RNA Research Program Continues to be a Valuable Tool in Variant Reclassification

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BACKGROUND

- Certain germline variants in cancer-associated genes have the potential to impair the mRNA splicing process, resulting in a non-functional protein.
- Although it is known that certain sequences at the splice donor and acceptor sites are almost always required for appropriate splicing, it is often unclear if other sequence changes near the splice sites are tolerated.

OBJECTIVE

- To aid in the complex process of interpreting and classifying cancer predisposition gene variants that may impact mRNA splicing, an IRB-approved RNA research protocol was established to gather more information about these variants and to help determine potential clinical impact.

METHODS

- Patients who were found, through clinical genetic testing, to carry selected variants of uncertain significance were contacted with their healthcare provider's permission and enrolled in the study.
- The variants were most often adjacent to splice junctions, or predicted to alter mRNA splicing.
- Eligible patients were contacted by a genetic counselor to discuss the risks and benefits of the study and to obtain verbal consent for enrollment.
- Patients wishing to participate submitted an additional blood sample for RNA analysis. The result of each splicing analysis was used in conjunction with other available evidence to aid in variant reclassification.
- If the collective evidence reached the threshold for reclassification, an amended report was sent to the ordering healthcare provider.

RESULTS

- Since December 2015, the RNA laboratory has processed 147 unique variants in 20 cancer-predisposition genes, leading to reclassification in 59.2% (87/147) of instances (Figure 1).
  - Of these reclassifications, 81.6% (71/87) were upgrades from uncertain to likely pathogenic/pathogenic, resulting in 611 amended patient reports.
  - The remaining 18.4% (16/87) were downgrades from uncertain to likely benign/benign.
- Classification of the other 40.8% (60/147) of processed variants remained unchanged due to inconclusive splicing analysis or because the collective evidence failed to meet the reclassification threshold (Figure 1).
- Figure 2 shows an example of one variant, *BRCA1* c.135-8A>G, that was reclassified after RNA analysis.
- *BRCA1* c.135-8A>G, creates a new splice acceptor that, if used, would result in the insertion of 7 base pairs in exon 5, resulting in a frameshift.
- Initially this variant was classified as uncertain as there was no evidence to indicate whether or not the new splice acceptor was used.
- Our laboratory received an RNA sample from a patient carrying the variant and an informative SNP (c.1067A>G).
- The RNA analysis showed use of the newly created cryptic acceptor 7 base pairs upstream of the exon.
- The informative SNP was used to determine penetrance of the splice defect.
  - All of the wildtype traces were produced by the wildtype allele and no wildtype traces were produced by the mutant allele (Table 1).
- RNA analysis showed that the c.135-8A>G variant results in a fully penetrant splice defect.
- Based on this, the variant was upgraded to likely pathogenic.

Figure 1. Variants reclassified by RNA research program

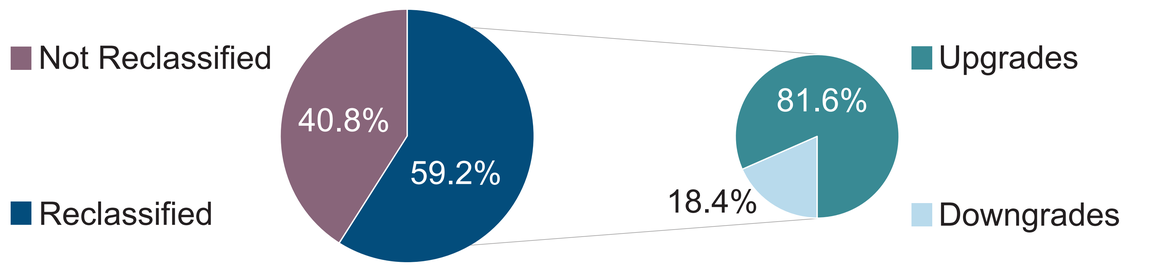

Figure 2. Possible aberrant splicing outcomes for the BRCA1 transcript

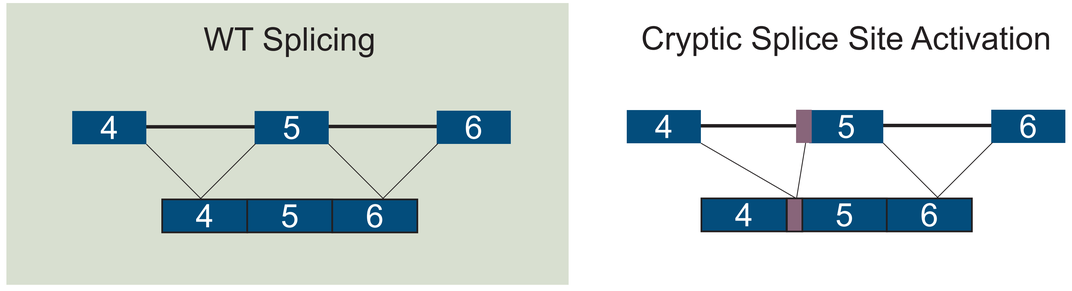

Table 1. PCR sequencing traces for the quantification of splice products

Patient's RNA Sample	Ex5+	Ex5ins7	ΔEx5	Total Traces
c.1067A	66 (69%)	0	1 (1%)	96
c.1067G	0	24 (25%)	5 (5%)	
Normal Ovarian Tissue	57 (100%)	0	0	57
Blood Control	71 (96%)	0	3 (4%)	74

CONCLUSIONS

- Functional RNA analysis provided direct evidence of the impact of selected variants on mRNA splicing and enabled reclassification.
- Many of these variants were upgraded to likely pathogenic/pathogenic, providing definitive evidence of clinical actionability and changing medical management recommendations.
- These RNA studies demonstrate the impact of one variant reclassification tool, and highlight the importance of developing and using robust reclassification tools.

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