Characterization of hereditary cancer predisposition in *BRCA1/2* negative patients with high-risk for breast and ovarian cancer

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INTRODUCTION

The next-generation sequencing (NGS) technology has revolutionized the clinical approach to genetic testing in medical oncology[1]. Instead of single gene testing interrogating, using a panel of multiple genes provides clinic information about genes involved in cancer susceptibility in a single test. It has been increased the detection of cancer predisposing variants offering advantages in time and cost compared with single gene testing[1,2]. A limited number of studies have investigated other genes but *BRCA1* and *BRCA2* for hereditary breast and ovarian cancer syndrome (HBOC) in Brazilian patients. These studies could clarify which genes are associated with this disease and how to counsel patients and their families regarding penetrance, screening, surveillance, and risk-reducing options. In this study, we sought to identify genetic variants in individuals who met current National Comprehensive Cancer Network (NCCN) high-risk criteria and previously tested negative for pathogenic *BRCA1* and *BRCA2* mutations.

MATERIAL AND METHODS

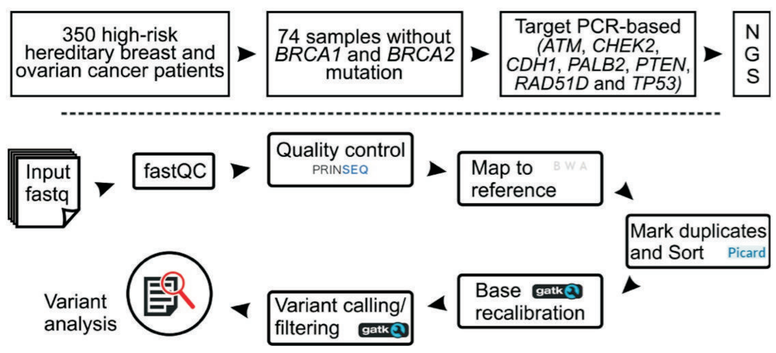

Figure 1. Workflow summary of experimental design and analysis pipeline.

RESULTS

Characteristics of study population are shown in table 1. Of the 74 high-risk women who had previously tested negative for *BRCA1* and *BRCA2* pathogenic mutations 24 were analyzed until now. Among these 24 women, 75% had at least one first- or second-degree relative with breast cancer. Age at diagnosis ranged from 27 to 74 years, with an average age of 44.76 years.

Table 1. Clinical features of patients analyzed.

Feature	n	%
Cancer type		
Breast cancer only (BC)	19	79.16%
Ovarian cancer only (OC)	4	16.66%
Ovarian and breast cancer	1	4.16%
Age at diagnostic		
≤50 years	22	91.66%
>50 years	2	8.33%
Tumor location		
Unilateral	19	79.16%
Bilateral	5	20.83%
Proband familial history of cancer		
Only first degree relatives	3	12.5%
1st and 2nd degree relatives	2	8.33%
Only 2nd degree relatives	2	8.33%
Only 3th degree relatives	3	12.5%
2nd and 3th degree relatives	7	29.16%
1st, 2nd and 3th degree relatives	7	29.16%

All coding exons and consensus splice sites of 7 cancer genes tested were screened for mutations. To date, twenty-four samples were sequenced and among 1403 target amplicons just 3% did not reach the coverage of at least 27x. Overall, 70 different genetic variants were identified, 22 (31%) occurred in *ATM*, 16 (23%) in *CDH1*, and 12 (17%) in *PTEN*, 10 (14%) in *RAD51D*, 6 (9%) in *TP53* and 4 (5.71%) in *PALB2* (Figure 2). Categorizing by molecular consequence we had: 23 intronic variations (were not localized into consensus splicing site), 21 missense, 15 localized into untranslated regions, and 11 synonymous. By clinical significance: 32 benign, 18 uncertain significance (VUS), 17 likely benign, 3 conflicting interpretation of pathogenicity.

Of the nineteen VUS discovered in this analysis (listed in Table 2), nine were novel, and just three of these were not predicted to be deleterious by standards and guidelines for interpretation of sequence variants provided by American College of Medical Genetics and Genomics. Following these criteria specific variants were classified in two distinct categories (Table 2): c.2131A>G, c.732G>A, c.997C>T. The variants c.*220_*221delTT and c.*221del were detected in a single patient and were distinct from the reference sequence. One of these was predicted to be pathogenic moderate and the other was predicted to be a VUS. This finding is consistent with the observation that many high-risk women who undergo testing for HBOC are found to be carriers of one or more VUS, generally missense, unannotated in respect to disease risk[3,4,5].

