# Classification of 15 new BRCA2 exons 2-9 splicing variants by hybrid minigenes

Fraile-Bethencourt E., Valenzuela-Palomo A., Díez-Gómez B., Acedo A., Velasco EA.
Institute de Biología y Genética Molecular (IBGM) – University of Valladolid/CSIC - Spain

## Introduction

Deleterious variants in *BRCA2* gene are involved in a higher risk of breast, ovarian, prostate and pancreatic cancer (Petrucelli et al., 2016). Moreover, biallelic mutations are associated with Fanconi anemia (Howlett et al., 2002). According to ClinVar, more than 9,000 variants have been identified, but approximately 50% of them are still classified as variants of uncertain significance (VUS). The classification is often made focused on the protein effects, thus nonsense or frameshift variants are considered pathogenic or likely pathogenic, while missense[EV1] and synonymous remain as VUS. However, other upstream gene-expression mechanisms can disrupt the protein. Here, we have studied the splicing effects of *BRCA2* exons 2-9 variants by functional assays in MCF7 cells using the minigene MGBR2_EX2-9 (figure 1).

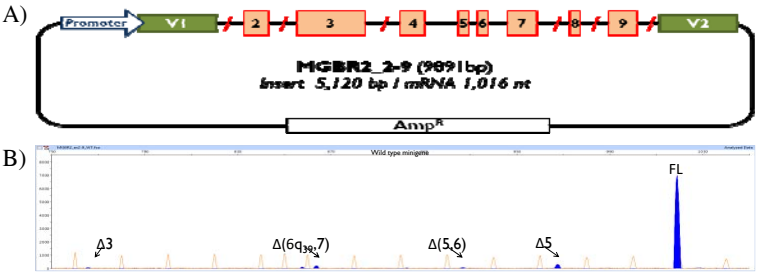


**Figure 1. Structure and functional analysis of MGBR2_2-9**. A) MGBR2_2-9 diagram. V1_exons2-9_V1 under SV40 promoter control in pSAD® splicing vector. Red lines indicate shortened introns. B) MGBR2_2-9 functional assay in MCF7-cells. Fluorescent capillary electrophoresis showed a majority of full length (FL) (92%) and other minority transcripts.

## Results

**Table 1. MGBR2_2-9 variant assay results.** After the *in silico* analysis of 302 variants, 83 were assayed. Among them, 36 severely altered the splicing (>2/3 of aberrant transcripts). Following ACMG/ENIGMA[EV3] criteria, we have classified 30 variants as pathogenic, 4 as likely pathogenic and 2 remain as VUS. * Previously classified as VUS.

| DNA variant | Main aberrant transcripts | ACMG/Enigma Classification[EV2] |
|---|---|---|
| c.67G>A | Δ2q$_4$ (r.64_67del) | Pathogenic |
| c.67+1G>T | Δ2 (r.-39_67del) | Pathogenic* |
| c.67+2T>C | Δ2 (r.-39_67del) | Pathogenic |
| c.67+3A>G | Δ2 (r.-39_67del) | Pathogenic* |
| c.97G>A | Δ3 (r.68_316del) | Likely pathogenic* |
| c.100G>A | Δ3 (r.68_316del) | Likely pathogenic* |
| c.316+2T>C | Δ3 (r.68_316del) | Pathogenic |
| c.316+3delA | Δ3 (r.68_316del) | Pathogenic |
| c.316+5G>C | Δ3 (r.68_316del) | Pathogenic |
| c.316+6T>C | Δ3 (r.68_316del) | Likely pathogenic |
| c.317-2A>G | Δ4;Δ(4,5);Δ(4,5,6) (r.[317_425del;317_475del;317_516del]) | Pathogenic |
| c.426-12del5 | Δ5 (r.426_475del) | Pathogenic* |
| c.426-2A>T | Δ5 (r.426_475del) | Pathogenic |
| c.441A>G | Δ5 (r.426_475del) | Pathogenic* |
| c.451G>A | Δ5;Δ(5,6) (r.[426_475del;426_516del]) | Pathogenic* |
| c.467A>G | Δ5q$_9$ (r.467_475del) | VUS* |
| c.470_474del | Δ5 (r.426_475del) | Pathogenic |
| c.475+1G>T | Δ5 (r.426_475del) | Pathogenic |
| c.475+3A>T | Δ5 (r.426_475del) | Pathogenic* |
| c.476-2A>G | Δ6;Δ(5,6) (r.[476_516del;r.426_516del]) | Pathogenic |
| c.476-3C>A | Δ(5,6);Δ6 (r.[476_516del;r.426_516del]) | Likely pathogenic* |
| c.516+1G>T | Δ6;Δ(5,6) (r.[476_516del;426_516del]) | Pathogenic |
| c.516+2T>C | Δ6;Δ(5,6) (r.[476_516del;426_516del]) | Pathogenic |
| c.516+4delAA | Δ(5,6), Δ6 (r.[426_516del;476_516del]) | Pathogenic* |
| c.517-2A>G | Δ7 (r.517_631del) | Pathogenic |
| c.517-1G>A | Δ7p$_1$;Δ7 (r.[517del;517_631del]) | Pathogenic* |
| c.517G>T | Δ7 (r.517_631del) | Pathogenic* |
| c.572A>G | Δ7q$_{60}$ (r.572_631del) | VUS* |
| c.631G>A | Δ7 (r.517_631del) | Pathogenic |
| c.631+1G>A | Δ7 (r.517_631del) | Pathogenic* |
| c.631+3A>G | Δ7 (r.517_631del) | Pathogenic* |
| c.632-3C>G | ▼8p$^2$;Δ(6q$_{39}$,7)▼8p$^2$(r.[631_632ins632-1_632-2;478_631delins632-1_632-2) | Pathogenic* |
| c.632-1G>C | Δ8p$_4$;Δ(6q$_{39}$,7,8p$_4$) r.[632_635del;517_635] | Pathogenic |
| c.681+4A>G | ▼8q$^4$ (r.681_682ins681+1_681+4) | Pathogenic |
| c.682-1G>C | Δ9 (r.682_793del) | Pathogenic |
| c.793+1G>T | Δ9 (r.682_793del) | Pathogenic |

