Identification of *BRCA1* biallelic pathogenic variants in a Fanconi anemia patient and the clinical implications of variant location

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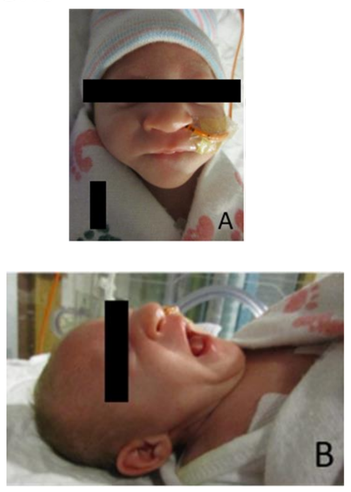
Background

- Pathogenic alterations in *BRCA1* are linked to the development of multiple cancer types including hereditary breast and ovarian cancer
- Biallelic pathogenic alterations in *BRCA1* cause Fanconi anemia subtype S (FA-S)[1]
- FA-S is characterized by dysmorphic features such as microcephaly and short stature, developmental delays, and a predisposition to developing cancers
- Full *BRCA1* knockout has been shown to be embryonic lethal in mouse models and the mechanism for viability of FA-S patients is unclear[2]

Clinical History of FA-S Patient

- 2-year-old female with history of microcephaly, poor growth, coloboma, duodenal webs, dysplastic corpus callosum, thrombocytopenia, high pitched cry, and hip dysplasia
- Small for gestational age, intrauterine growth restriction, and microcephaly (<3rd percentile) noted prenatally on ultrasound
- Skeletal survey, renal ultrasound, SNP-array, karyotype, and FISH assays were normal
- Family history remarkable for MGM who passed at 38y from breast cancer and PGM with "female cancer"
- At 2 years patient's physical exam was remarkable for café-au-lait macules (10 >5mm), microcephaly (<2 SD), and growth failure



Molecular Confirmation

- Diepoxybutane (DEB)-induced chromosomal breakage test returned positive for Fanconi anemia
- Exome sequencing at another diagnostic laboratory identified *BRCA1* c.3991C>T, p.Q1331* as the only pathogenic alteration in the Fanconi anemia-associated genes (*BMP4*, p.V117V - VUS)
- Follow-up *BRCA1* genetic testing identified the patient to also be a carrier of *BRCA1* c.191G>A, p.C64Y (pathogenic)
- Parental testing to confirm phase is still pending parental participation, however clinical and molecular phenotype is consistent with FA-S[3]

