READTHROUGH COMPOUND AS SUPPRESSION AGENT OF NONSENSE MUTATIONS IN *BRCA1*

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Mutations in *BRCA1* are responsible for most cases of hereditary breast and ovarian cancer syndrome (HBOC). Nonsense variants account for ~ 13% of mutations in *BRCA1* gene; they are characterized by a premature stop codon (PTC) that encodes a truncated protein. Different studies have shown that some compounds, like aminoglycosides, can induce readthrough of PTCs, restoring the function of the protein. The use of these compounds may represent an important strategy for the prevention of hereditary breast and ovarian cancer in HBOC patients. Our study intends to evaluate the use of aminoglycosides on the restoration of tumor suppressor activity of nonsense variants of the *BRCA1* gene. Twelve variants coding PTC in the BRCA1 C-terminus were generated and cloned into pQCXIH in a fusion with EGFP or GAL4 DBD. HeLa cells constitutively expressing the nonsense variants were tested in the presence and absence of G418 and evaluated for full-length protein synthesis restoration using flow citometry. However, restoration of full-length protein levels does not reflect their biological functional status. BRCA1 ability to interact with CtIP was used to evaluate this issue. Functional restoration was observed for a limited group of variants. *BRCA1* missense variants representing the most probable acquired mutations consequence of the readthrough event of the original nonsense mutants were identified. Their impact in BRCA1 biological function was evaluated by the transcription activation assay. The results corroborate BRCA1-CtIP interaction data. This is the first study that evaluates the readthrough of nonsense variants with clinical relevance in *BRCA1* gene.

