative	Negative	No	Yes
16	Negative	Negative	Triple negative breast cancer	Yes
17	Negative	Negative	Luminal A breast cancer	Yes
18	Negative	Negative	Triple negative breast cancer	Yes
19	Negative	Negative	Triple negative breast cancer	Yes
20	Negative	Negative	ovarian cancer	No
21	Negative	Negative	Triple negative breast cancer	No
22	Negative	Negative	No	Yes
23	Negative	Negative	No	Yes
24	Negative	Negative	Luminal A breast cancer	Yes

4. CONCLUSION

Although there are recurrent mutations, which appear repeatedly in unrelated families, with peak prevalence, as in the Jewish ethnic, this is the first study on the population of the province of Córdoba, Argentina. Finally, we have found polymorphisms, sequence variations that involves a nucleotide substitution and the resulting amino acid change, but with no clinical relevance relating to BC, due to their very low frequency ≤ 1% in the general population.

According to Table 3 results, we observe that patients with BC / OC with and without a family history have the highest percentage of negative BRCA. Same for patients without BC / OC with a family history have a high percentage of negative BRCA. Patients with and without BC / OC with family history and positive BRCA show a low percentage of this gene mutated. Regarding the phenotypic changes the most predominant in this study was the triple negative subtype. Of the two patients with positive BRCA1, do not have tumor and the other patient has Triple negative breast cancer.

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