Searching for new genes associated to hereditary breast cancer using whole exome sequencing.

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BACKGROUND: Hereditary breast cancer (HBC) represents 5-10% of all the breast cancer cases and it is associated with mutations in certain genes, mainly BRCA1 and BRCA2 (BRCA1/2). Both genes are involved in DNA double-strand break repair. Around 20% of Brazilian women with clinical criteria for HBC are BRCA1/2 mutation carriers (Silva et al., 2014). The remaining 80% are women who either are carriers of mutation in other less prevalent genes like TP53, CHEK2, ATM, BRIP1, PALB2, among others, or are negative for all known genes associated to HBC. Thus, more efforts are still necessary for the identification of new predisposing genes in HBC patients negative for the BRCA1/2 mutations.

HYPOTHESIS: By using whole exome sequencing (WES) of unrelated BRCA1/2 negative patients and family-based analysis, new putative genes associated to HBC can be identified.

METHODS: We investigated 17 women (16 unrelated patients and one affected sister) diagnosed with breast cancer before 40 years old, negative for pathogenic mutations in BRCA1/2 and with a well-established family history of cancer. Additionally, five germline BRCA1-mutation carriers were included as controls. All participants signed an informed consent term. DNA from 21 blood-samples was submitted to WES using the SOLiD and Ion Proton platforms. For variant calling, the criteria of 10X minimum coverage and minimum frequency of 20% were used. Variants present in dbSNP with frequencies higher than 1% were excluded. First, we selected all genes with frameshift or nonsense mutations as potential candidates. Additionally, among those genes with missense mutations, we selected those involved either in homologous recombination repair process or associated to cancer. Next, the selected variants were validated in the same samples by using a customized panel (Ion AmpliSeq™). In order to remove common variants, a group of 25 health women, with no personal history of cancer, was used for filtering.
Additionally, for three unrelated patients we carried out co-segregation analysis to find strong candidate genes for HBC.

RESULTS:
From the WES analysis we identified 642 variants detected among the 17 patients and absent in any of the 5 control samples. Then, we applied filters based on the gene functions and looked at each case individually to select a total of 361 distinct variants, being 63 frameshift indels and 298 SNVs. Of these, 320 (88,64%) were validated by using target sequencing – 122 loss of function (frameshift and nonsense) and 198 missense variants, being 41 in genes belonging to homologous recombination process and 157 in cancer-associated genes. After removing the common variants and selecting the co-segregating variants, 273 genetic variants in 220 genes constitutes our final list of candidate genes for breast cancer predisposition and are currently under analysis.