Practical applications of RNA genetic testing for variant detection and interpretation: A case series

Improvements in genetic testing technology and increased adoption of large panel testing has led to better identification of individuals with hereditary predisposition to cancer. However, the source of inherited cancer risk remains unresolved in many individuals who undergo genetic testing, due in part to limitations in the analytical range of cDNA sequencing and in the availability of data for classification of inconclusive results in genes with common disease prevalence or reduced penetrance. Here we present a case series to demonstrate a variety of applications for paired RNA and DNA genetic testing (RGT-DGT) in mitigating these limitations.

The study cohort consists of the first 2,500 consecutive patients referred for concurrent RNA genetic testing (RGT) alongside DNA hereditary cancer panels at Ambry Genetics® by ordering clinicians from 17 collaborating medical centers across the United States. DNA results, RNA results, classification history, and clinical data were curated to determine the impact of RGT on overall results.

Data from paired RGT-DGT resulted in a relative increase in diagnostic yield of 6.7% and relative decrease in inconclusive rate of 4.5%. The two following cases demonstrate use of evidence from RGT to identify pathogenic alterations. In Case 1, identification of aberrant transcript (r.2466_2467ins2466+1555_2466+1649) prompted the development of unique primers and led to the detection of a novel deep intronic alteration (*ATM* c.2466+1552G>C) not detectable by standard DGT. In Case 2, an exonic missense alteration (*ATM* c.3065T>G p.I1022S) with no available data on a protein level was revealed as a splicing alteration resulting in the creation of a cryptic donor site. In Cases 3 through 5, RGT was used as evidence towards benign classification. Aberrant transcript was detected in the proband of Case 3, who carried *BRIP1* c.1936-4C>T, however, the same transcript was also found in nearly all wildtype controls, thereby indicating that the alternative transcript was well tolerated in the population and not attributable to this alteration. In Cases 4 and 5, aberrant transcript was absent in probands carrying *ATM* c.3077+4G>A and *BRCA2* c.476-3C>T, respectively, demonstrating that the alteration does not lead to abnormal splicing.

Our data indicate that RGT improves the accuracy of hereditary genetic testing via better detection and interpretation of variants. Presence and absence of alternative RNA transcript was used in a variety of ways when paired with DGT results and other lines of evidence, both as support towards pathogenicity and clarification of benign variants. RGT is an especially powerful tool for classification in moderate penetrance genes or those that cause common phenotypes, as well as in the identification of novel pathogenic variants.

	Alteration	RNA Application	Classification Impact	Clinical History
Case 1	*ATM* c.2466+1552G>C	Identification of deep intronic alteration out of analytical range	Initial classification of novel alteration as likely pathogenic	44yo female, with breast cancer
Case 2	*ATM* c.3065T>G p.I1022S	Identification of an exonic cryptic splice site	Reclassification from variant of uncertain significance (VUS) to likely pathogenic	33yo female with breast cancer diagnosed 33y
Case 3	*BRIP1* c.1936-4C>T	Existence of aberrant transcript in cases and controls	VUS to variant likely benign (VLB)	45yo unaffected female with family history of breast cancer
Case 4	*ATM* c.3077+4G>A	No aberrant transcript detected	VUS to VLB	38yo unaffected female with family history of breast and colon cancer
Case 5	*BRCA2* c.476-3C>T	No aberrant transcript detected	VUS to VLB	59yo female with breast cancer 47y and 49y