

RNA RESEARCH PROGRAM TO AID IN THE RECLASSIFICATION OF GENETIC VARIANTS THAT ALTER SPLICING

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OBJECTIVES

- RNA splicing is the process by which non-coding intronic regions of a gene are removed.
- Germline variants can impair splicing, giving rise to a non-functional protein and potential disease risk.
- Specific splice junctions may tolerate wide variation and still splice correctly, while other junctions may become impaired by only minor changes.
- We recently established an IRB-approved research program to enroll selected patients, after clinical testing, that carry variants that may alter splicing.
- Here we present two case studies to demonstrate how functional RNA analysis aids in the potential reclassification of variants with unknown cancer risk.

METHODS

- Additional blood samples were collected from individuals who underwent hereditary cancer testing and were found to carry a variant of uncertain significance that may impair RNA splicing.
- RNA was extracted, cDNA was synthesized, and PCR was performed to amplify portions of the gene of interest.
- Splicing patterns were visualized on an agarose gel and splice products identified by sequencing. The wild-type splicing pattern was confirmed in age/gender matched blood controls, and in normal tissue (breast and/or ovarian).
- Further experiments were performed to determine if the mutant allele produces any wild-type splice product.

RESULTS

Table 1. Case Studies of Variants in BRCA1 and CDH1

Variant (Location)	BRCA1 c.4484G>A (last base of exon 14)	CDH1 c.715G>A (28 bases into exon 6)
Classification and # of Observations	Uncertain 22 observations	Uncertain 4 observations
Proband(s) Studied	Mother and daughter both with invasive breast cancer	Woman with lobular breast cancer, no family history of gastric cancer
Notes	Previous observation of variant at this position (c.4484G>T) in >350 individuals Clinical and functional splicing data were used to classify that variant as Pathogenic	Predicted to create a cryptic splice acceptor, which would create an out-of-frame mRNA, if used
Gel Results (Splicing Patterns)	Skipping of exon 14 in both patient samples, but not in RNA from normal breast tissue or RNA from blood from 5 age/gender matched controls (Figure 1A)	Aberrant splicing product showing usage of the cryptic acceptor only in the patient sample, but not in RNA from blood in 5 age/gender matched controls (Figure 2A)
Digital PCR Results (Splicing Products)	117/214 (55%) digital traces showed skipping of exon 14 (Figure 1B) 0/94 digital traces of the WT splice product were produced by the mutant allele 0/171 traces from a negative blood control as well as breast and ovarian tissue controls showed skipping of exon 14	53/130 (41%) of all traces showed activation of the cryptic acceptor (Figure 2B) 11/77 (14%) traces of WT splice product were produced by the mutant allele 85 traces from a negative control blood sample and 90 traces from normal breast tissue showed no activation of the cryptic splice acceptor
Re-classification	Pathogenic	Uncertain

- We have now analyzed many variants within genes in which pathogenic variants cause an increased risk of breast and/or ovarian cancer, such as BRCA1, BRCA2, and CDH1.
- In some cases, a variant was observed to fully disrupt splicing and was reclassified; in other cases, a variant was observed to only partially disrupt splicing and remained classified as uncertain.
- Representative cases are shown in Table 1 and Figures 1 and 2.

Figure 1. BRCA1 c.4484G>A
A. Gel of splice products in control and 2 variant carriers B. Representative digital sequencing trace of aberrant splicing product

