# Functional Analysis of BRCA1 variants: a snapshot of the linker region

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**INTRODUCTION:** *BRCA1* is a tumor suppressor gene crucial for the maintenance of genome integrity. Germline mutations that lead to a dysfunctional protein can increase the risk of breast and ovarian cancer. Functional assays represent an important tool to contribute to pathogenicity classification of low frequency variants, mostly missense. **OBJECTIVES:** Since 2007, our group have been working a functional assay that correlates BRCA1 transcriptional activity and its C-terminus integrity (TA assay). In this work, we focused on the region that links the two tandem BRCT domains (linker). **MATERIAL AND METHODES:** Using bioinformatics strategies (Align-GVGD, SFIT and PolyPhen-2) we predicted and scored all possible mutations located in the linker region (136 mutants) but variants already studied/classified (16 mutants). Only 23 variants were predicted in *silico* as cancer associated by all methods. We generated and cloned the 23 variants predicted as pathogenic into pCDNA3 vector, coding a fusion protein (DNA binding domain fused to BRCA1 C-terminus). The constructs were used to assess functional data through the TA assay (using Dual-Luciferase Reporter Assay System, Promega). **RESULTS AND DISCUSSION:** Assays were originally conducted at 37ºC. Most variants (20 mutants) behaved similar to pathogenic controls, whilst 3 variants (all in 1740 position) appear to behave as positive controls. Variants in 1740 position demonstrate a significant variation in transcription activity (replicates and in different experiments), featuring a possible thermosensitive property. Therefore, all variants were tested at 30ºC. The 1740 variants sustained a non-pathogenic behavior, however other 14 variants displayed an increase in transcriptional activity. We did not observe any significant change in the linker secondary structure due to nucleotide change using the Ramachandran diagram approach. **CONCLUSIONS:** The differences in transcription activity observed 37ºC and 30ºC suggest that the linker region is possibly a thermosensitive segment in BRCA1 structure. We also generated data that will support mathematical models to predict the pathogenicity of BRCA1 variants.

**Keyword:** BRCA1, variants, functional assay
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