Sequencing analysis of germline breast cancer mutations in Brazilian population

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ABSTRACT

Aims: Next-generation sequencing (NGS) allows sequencing the wide variety of cancer susceptibility genes. Hereditary breast cancer (BC) is found in 5 to 10% of the cases. Germline mutations are associated with the development of BC in younger women. The aim of this work is to describe germline mutations found in young Brazilian patients diagnosed with BC.

Methods: DNA samples of peripheral blood cells from 32 young patients (<45 years) submitted to neoadjuvant therapy with no family history of BC were sequenced for the exons of 16 genes related to hereditary BC. Pathogenic mutations were analyzed based on known databases.

Results: Pathogenic mutations were identified in 5 patients. Two patients were *BRCA1* (6%), one *BARD1* (3%) mutated, one patient (3%) harbours mutations in both genes and another has *BARD1* and *BRIP1* mutations (3%). *BRCA1* mutations were only detected in Triple Negative (TN) patients, including one with *BARD1* mutation. Two of them presented pathologic partial response (one with and another without relapse after two years). Luminal A patients analysis detected *BARD1* mutation (pathologic complete response) and another with *BRIP1* and *BARD1* mutations (relapse after two years). Variants not yet described were found in three HER2 patients: two with pathologic partial response (*BRCA2* and *CHEK2*) and one with pathologic complete response (*NBN*).

Conclusions: Mutations in *BRCA1* were related to TN subtypes of BC. Pathologic complete response was not detected in patients with only *BRCA1* mutations. This study may contribute to the screening of germline mutations in patients with BC in Brazil.

INTRODUCTION

Breast cancer (BC) is the leading cause of cancer death in women. About 5 to 10% of these cases are associated with germline mutations in specific genes that are strongly related to hereditary breast cancer (HBC). Studies on HBC in Brazil are important to identify specific mutations related to the Brazilian population. Next-generation sequencing (NGS) is a revolutionary tool which allows sequencing a large amount of DNA sequences in reduced time.

MATERIAL AND METHODS

DNA samples from peripheral blood cell of 32 young women (<45 years) submitted to neoadjuvant therapy with or without family history of BC were extracted and target sequencing of the exons of 17 genes (*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, PALB2, PTEN, RAD50, RAD51C, STK11* and *TP53*) related to hereditary BC were performed by the PGM Ion Torrent NGS Platform (Fig. 1). The Human reference genome (hg19) was used as reference with Q-score>20 (Q20).

Intronic regions including 25 nucleotides upstream and downstream of the exon were also analyzed. Pathogenic mutations were described based on online tools. Sanger sequencing was used to confirm the results.

BRCA1	*BRCA2*	*TP53*	*ATM*	*BARD1*	*BRIP1*
CDH1	*CHEK2*	*PALB2*	*PTEN*	*RAD50*	*RAD51C*
	STK11	*MRE11A*	*MUTYH*	*NBN*	*TWIST1*

Figure 1. Hereditary Breast Cancer Panel covers 17 known genes related to hereditary breast cancer (HBC).

RESULTS

Young women (<45 years) were selected according with the subtype and pathologic response. The group 1-19 are patients from Hospital Ophir Loyola, Belém, Brazil; and patients 20-32 were HER2+ selected from the Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil (Table 1).

The pathogenic described mutations were identified in 5 patients (Table 2). Two Patients (2 and 11) were *BRCA1* (6%) with pathologic partial response (PPR). In addition, the *BARD1* (3%) mutation was found in one patient (14) (3%) with pathologic complete response (PCR). The Patient 9 harbours mutations in both genes (3%) and the patient 16 presented *BARD1* and *BRIP1* mutations (3%) and PPR. All *BRCA1* mutations were detected only in Triple Negative (TN) patients, including one with *BARD1* mutation (9). Two of them presented pathologic partial response (one with and other without relapse after two years). Luminal A patient analysis detected *BARD1* mutation with PCR in the patient 14 and other (16) with *BRIP1* and *BARD1* mutations (relapse after two years and PPR). These *BARD1* variants are probably polymorphisms, because all of them are at the same locus and recent analysis published in Clinvar has classified them as benign. Variants not yet described were found in three HER2 patients: *BRCA2* (24) and *CHEK2* (25) with PPR and one *NBN* mutant with pathologic complete response (21) (Table 2). This *BRCA2* sequence change (24) generated a stop codon at position 616 of the protein and the *NBN* frame shift created a termination codon at position 719. In both cases, such mutations are probably deleterious.The mutations of the first 5 patients were confirmed by Sanger Sequencing (fig. 2).The *NBN* and *BRCA2* mutations will be confirmed.