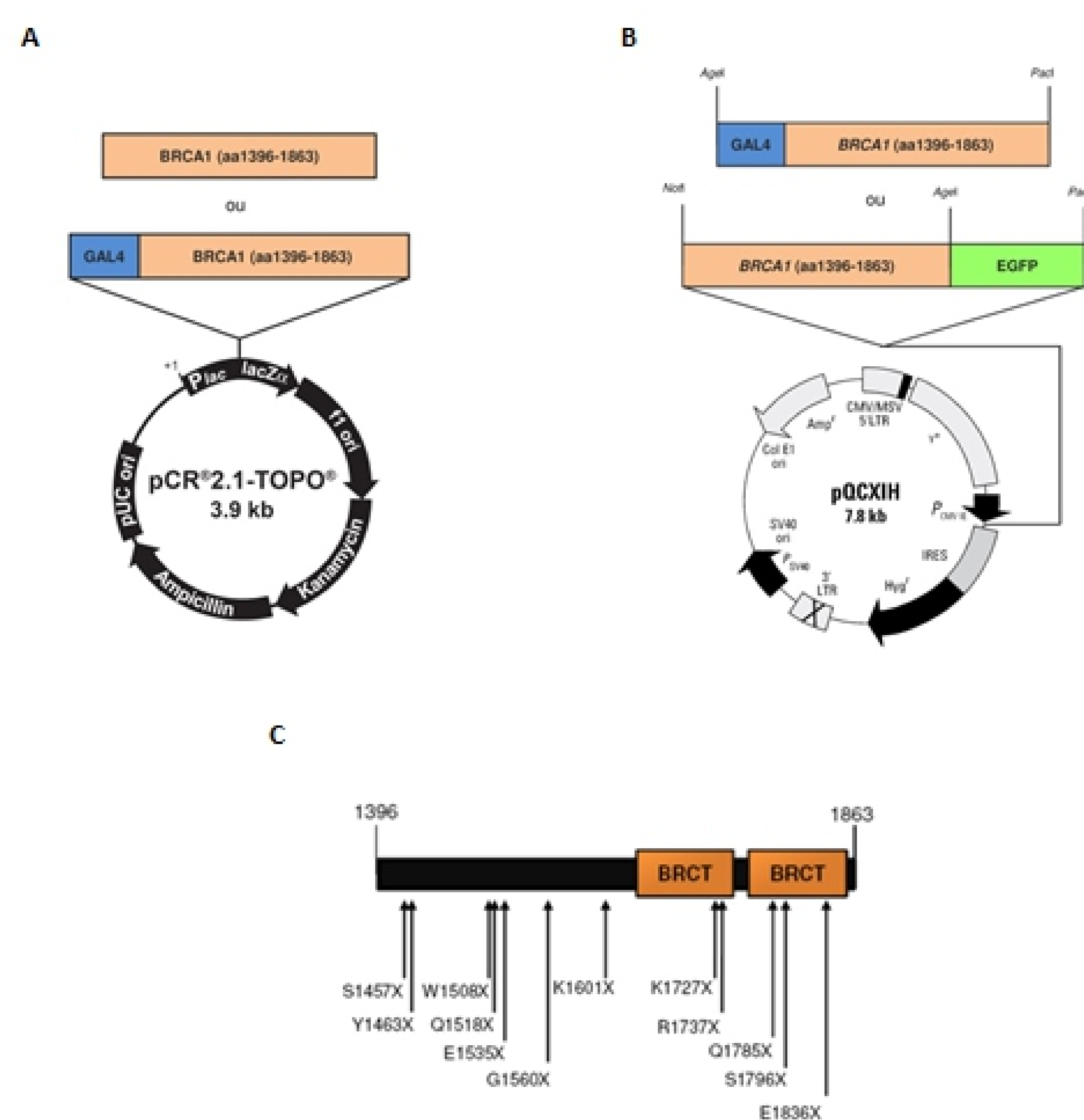

Figure 1: Schematic representation of cloning vectors and BRCA1 variants selected for this study. The coding sequences for fusion proteins (EGFP in green, BRCA1 aa1396-1863 in orange and GAL4DBD in blue), including selected variants **(C)**, were initially cloned using TOPO® Cloning System **(A)**. Subsequently, they were subcloned into pQCXIH **(B)**.

