As a result, both variants were reclassified to variants of uncertain significance.

Additional functional RNA analysis performed by our laboratory indicated that some functional transcript may be produced by the variant allele.

The role of allele-specific RNA analysis in hereditary cancer variant classification

BACKGROUND

DNA variants that result in abnormal mRNA processing are pathogenic if the resultant transcripts are unable to produce functional protein.

In silico RNA splice predictors are informative for variant classification, but are not comprehensive. Therefore, it is often useful to perform functional RNA studies to confirm a predicted splice defect.

INITIAL VARIANT CLASSIFICATION

BRCA2 c.8331+2T>C and BRCA2 c.425G>T were initially classified as pathogenic in accordance with ACMG/AMP guidelines based on:

- Variant position
- In silico models predicting the variant would abolish (c.8331+2T>C) or significantly reduce the strength (c.425G>T) of the wildtype splice donor
- Published RNA analysis showing the production of aberrant splice products

VARIANT RECLASSIFICATION

Data from our laboratory’s history weighting algorithm was consistent with BRCA2 c.8331+2T>C and BRCA2 c.425G>T being benign.

In addition, functional RNA analysis performed by our laboratory indicated that some functional transcript may be produced by the variant allele.

As a result, both variants were reclassified to variants of uncertain significance.

CONCLUSION

RNA analysis is an effective classification tool; however, the data produced from RNA studies must be evaluated in the context of other classification tools as they may not provide sufficient evidence for classification alone. Presented at NSGC on November 18-21, 2020

The role of allele-specific RNA analysis in hereditary cancer variant classification

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VARIANT RECLASSIFICATION

- BRCA2 c.8331+2T>C impacts the canonical splice donor site of exon 18.
- Published RNA analysis showing skipping of exon 18 and other aberrant transcripts that are predicted to cause protein truncation.

B. Schematic representation BRCA2 regions amplified and digital electrophoresis of control and carrier samples.

FIGURE 1. BRCA2 C.8331+2T>C

A. History weighting algorithm analysis based on 18 observations.

C. Fraction of total transcript detected in control and carrier samples determined by quantification of transcripts from 119 isolated traces.

FIGURE 2. BRCA2 C.425G>T

A. History weighting algorithm analysis based on 10 observations.

C. Fraction of total transcript detected in control and carrier samples determined by quantification of transcripts from isolated traces.

Allele-specific RNA analysis for c.425G>T revealed the production of several aberrant transcripts that remain in-frame and may retain function.