FUNCTIONAL EVALUATION OF BRCA1 VARIANTS IN THE COILED-COIL REGION USING TRANSCRIPTION ACTIVATION AND PALB2 INTERACTION: A NEW APPROACH

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Introduction and objectives. Germline mutations in BRCA1 are responsible for the majority of cases involving hereditary breast and ovarian cancer. Genetic counseling based on comprehensive analysis of alterations in BRCA1 may assist affected individuals to adopt preventive procedures. However, many of the mutations found in patients do not have a clear designation as either pathogenic or not, remaining as variants of uncertain clinical significance (VUS). Functional assays provide an approach that may circumvent this problem, generating complementary data to support classification. In this work we have assessed the functional implication of missense VUS located on BRCA1 coiled-coil region (amino acids 1293-1560). Materials and methods. In order to test BRCA1 VUS functional effects we have performed the transcriptional activation (TA) assay and a mammalian two-hybrid (M2H) approach using BRCA1 C-terminus coding plasmid constructs (aa 1315-1863). The TA assay is used to evaluate structural integrity of BRCA1 C-terminus based in its transcriptional activation ability. The M2H system was conducted to evaluate BRCA1/PALB2 interaction using the BRCA1 constructs as baits for the N-terminal region of PALB2 (aa 1-315) - PALB2 interacts with BRCA1 coiled-coil region and abrogation of the this interaction has been shown to compromise homologous recombination (HR). Results. Seven VUS already described in population were evaluated. A non-pathogenic behavior (TA and PALB2 interaction similar to wildtype BRCA1) was observed for Q1395R, M1400I, I1405V, E1419Q and H1421Y variants. In our assessment, L1404P and M1411T variants had a pathogenic behavior and may be cancer-associated variants. Conclusions. We have shown that the TA assay is capable of predicting the functional impact of VUS in the coiled-coil region of BRCA1 and the use of a secondary functional approach (PALB2 interaction) may be an interesting way to better understand the impact of VUS harbored in this region and its vicinity.

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