BRCA1 MUTATION SCREENING IN BRAZILIAN HEREDITARY BREAST CANCER AND OVARY SYNDROME USING HIGH RESOLUTION MELTING

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INTRODUCTION

Breast cancer is the leading cause of deaths in the female population between 40-69 of years age, whereas ovarian cancer is the eighth most commonly diagnosed malignancy in women of any age.

About 10% of cases of breast and/or ovaries cancer are characterized as hereditary or familial, where the presence of cases of cancer in the family is the single most important risk.

Germline mutations in two susceptibility genes called BRCA1 and BRCA2 increase the risk of developing breast or ovarian cancer during the woman's lifetime.

BRCA1 is a tumor suppressor gene involved in DNA damage response, cell cycle control, chromatin remodeling, ubiquitination and transcriptional regulation.

The present study aims to characterize BRCA1 gene mutations that may be associated with Hereditary Breast/ovary Cancer Syndrome (HBOC).

PATIENTS AND METHODS

PATIENTS:

We have analyzed 40 samples of patients from the Cancer Genetic Counseling Service of the General Hospital of the Medical Scholl of Ribeirão Preto, University of São Paulo (USP-HCFMRP) that fulfilled the criteria for genetic testing according to NCCN (National Comprehensive Cancer Network) Clinical Practice Guidelines in Oncology v.1.2010 specific for HBOC.

METHODS:

DNA Extraction~ → High Resolution Melting (HRM) → DNA Sequencing

RESULTS

The previous results revealed one deletion (S616del), eight missense mutations in exon 11 (Gln356Arg, Asp693Asn, Pro871Leu, Glu1038Gly, Ser1040Asn, Val1117Ile, Ser1140Gli, and Lis1183Arg), and two in exon 16 (Ser1613Gli and Met1652Ile). Four synonymous mutations (three in exon 11, Ser694Ser, His771His, and Glu1004Glu and one in exon 13, Ser1436Ser) and four intronic mutations were also identified (Table 1).

The mutation (S616del) was characterized as "pathogenic" by ELLIS et al. (2000) because it fulfills the criteria for pathogenic status such as segregation with the disease, absence in health controls, and the residue is conserved among ortologues of BRCA1. However, other studies do not consider this mutation as pathogenic.

Despite the eight non-synonnymous mutations have already been found in women with breast or ovarian cancer, there is no evidence in the literature that these mutations are pathogenic.

The figure 1 shows the results of the exon 16 analysis by HRM. We can see five melt curves pattern related with the Ser1613Gli and Met1652Ile mutations. All pattern were confirmed by sequencing technique (Figure 1C).

The mutation, Met1652Ile, was identified in one of the BRCT domain of BRCA1. This domain is responsible for the BRCA1 protein interaction with other DNA repair proteins and also with proteins that participate in cell cycle checkpoint. This interaction facilitates DNA repair by BRCA1 protein by homologous recombination.

Table 1. BRCA1 mutations identified in familial breast cancer patients

Number of patients	Exons	Nucleotide *	Protein
13	Íntron 7	26456 C>T	No effect
7	Íntron 8	29023 del T	No effect
2	11	31906 A>G	Gln356Arg
1	11	32684-32686 del TCT	S616del
2	11	32916 G>A	Asp693Asn
22	11	32921 C>T	No effect
19	11	33150 T>C	No effect
26	11	33451 C>T	Pro871Leu
1	11	33851 G>A	No effect
21	11	33 952 A>G	Glu1038Gli
5	11	33958 G>A	Ser1040Asn
1	11	34188 G>A	Val1117Ile
2	11	34257 A>G	Ser1140Gli
20	11	34387 A>G	Lis1183Arg
20	13	43917 T>C	No effect
20	16	55292 A>G	Ser1613Gli
2	16	55412 G>A	Met1652Ile
3	Íntron 17	62366 C>T	No effect
1	Íntron 21	75322 G>C	No effect

* The position of mutated nucleotides followed the numbering of the reference sequence : NM_007294.3

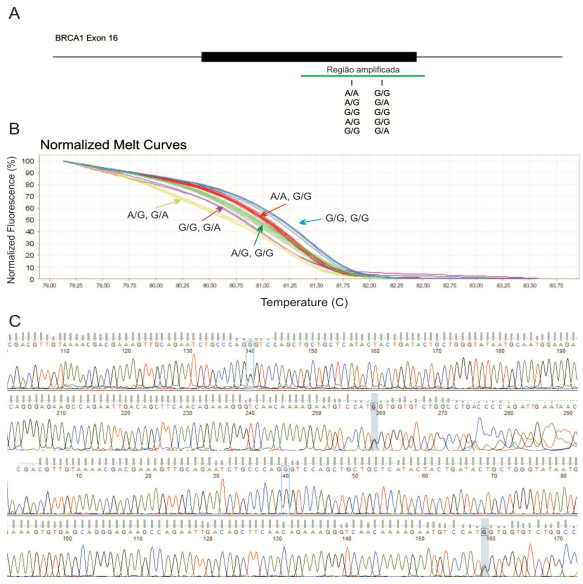

Figure1: A) Schematic representation of the amplified region of exon 16 containing the mutations Ser1613Gli and Met1652Ile. B) Specific melting curves for each genotype identified. C) Sequencing results of samples with genotype A/G, G/A e G/G, G/A.

REFERENCES

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ELLIS D, *et al*. Low prevalence of germline BRCA1 mutations in early onset breast cancer without a family history. J Med Genet. 2000; 37(10): 792–794.

CAPES CNPq Hemocentro RP