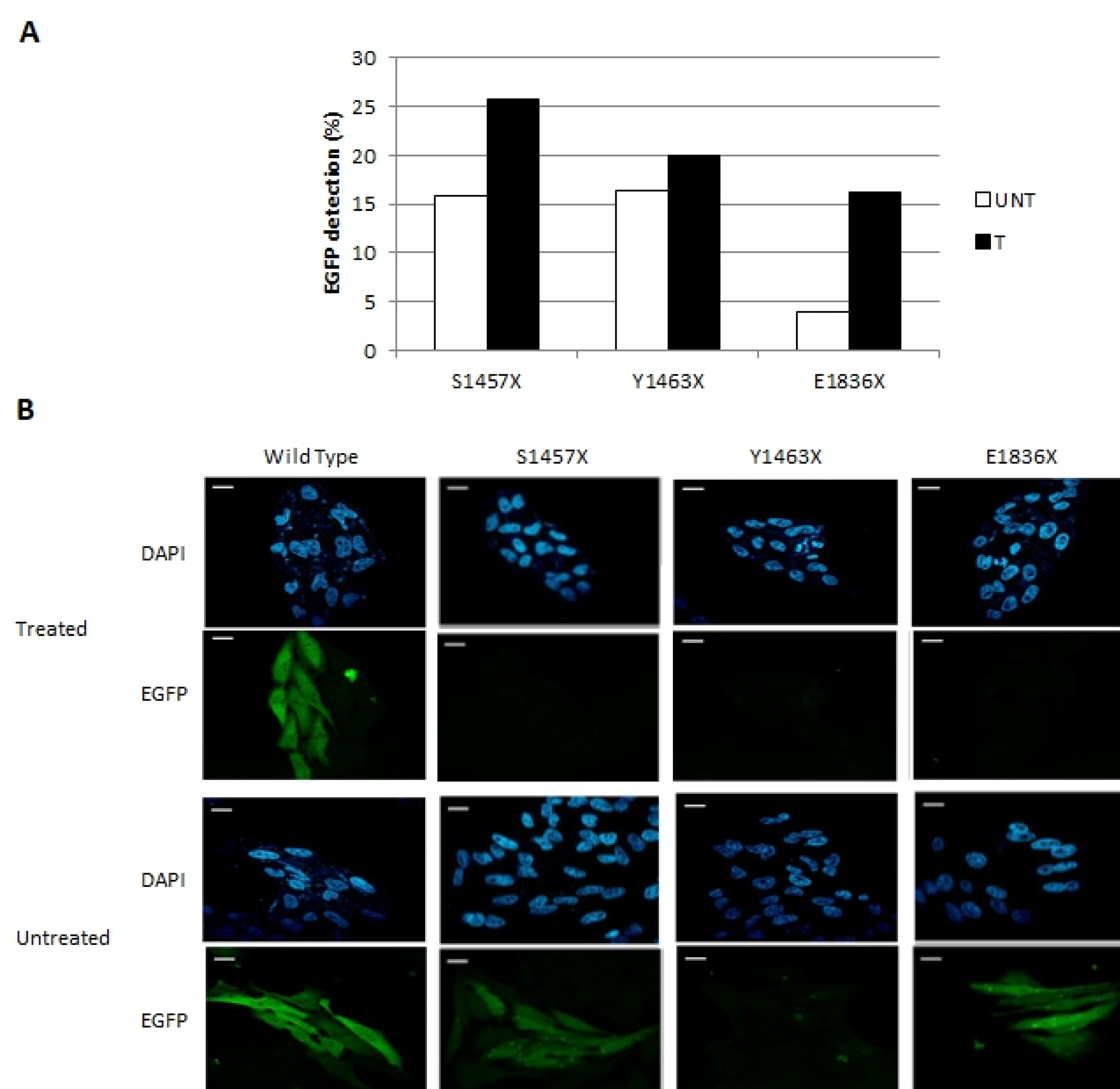

Figure 2: Readthrough analysis by flow cytometry and confocal microscopy. (A) EGFP detection in HeLa cells expressing BRCA1 variants, treated (T) or not (UNT) with G418 (300mg/mL); (B) The panel displays an experiment of induced readthrough 48 hours after treatment. HeLa cells expressing BRCA1 wild type construct show a constitutive expression of BRCA1:EGFP. Cells expressing BRCA1 variants only reveal EGFP signal in the presence of G418.

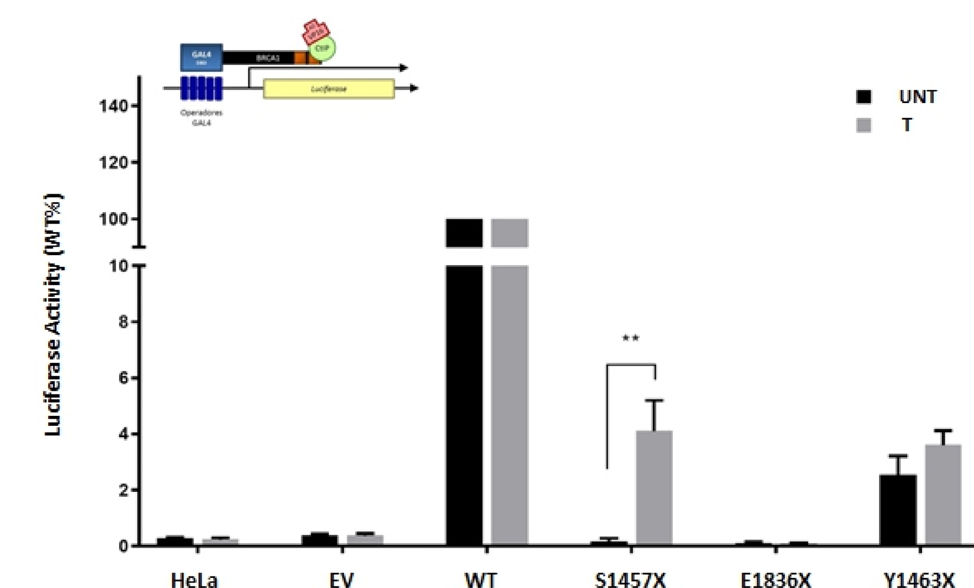

Figure 3: Readthrough analysis by transcriptional activation assay. Activity of wild type (WT) or variants GAL4:BRCA1 constructs was assessed by its interaction with CtIP fusioned with VP16AD. All variants showed very low activity in the abcense of G418 (UNT), in contrast with treated cells (T).

