RNA Analysis from Residual Blood Aids the Interpretation of VUS Identified in Individuals Undergoing Hereditary Cancer Genetic testing

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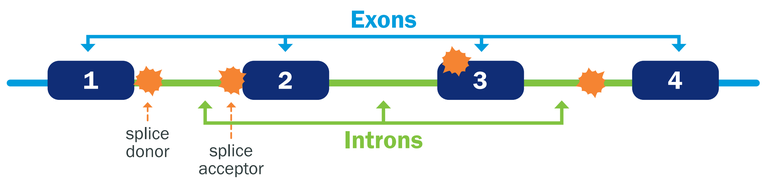
Background

- Functional RNA studies are a useful tool to help resolve the classification of variants of uncertain significance (VUS) suspected to have an impact on mRNA splicing.



  - Splicing mutations are a possible cause of gene disruption
  - Functional RNA studies help determine the level of aberrant splicing that a variant might cause

- RNA studies may also help establish the phase of co-occurring variants in cases where clinical management differs depending on whether variants occur *in cis* or *in trans.*
- We recently validated the use of blood samples collected in EDTA tubes for RNA studies.
- Residual blood submitted for hereditary cancer (HC) DNA testing can yield sufficient material for RNA isolation and functional analysis by RT-PCR and cDNA sequencing.

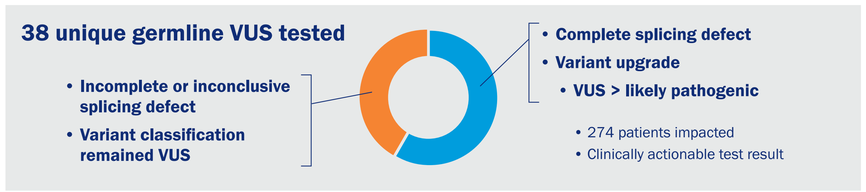
Methods

- RNA analysis was performed on residual EDTA blood samples submitted for HC panel testing that carried a heterozygous VUS predicted to impact splicing by in silico models or carried co-occurring variants where phase could not be determined by NGS sequence or haplotype review.
- Total RNA was isolated and subjected to RT-PCR and cDNA sequencing.
- Allele-specific transcripts were reviewed to identify and quantify RNA isoforms produced by either the wild-type or the variant allele.
- These data were in turn compared to RNA from control blood and relevant tissue samples.

Results

- We performed RNA analysis on 56 selected cases between May 2024-April 2025.
- RNA and cDNA of sufficient quantity and quality were isolated from 41/56 (73%) residual EDTA blood samples.
- 38 samples were analyzed for splicing effects and 3 samples were analyzed for variant phase for a total of 41 samples tested.

Figure 1. Allele-specific RNA studies from residual blood help interpret VUS identified in a patient's germline DNA test



Example 1: *ATM* c.3078-5T>C

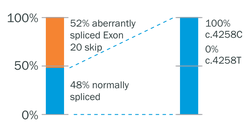
Complete splice defect
(VUS > likely pathogenic)

SpliceAI acceptor loss 0.81

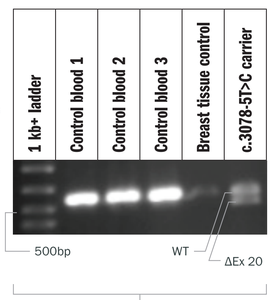
Quantification of *ATM* splice products

Exon 20 flanking primers: E17F-E22R

Allele-specific analysis using tag-snp c.4258C>T in exon 28: E20F-E28R

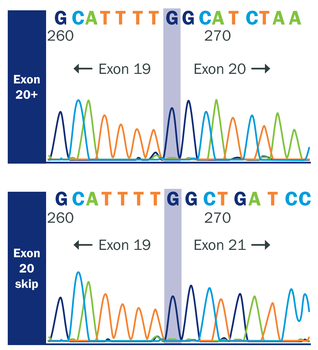


The c.3078-5C allele only produces aberrantly spliced transcript causing frameshift and premature truncation



Agarose gel electrophoresis of control and carrier samples amplified by E17F-E22R. Control samples only amplify normally spliced transcript while the carrier sample shows an additional smaller band consistent with skipping of exon 20.

