Functional classification of selected exonic splicing variants occurring outside of 5’ and 3’ exon boundaries in cancer-predisposing genes

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BACKGROUND AND METHODS

- Sequence variants affecting mRNA splicing account for 10-15% of disease-causing mutations in cancer-risk genes.
- Most occur at 5’ and 3’ exon-intron boundaries. Other variants outside of canonical splice sites may alter mRNA processing through use of cryptic splice sites or by altering exonic splicing enhancers and silencers.
- Variants outside of canonical splice sites are less predictable and require experimental verification for accurate classification.
- Here, we assessed the effects of 8 variants of uncertain significance (VUS) identified during clinical genetic testing.
- RNA extracted from blood of VUS carriers was reverse transcribed for splicing analysis.

RESULTS

- 7 of the 8 variants analyzed occur in exonic regions, outside of exon-intron boundaries. Of these, 5 variants were shown to produce a complete splice defect and were upgraded to likely pathogenic mutations (Table 1).

Table 1. RNA analysis of 7 variants in cancer-predisposing genes

Gene	Variant	Predicted effect on splicing	RNA analysis*			Myriad classification
BRCA2	c.9057A>G (p.Lys3019Lys)	Strengthens existing cryptic donor 65bp from end of exon 23	**Ex23+**	**Ex23Δ65**	**Full splice defect**	Pathogenic
			c.9057A: 61 (53%) c.9075G: 0	54 (47%)	Deletion of 65 bases from the end of exon 23	
PALB2	c.2559C>T (p.Gly853Gly)	New donor created	**Ex6+**	**Ex6Δ29**	**Full splice defect**	Likely Pathogenic
			c.2559C: 109 (86%) c.2559T: 0	18 (14%)	Deletion of 29 bases from the end of exon 6	
PALB2	c.18G>T (p.Gly6Gly)	New donor created	**Ex1+**	**Ex1Δ32**	**Full splice defect**	Likely Pathogenic
			c.18G: 70 (30%) c.18T: 0	165 (70%)	Deletion of 32 bases from the end of exon 1	
MSH6	c.3417C>T (p.Gly1139Gly)	New donor created	**Ex5+**	**Ex5Δ23**	**Full splice defect**	Likely Pathogenic
			c.3417C: 103 (80%) c.3417T: 0	25 (20%)	Deletion of 23 bases from the end of exon 5	
BRCA1	c.5408G>C (p.Gly1803Ala)	2nd base of exon 23 - no prediction	**Ex23+**	**ΔEx23**	**Full splice defect**	Likely Pathogenic
			c.5408G: 74 (57%) c.5408C: 0	55 (43%)	Skipping of exon 23	
APC	c.541C>G (p.Gln181Glu)	Strong acceptor created 10bp into exon 5	**Ex5+**	**Ex5Δ10**	**No splice defect**	VUS
			c.541C: 45 (45%) c.541G: 55 (55%)	0	No use of cryptic acceptor observed	
MLH1	c.996C>T (p.Ser332Ser)	New donor created 43bp from end of exon 11	**Ex11+**	**Ex11Δ43**	**No splice defect**	Benign
			c.996C: 73 (65%) c.996T: 38 (34%)	1 (<1%)	-	

*Results from patient sample - number of transcripts observed for each splice product and the allele encoded at the indicated position (% of total transcript)

- The 8th variant, *BRCA2* c.68-2A>G, occurs at the consensus exon 3 splice acceptor and could be classified as likely pathogenic based on its position and splicing predictions. Our results raise the possibility that significant functional transcript may be produced due to a partial splice defect (both out-of-frame exon skipping and use of in-frame cryptic acceptor 6bp into the exon). Therefore, the classification remains VUS (Table 2).

Table 2. RNA analysis of BRCA2 c.68-2A>G

Predicted effect on splicing	RNA analysis	Ex3+	ΔEx3^	Ex3Δ6
Abolishes splicing at the native exon 3 acceptor	**Patient sample**	42 (31%)	36 (27%)	51 (38%)
	Normal breast tissue	61 (85%)	10 (14%)	0
	Control blood	69 (99%)	1 (1%)	0

^Exon 3 skipping occurs naturally in normal tissue (Fackenthal 2016, Davy 2017)

CONCLUSIONS

- 6 of 8 variants were reclassified from VUS, allowing 91 patients to receive a clinically actionable result. Additionally, 21 patients carrying *BRCA2* c.68-2A>G may avoid unnecessary surveillance and surgical interventions.
- Continued research on RNA splicing defects provides additional data for a robust variant classification program.

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