The dual nature of alternative splicing: How alternative transcripts rescue or attenuate the tumor suppressor activity of *PALB2* variants

Authors:
Romy L.S. Mesman[1], Sami Belhadj[2], Suzanne J.S. Musters[1], Sylvia Gelpke-Vermeulen[1], Terra Brannan[2], Rachid Karam[2], Fergus J. Couch, Marcy E. Richardson[2], Haico van Attikum[1], Maaike P.G. Vreeswijk[1]*
*presenting author

Affiliations:
1) Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands
2) Ambry Genetics, Aliso Viejo, Los Angeles, the United States

Naturally occurring alternative transcripts of *PALB2* may encode (partially) functional protein isoforms. Consequently, clinical interpretation of predicted loss-of-function variants, such as variants in the canonical splice sites, may be challenging as their functional impact depends on the functionality and relative expression of these isoforms. To address this, we developed a mouse embryonic stem (mES) cell-based assay that allows robust and quantitative functional characterization of human *PALB2* variants, focusing on their effect on mRNA splicing and the functional impact on homologous recombination (HR).

First, we mapped the expression of naturally occurring transcripts in mESCs transfected with the human wild-type *PALB2* gene and compared it to the transcript profiles observed in human (lymphoblastoid) cells. The transcript diversity and relative expression levels were highly similar, demonstrating the suitability of the mESC model for assessing the functional impact of variants affecting RNA splicing. We assessed the functionality of the different isoforms and identified four functional isoforms encoded by transcripts Δ(E4), Δ(E5p24), Δ(E6) and Δ(E10p3).

Next, we analyzed the functional impact of 30 canonical splice site variants across all *PALB2* exons on HR functionality. Variants affecting the exon 4 and exon 6 acceptor sites retained HR activity, most likely due to the expression of Δ(E4) and Δ(E6) rescue transcripts, as determined by quantitative single molecule sequencing. Exon 5 and exon 10 acceptor site variants, despite producing Δ(E5p24) and Δ(E10p3) transcripts, exhibited insufficient expression to retain HR activity. Variants affecting the donor and acceptor sites of other exons were all damaging. Transcript profiles from blood-derived RNA of 13 carriers of functionally characterized splice site variants will be analyzed to validate these findings.

This work underscores the importance of quantitative functional assays that assess the impact of variants on mRNA splicing and protein function and provides a framework for improved clinical variant classification.