ATM splice products in the patient sample



Example 2: *BRCA1* c.4358-31_4358-27del

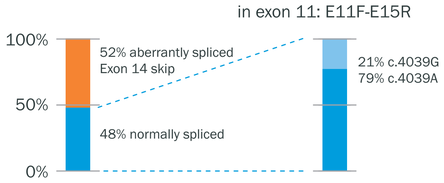
Incomplete splice defect (remains VUS)

SpliceAI acceptor loss 0.37

Quantification of *BRCA1* splice products

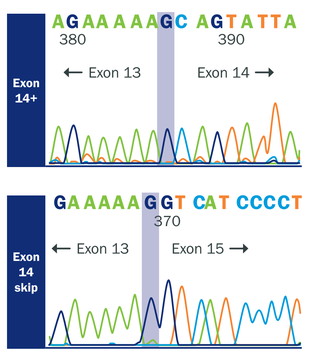
Exon 14 flanking primers: E11F-E15R

Allele-specific analysis using tag-snp c.4039A>G in exon 11: E11F-E15R



21% of the normally spliced transcript is produced by the c.4358-31_4358-27del allele

BRCA1 splice products in the patient sample



- Of these, 22/38 (58%) demonstrated a complete splice defect and were upgraded from VUS to likely pathogenic, while 16/38 (42%) showed incomplete or inconclusive splice defects and retained a VUS classification.

- Three samples each carried two variants in *MUTYH, MLH1,* and *CHEK2,* respectively.
  - RNA analysis determined the cis or trans orientation in each case, ultimately providing more specific clinical management to the patient.

Figure 2. Confirming variant phase provides more precise cancer risk estimate

Patient 1: Carrier of two *CHEK2* mutations

Exon 12: c.1420C>T (p.Arg474Cys) Likely Pathogenic

Exon 14: c.1555C>T (p.Arg519*) Pathogenic

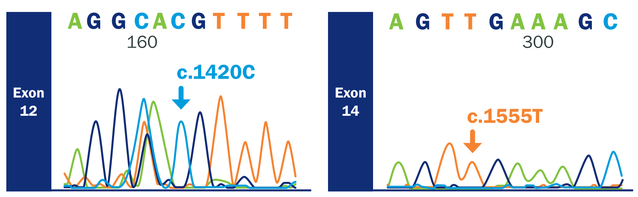
in cis

- **20-31% risk** of female breast cancer to age 80

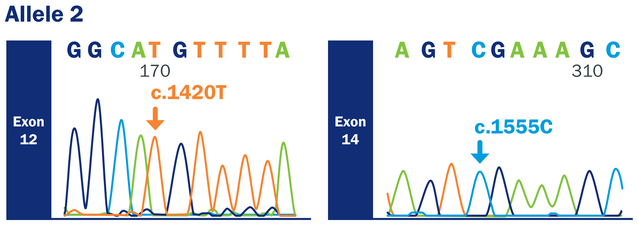
in trans

- **Up to 89% risk** of female breast cancer to age 80

Allele 1



Allele 2



Variants are determined to be *in trans*

Conclusions

- Under certain conditions, residual samples can be used for RNA analysis, thereby avoiding the burden of secondary sample submission.
- This pathway facilitates both qualitative and quantitative analysis of allele-specific transcripts.
- The addition of residual blood RNA studies has resulted in a 5-fold increase in the number of variants analyzed in our laboratory during a 12-month period.
- These functional studies contribute substantially to the reclassification of splicing variants, providing definitive test results to more patients in a streamlined manner.

Disclosures: All authors were employees of Myriad Genetics, Inc. at the time of this study and received salaries and stock as compensation.

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