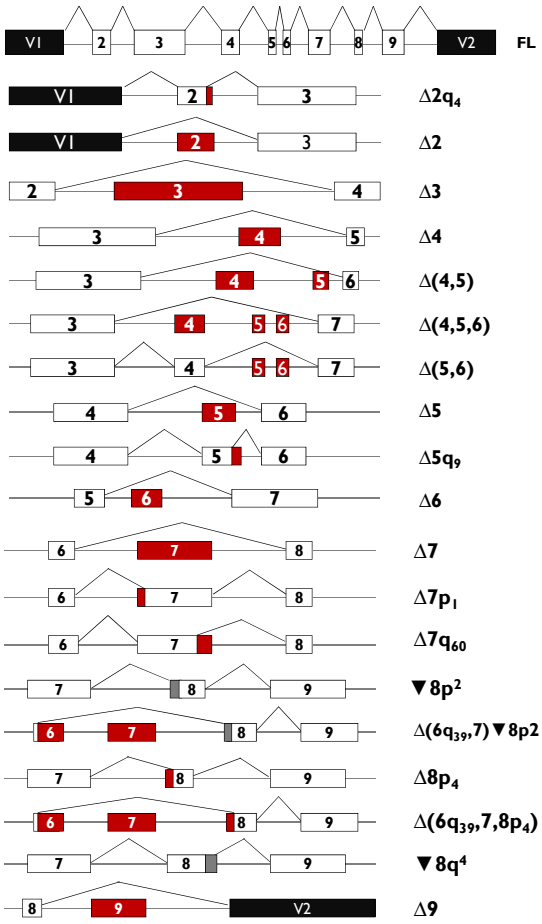


**Figure 2. Main transcripts produced by spliceogenic variants.** Capillary electrophoresis revealed more than 27 different transcripts, being exon skipping the most common event. Here, 19 of them are represented(the main ones). Black boxes indicate V1 and V2 exons, white boxes are *BRCA2* exons, red and grey boxes indicate exon skipping and insertions, respectively. Transcripts were annotated according to the ENIGMA consortium (Colombo et al 2014). Δ: skipping; ▼: insertion; p: alternative acceptor site; q: alternative donor site; subscript number: number of deleted nt; superscript number: number of inserted nt.

## Conclusions

*BRCA2* spliceogenic variants are more common than supposed and not only affect the splice sites sequences but other regulatory motifs, such as enhancers or silencers, may be altered. Here, we found 36 variants from eight exons that critically disrupted the splicing. Among them, we have catalogued 34 as pathogenic or likely pathogenic. It is important to note that 15 were previously classified as VUS. Our results also suggest a highly inter-regulation between exons, since one variant can affect processing of neighbour exons. In this context, RNA data provides essential information for the variant classification, ~~which poses a challenge in Medical Genetics~~[EV4]. However, patient RNA is not always available and minigenes can be used as a reliable alternative approach.

**Acknowledges**: Velasco EA's lab was supported by the Spanish Ministry of Economy and Competitiveness, the Plan Nacional de I+D+I 2013-2016, the ISCIII (PI13/01749 and PI17/00227) co-funded by FEDER from Regional Development European Funds (European Union), and CSI090U14 from the Consejería de Educación (ORDEN EDU/122/2014), Junta de Castilla y León. EFB was supported by a pre-doctoral fellowship from the University of Valladolid and Santander Bank..