The Role of Allele-Specific RNA Analysis in Hereditary Cancer Variant Classification

Erin Mundt, Paola Nix, Eric Rosenthal, Susan Manley, Karla Bowles, Thomas Slavin, Benjamin Roa

All authors are current or former employees of Myriad Genetics, Inc.

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.

Objective

- Here we provide examples of two variants that illustrate how allele-specific RNA analysis, interpreted in the context of additional lines of evidence, contributed to the classification of variants in hereditary cancer genes.

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.
- Additional 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

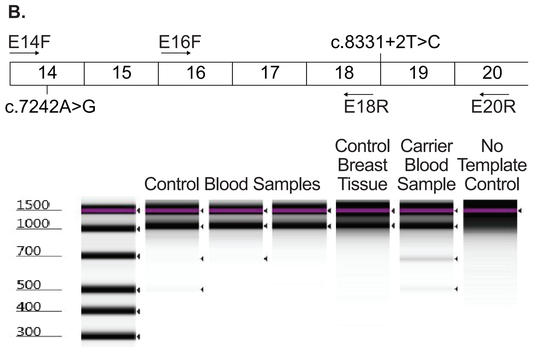
- RNA analysis is an effective classification tool; however, the data produced from RNA studies must be evaluated in the context of other classification tools as they may not provide sufficient evidence for classification alone.

Presented at NSGC on November 18-21, 2020

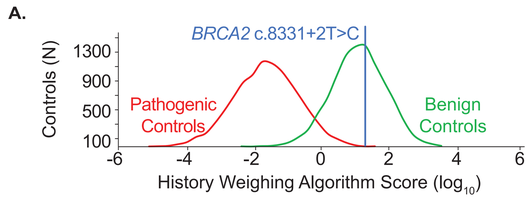
FIGURE 1. BRCA2 C.8331+2T>C

- *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.

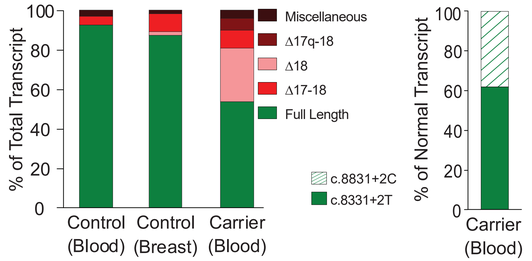


A. History weighting algorithm analysis based on 18 observations.



The history weighting algorithm called *BRCA2* c.8331+2T>C **benign** based on the severity of cancer history among pathogenic and benign controls.

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



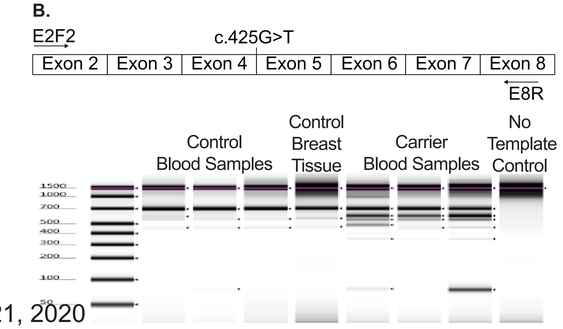
RNA analysis with allele-specific quantification for c.8331+2T>C found that the variant allele produced some wildtype transcript.

This is likely due to utilization of the GC donor created by the variant.

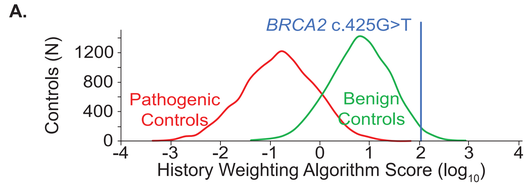
FIGURE 2. BRCA2 C.425G>T

- *BRCA2* c.425G>T impacts the last base of exon 4.
- Published RNA analysis showed that c.425G>T caused a fully penetrant splice defect (i.e. no wildtype transcript was produced by the variant allele).

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

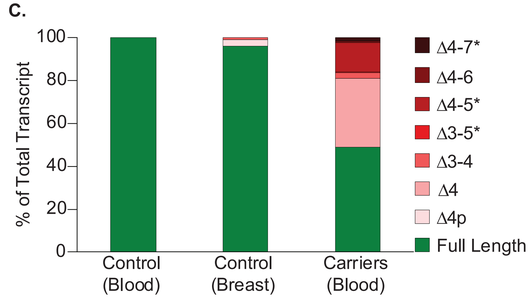


A. History weighting algorithm analysis based on 10 observations.



The history weighting algorithm called *BRCA2* c.425G>T **benign** based on the severity of cancer history among pathogenic and benign controls.

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.