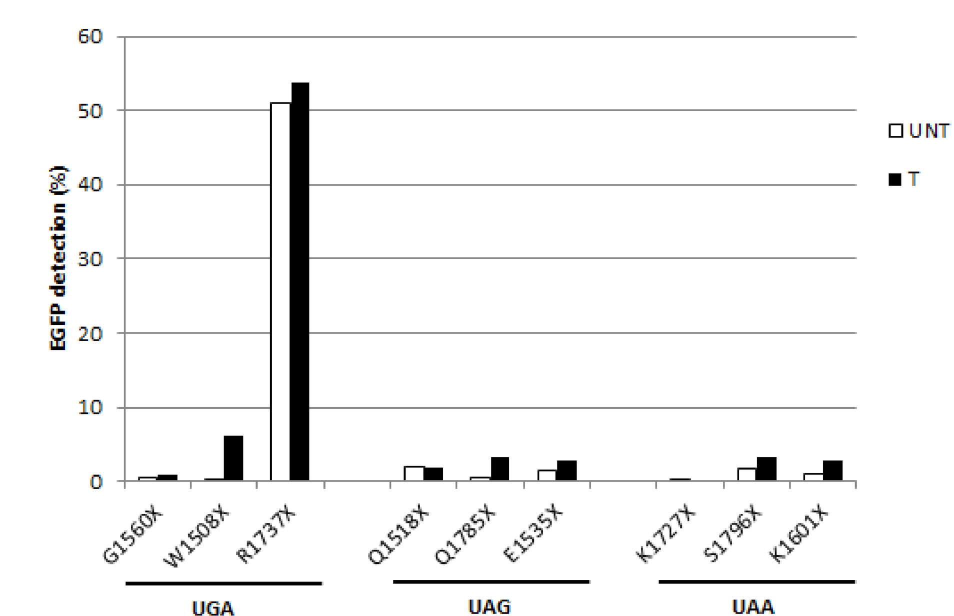

Figure 4: Readthrough analysis by flow cytometry. Graphic displays EGFP detection in HeLa cells expressing BRCA1 variants, treated (T) or not (UNT) with G418 (300mg/mL)