Table 2. Variants of uncertain significance (VUS) report detected in this work and its pathogenicity classification

Times reported	Gene	HGVSc	ID	HGVSp	Consequence	ClinVar Classification	ACMG classification
1	ATM	c.2130A>G	Novel	p.Thr710=	Synonym	VUS	Pathogenic moderate
1	ATM	c.2131A>G	Novel	p.Asn711Asp	Missense	VUS	Pathogenic moderate, benign supporting
1	ATM	c.3256C>G	Novel	p.Arg1086Gly	Missense	VUS	Pathogenic moderate
1	ATM	c.7788+8G>T	rs112775908	na	Intronic	VUS	Benign supporting
1	ATM	c.4362A>C	rs148993589	p.Lys1454Asn	Missense	VUS	Benign supporting
1	ATM	c.1595G>A	rs35963548	p.Cys532Tyr	Missense	VUS	Likely pathogenic
*1	CDH1	c.*220_*221delTT c.*221del	Novel rs369254048	na	3'UTR	VUS	Pathogenic moderate na
21	CDH1	c.*221del	rs369254048	na	3'UTR	VUS	na
22	CDH1	c.*475del	rs113202135	na	3'UTR	VUS	na
1	PTEN	c.165-13_165-10del	Novel	na	Intronic	VUS	Benign supporting
1	PTEN	c.253+93_253+94ins30	Novel	na	Intronic	VUS	Pathogenic moderate
1	PTEN	c.492+14del	Novel	na	Intronic	VUS	Pathogenic moderate
1	PTEN	c.802-674_802-672del	Novel	na	Intronic	VUS	na
1	PTEN	c.802-278G>A	rs115962293	na	Intronic	VUS	na
2	PTEN	c.802-359T>A	rs35755883	na	Intronic	VUS	Benign supporting
5	PTEN	c.802-400T>C	rs17431184	na	Intronic	VUS	Benign supporting
1	RAD51D	c.732G>A	Novel	p.Leu244=	Synonym	VUS	Pathogenic moderate, benign supporting
1	TP53	c.997C>T	Novel	p.Arg333Cys	Missense	VUS	Pathogenic moderate, pathogenic supporting
22	TP53	c.96+41_97-54del	rs150200764	na	Intronic	VUS	Benign supporting

ID – Identification of variant in National Center for Biotechnology Information (NCBI); na - not available or does not apply; * homozygous alternate.

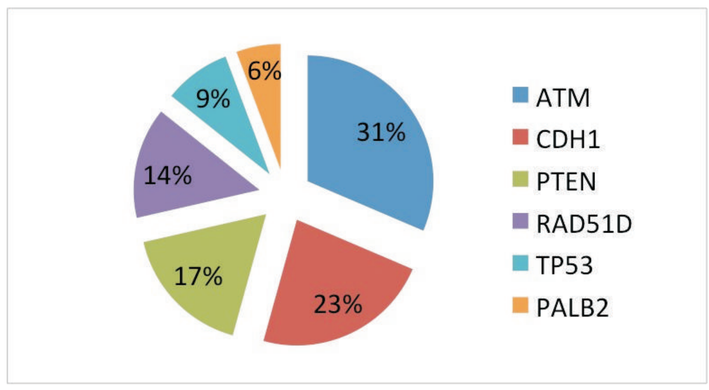

Figure 2. Pie chart showing the Percentage of mutations across 7 genes analyzed in this work.

DISCUSSION AND CONCLUSION

Multiple-gene testing studies have been reporting a prevalence of 10.2% to 13% deleterious or likely deleterious variant in other genes associated with breast and ovarian cancer but *BRCA1* and *BRCA2*[3,4,5]. Despite selecting for patients with high risk of genetic inheritance, none of the subjects in our sample were found to harbor a pathogenic variant. However, our sample size was too limited, and NGS approach have limitations in the identification of large genomic rearrangements, therefore, these types of variants could still be present in our patient population. The method developed by our group to obtain all coding regions using PCR-based target sequencing was efficient due to high coverage achieved and potential to identify distinct genetic variants.

References

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