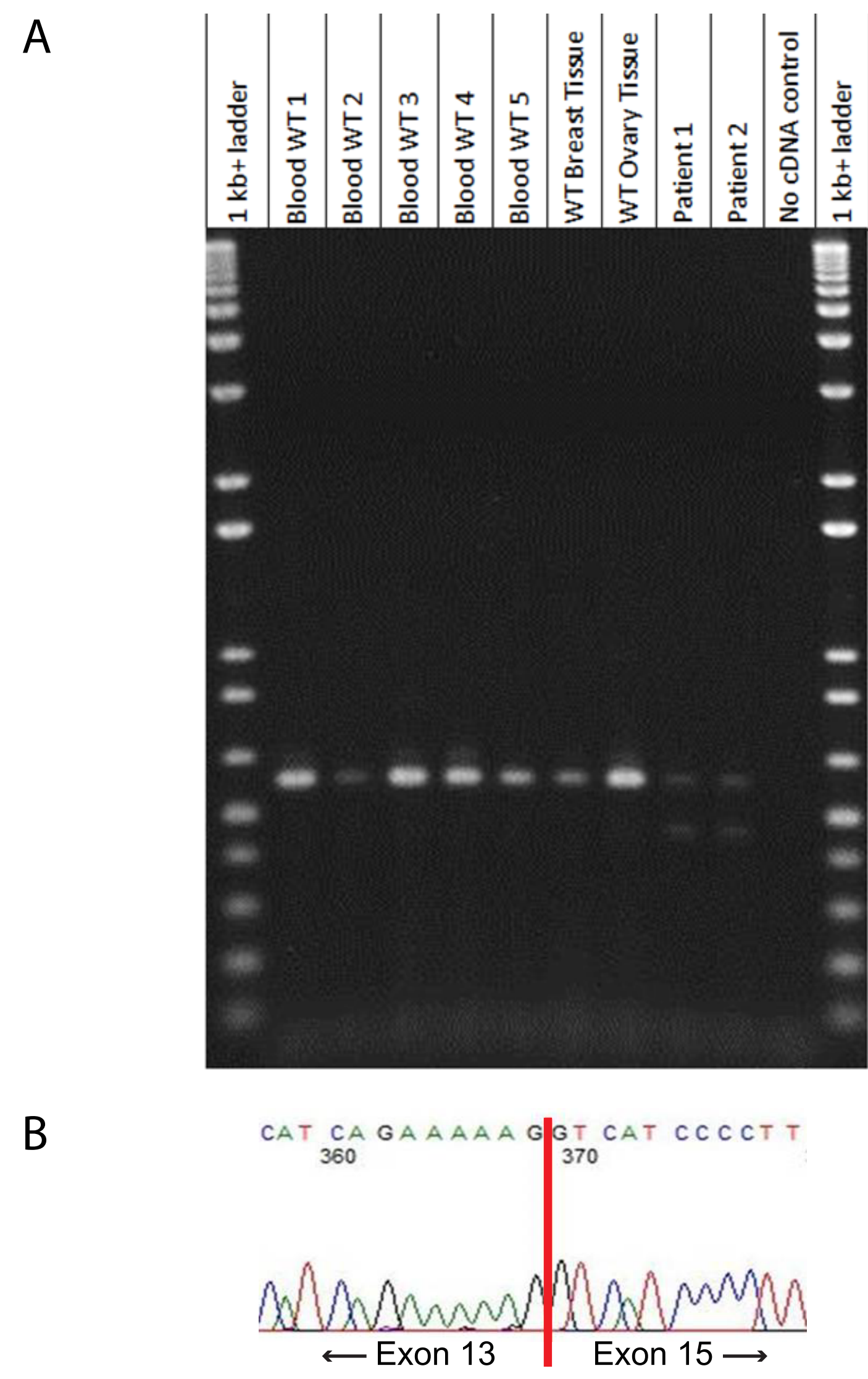
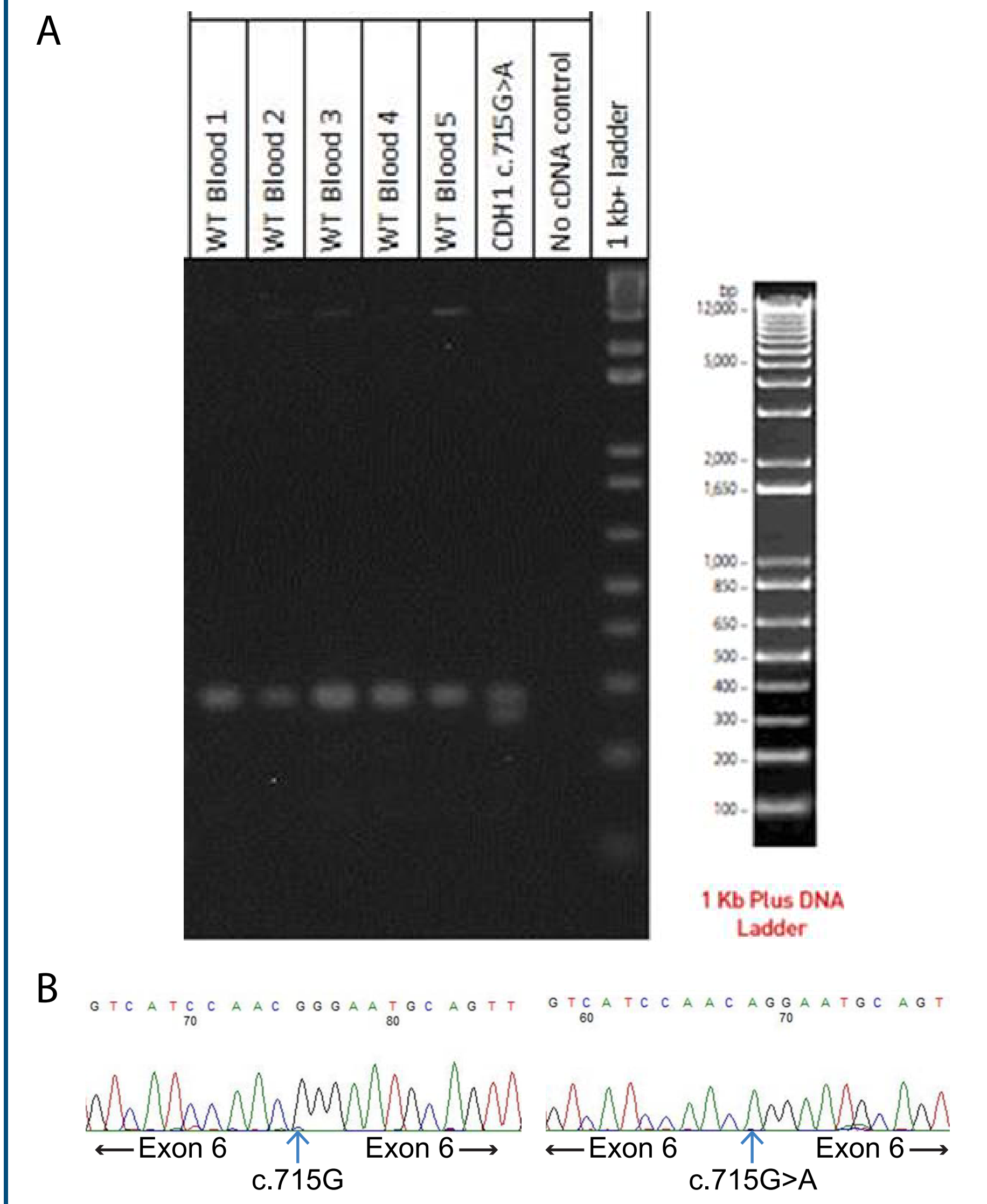


Figure 2. CDH1 c.715G>A
A. Gel of splice products in control and variant carrier B. Representative digital sequencing trace of normal splice product, as produced by both the normal (left) and mutant (right) alleles



DISCUSSION

- These studies demonstrate that RNA studies are very helpful in the reclassification of variants that alter splicing.
- Variants that fully impair splicing can be reclassified, while variants that cause intermediate splicing defects may require additional data to determine if the variant causes an increased cancer risk.