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# Practical Applications of RNA Genetic Testing for Variant Detection and Interpretation: A Case Series

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## BACKGROUND

- The source of cancer risk remains unresolved in a large proportion of individuals who undergo genetic testing.
- This is due, in part, to limitations in analytical range of DNA sequencing and challenging variant interpretation when common phenotypes cannot be leveraged for classification purposes.
- 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.

## Table 1. Case Details

| | Alteration | RGT Results | Classification Impact | Overall Result Impact | Case Count* |
|---|---|---|---|---|---|
| Case 1 | *ATM* c.2466+1552G>C | Identification of deep intronic alteration out of analytical range | Initial classification of novel alteration as likely pathogenic (VLP) | Negative to Positive | 2 |
| Case 2 | *ATM* c.3065T>G | Identification of an exonic cryptic splice site | Reclassification from variant of unknown significance (VUS) to VLP | Inconclusive to Positive | 2 |
| Case 3 | *BRIP1* c.1936-4C>T | Existence of aberrant transcript in cases and controls | Reclassification from VUS to variant likely benign (VLB) | Inconclusive to Negative | 11 |
| Case 4 | *ATM* c.3077+4G>A | No aberrant transcript detected | Reclassification from VUS to VLB | Inconclusive to Negative | 31 |
| Case 5 | *BRCA2* c.476-3C>T | No aberrant transcript detected | Reclassification from VUS to VLB | Inconclusive to Negative | 9 |

*Case Count includes patients who received inconclusive results prior to the availability of RGT

## METHODS

- Five cases were selected from the first 2,500 consecutive patients referred for concurrent RNA genetic testing (RGT) and DNA hereditary cancer panels to demonstrate applications of RGT.
- Testing was submitted to Ambry Genetics® by ordering clinicians from 17 collaborating medical centers across the United States.
- DGT and RGT results, classification history, clinical data, and transcripts from controls were curated to determine the impact of RGT on overall results.

## TAKE-HOME POINTS

- RGT improved the accuracy of hereditary genetic testing via better detection and interpretation of variants.
- RNA data can be used as evidence both in support of pathogenicity and as clarification of benign variants.
- RGT is an especially powerful tool for classification in moderate penetrance genes or those that cause common phenotypes, and in the identification of novel pathogenic variants.

## REFERENCES

1. Landrith, T., Li, B., Cass, A.A. *et al.* Splicing profile by capture RNA-seq identifies pathogenic germline variants in tumor suppressor genes. *npj Precis. Onc.* **4,** 4 (2020). https://doi.org/10.1038/s41698-020-0109-y

## Figure 1. Pathogenic Applications

### Detect Deep Intronic Alterations
### VLP *ATM* c.2466+1552G>C

Figure 1a. Case 1 Pedigree

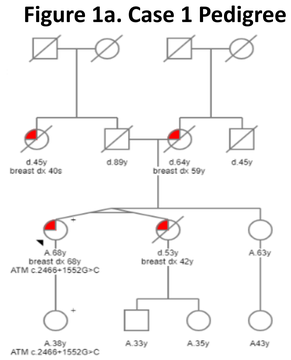
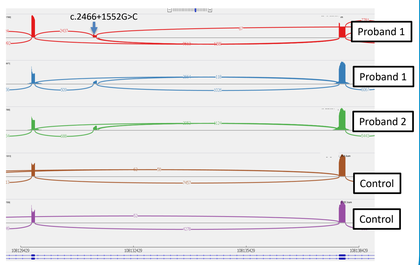



Figure 1b. Sashimi Plot




### Identify Mid-Exonic Splice Sites
### VLP *ATM* c.3065T>G

Figure 1c. Case 2 Pedigree

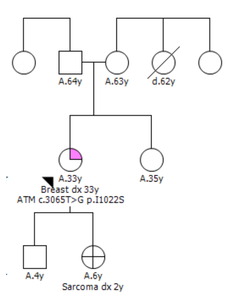
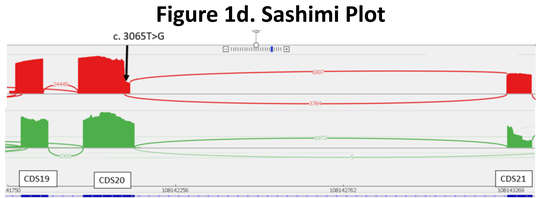



Figure 1d. Sashimi Plot




## Figure 2. Benign Applications

### Leverage Control Transcripts
### VLB *BRIP1* c.1936-4C>T

Figure 2a. Case 3 Pedigree

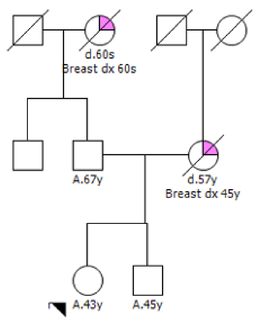
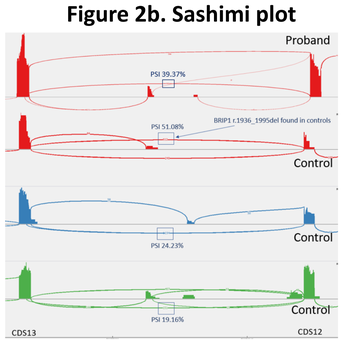



Figure 2b. Sashimi plot




### Lack of Abnormal Transcripts
### VLB *ATM* c.3077+4G>A

Figure 2c. Case 5 Pedigree

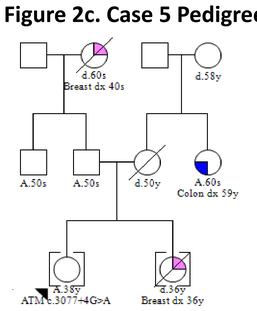
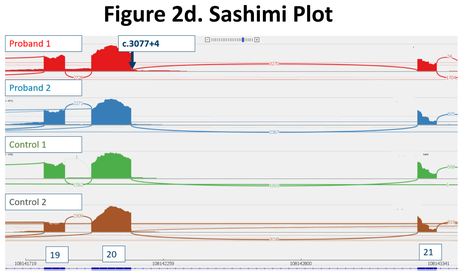



Figure 2d. Sashimi Plot

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