Alternative Splicing in *BRCA1* May Rescue Function in FA-S Patients

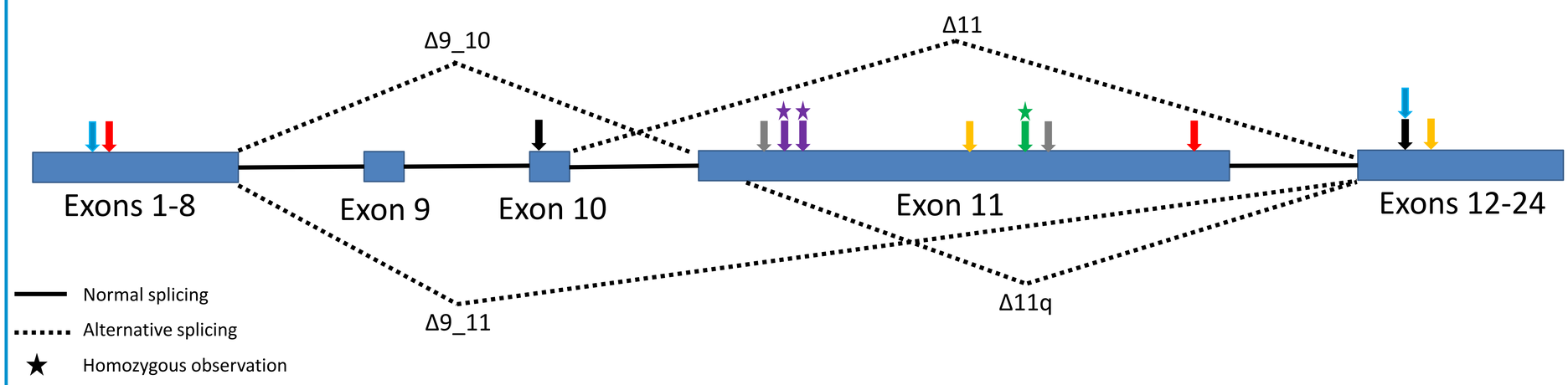


Table 1

Study	Allele 1	Allele 2
Sawyer 2015 PMID 25472942	p.S198Rfs*35	p.R1699W
Keupp 2019 PMID 31347298	p.C61G	p.R1699Q
Domcheck 2013 PMID 23269703	p.D821Ifs*25	p.V1736A
Freire 2019 PMID 29133208	p.C903*	Homozygous
Seo 2018 PMID 29712865 (2 probands)	p.W372*	Homozygous
Seo 2018 PMID 29712865 (2 probands)	p.L431*	Homozygous
Chirita-Emandi 2021 PMID 32843487	p.Y978*	p.S282Yfs*15
This study	**p.C64Y**	**p.Q1331***

Figure 1: Schematic representation (not to scale) of *BRCA1* highlighting exons 9, 10, and 11 (protein coding exons 7, 8, and 9). This region is characterized by naturally-occurring, in-frame alternative splicing isoforms (dashed lines) which remove exons 9 and 10 (Δ9_10), exons 9-11 (Δ9_11), a majority of exon 11 (Δ11q), and exon 11 (Δ11)[4] . The colored arrows represent the approximate locations of *BRCA1* alterations observed in FA-S patients in the published data summarized in **Table 1**. Of the 8 identified FA-S probands, 7 of them have a nonsense or frameshift alteration in an exon which is known to be removed in alternatively spliced transcripts, including 3 homozygous observations. As full *BRCA1* knockout has been shown to be embryonic lethal in mouse models[2], the alternatively spliced transcripts may retain residual BRCA1 function that leads to viability for life in FA-S patients. This suggests that nonsense and frameshift alterations in exons 9 through 11 may not be total loss-of-function alleles.

Discussion

- The proband in Keupp et al. is the only FA-S patient identified to have alterations outside exons 9-11, but p.R1699Q is known as a hypomorphic variant with lower cancer risks. The proband did not have a positive result from a chromosomal breakage test despite presenting with FA-S consistent features
- The deletion of exon 11 alone (p.A224_L1365) removes over 60% of the total BRCA1 protein
- Alternative splicing impacts in exon 11 have been proposed previously as a potential explanation for FA-S viability and the relative rarity of these patients[5,6]
- Functional studies on Δ11 and Δ11q have shown the capacity to perform homology-directed repair (at a reduced level) and to result in an increase in murine embryonic viability[7,8]

Clinical Implications

- The *BRCA1/2* Variant Curation Expert Panel classification guidelines for *BRCA1* (unpublished, personal communication) do not apply PVS1 weight for loss-of-function variants in exon 9 or exon 10 due to the presence of in-frame alternative splicing. Nonsense and frameshift alterations in Exon 11 are recommend to be eligible for PVS1
- The presence of an FA-S patient with a frameshift variant in exon 10 provides evidence that nonsense or frameshift variants in exon 10 may present with similar clinical risks as those in exon 11
- The presence of alternatively spliced transcripts which remove nonsense or frameshift variants may indicate that these variants are hypomorphic when compared to loss-of-function variants in exons that are conserved in all transcript forms
- The cancer risk for carriers of a nonsense or frameshift variant in exon 11 is likely still elevated, the cancer profile and overall lifetime risks may be distinct from other full loss-of-function variants[7,9-10]

TAKE-HOME POINTS

- A tenth FA-S patient has been identified with two *BRCA1* pathogenic mutations (phase confirmation pending)
- Alternative splicing between exons 9 through 11 is implicated in maintaining BRCA1 function that may result in viability for FA-S patients
- Nonsense and frameshift variants in exon 11 may present with a different cancer profile and overall lifetime risks to fully penetrant loss-of-function alleles

REFERENCES

1. Mehta et al. PMID 20301575
2. Gowen et al. PMID 8563759
3. Chirita-Emandi PMID 32843487
4. Colombo et al. PMID 24569164
5. Domcheck PMID 23269703
6. Seo PMID 29712865
7. Wang et al. PMID 27197267
8. Huber et al. PMID 11359908
9. Rebbeck et al. PMID: 25849179
10 Ruiz de Garibay et al. PMID 34420246