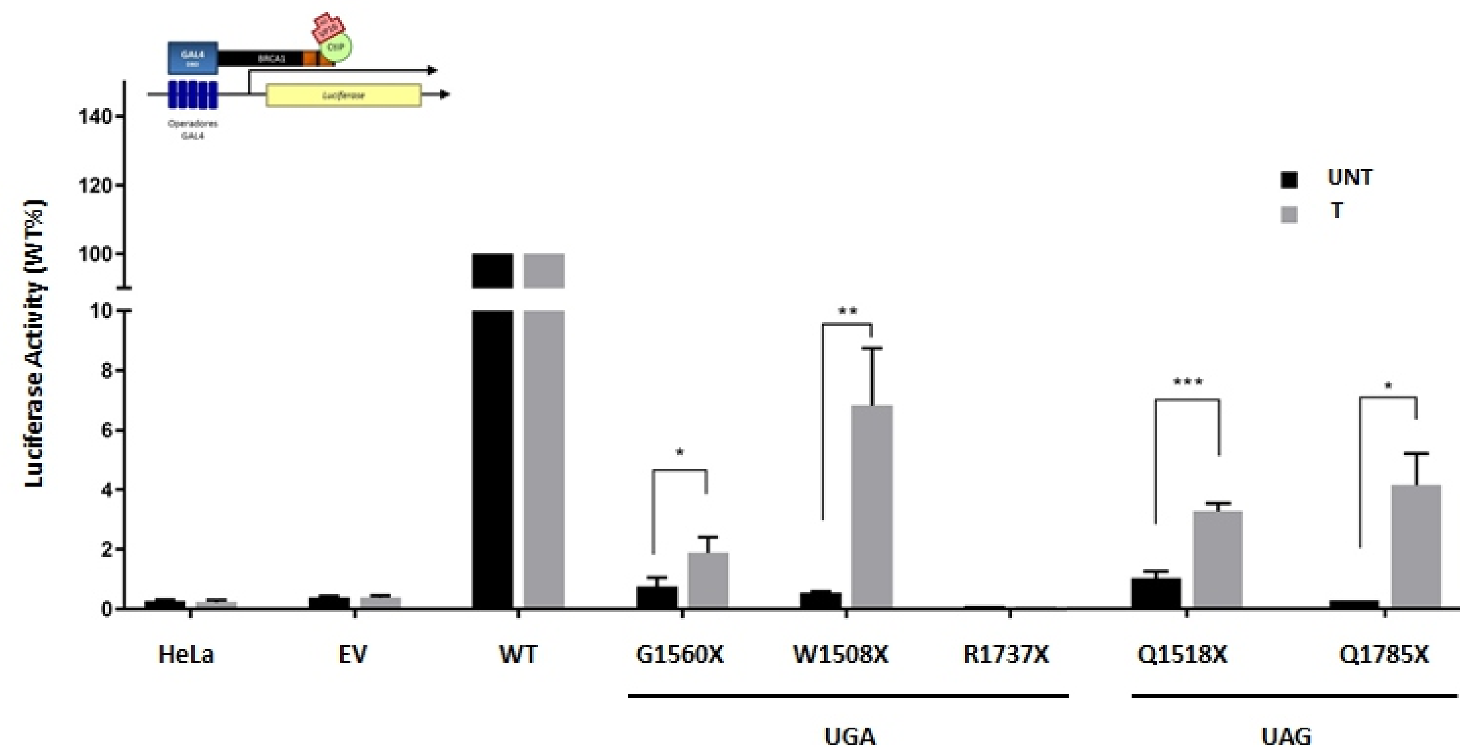

Figure 5: Readthrough analysis by transcriptional activation assay. Activity of wild type (WT) or variants expressing the fusion protein GAL4:BRCA1 was assessed by its interaction with CtIP fusioned with VP16AD. All variants showed very low activity in the abcense of G418 (UNT), in contrast with treated cells (T).

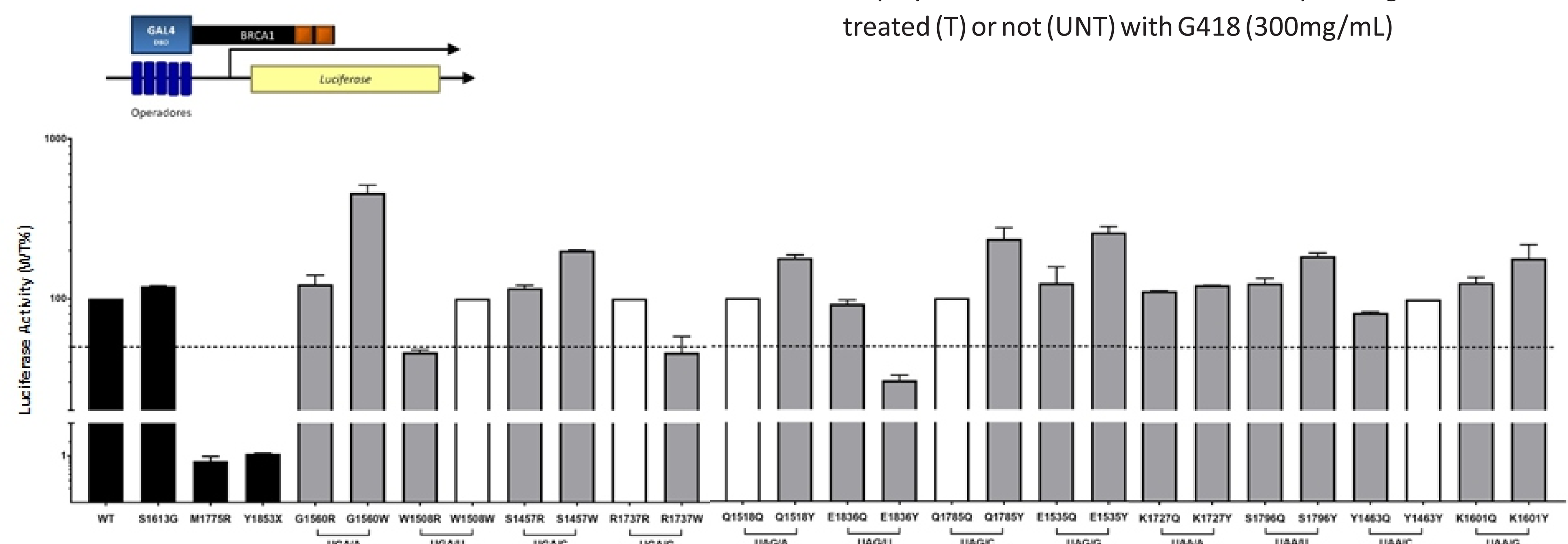

Figure 6: BRCT integrity assessement by transcriptional activation assay. Activity of wild type (WT) or variants expressing the fusion protein GAL4:BRCA1 was assessed by a luciferase reporter activity. All variants presented diferent activity levels, showing distinct impacts on BRCT's structure.

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