Table 1 - Description of the patients analyzed in this study

Patient	age	subtype	response	Patient	age	subtype	response
1	25	Luminal/HER2	P	18	36	TN	P
2	37	TN	P	19	34	HER2	P
3	33	Luminal B	C	20	36	HER2	P
5	39	Luminal B	P	21	40	HER2	C
6	31	TN	P	22	42	HER2	P
7	35	Luminal A		23	33	HER2	P
8	30	Luminal/HER2	C	24	42	HER2	P
9	37	TN	WC	25	32	HER2	P
10	39	Luminal B		26	26	HER2	P
11	28	TN	P	27	44	HER2	P
12	35	Luminal B	P	28	44	HER2	C
13	38	Luminal//HER2	C	29	36	HER2	C
14	37	Luminal A	C	30	44	HER2	P
15	35	Luminal A	C	31	37	HER2	P
16	30	Luminal A	P	32	43	HER2	C
17	37	Luminal A	P				

C - pathologic complete response
P - pathologic partial response
WC: without chemotherapy
TN: Triple Negative

Table 2. Pathogenic Variants found in this study

Patient	gene	position	protein
2	*BRCA1*	g.41242980_41242981delCT	p.Ser1389Terfs
9	*BRCA1*	g.41219623A>G	splice site
9	*BARD1*	g.215645503_215645523del21	p.Leu340_Pro346del
11	*BRCA1*	g.41209082dup	p.Gln1756Profs
14	*BARD1*	g.215645503_215645523del21	p.Leu340_Pro346del
16	*BRIP1*	g.59886018A>G	p.Ile243Thr
16	*BARD1*	g.215645503_215645523del21	p.Leu340_Pro346del
21	*NBN*	g.90994963delAA	p.Ser53CysfsTer719
24	*BRCA2*	g.32907462delT	p.Cys616X

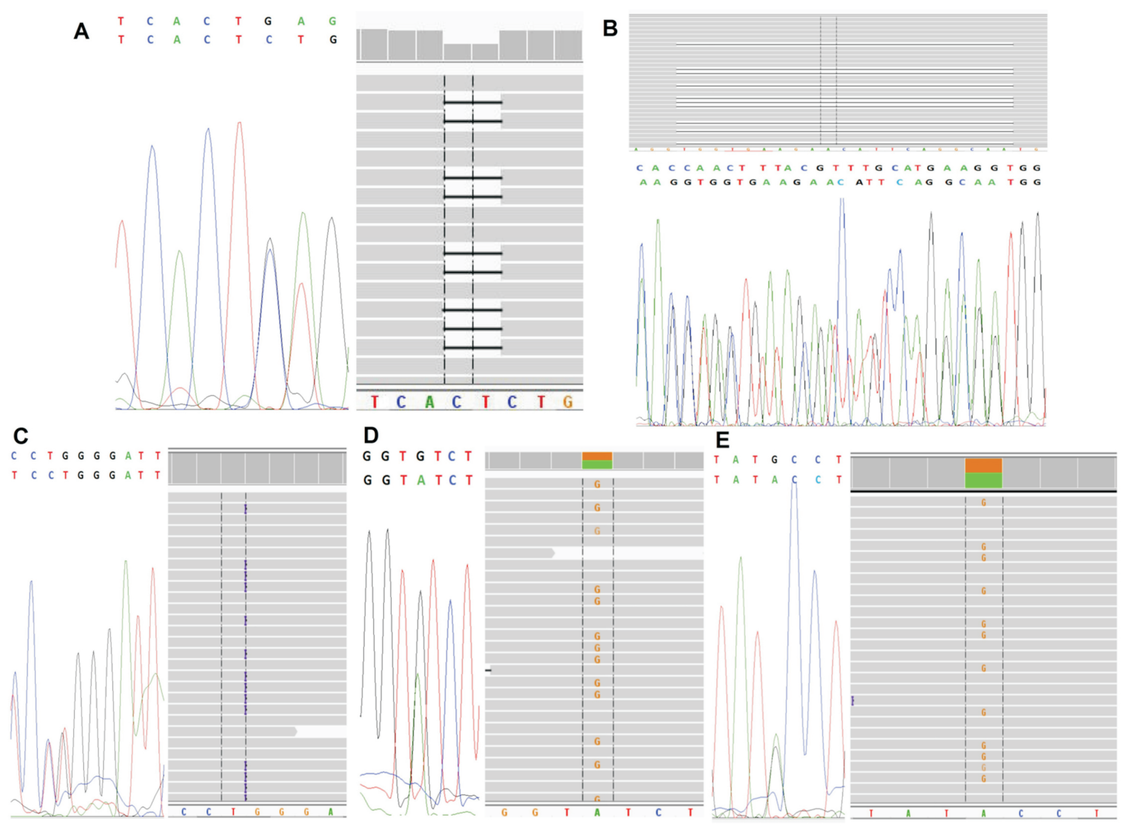

Figure 2. Sanger sequencing electropherogram. The upper sequence represents the reference genome and below the variant sequence. The other image shows readings generated from the IGV software that represent the fragments (reads) sequenced by NGS (A-E). A. Patient 2: *BRCA1*; B. Patients 9, 14 and 16: *BARD1;* C. Patient 11: *BRCA1*; D. Patient 16: *BRIP1*; E. Patient 9: *BRCA1*.

CONCLUSION

Mutations in *BRCA1* were related to TN subtypes of BC. Pathologic complete response was not detected in patients with only *BRCA1* mutations. These data confirm the correlation between the most aggressive subtype of BC (TN) with *BRCA1* mutations. This study may contribute to the screening of germline mutations in patients with BC in Brazil.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA