Expanding the detection range of DNA multigene panel testing with concurrent RNA sequencing

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Background: There have been numerous reports of deep-intronic pathogenic variants in hereditary cancer genes. However, most hereditary cancer genetic tests have traditionally focused on protein-coding regions (exons) due to the size and limited interpretability of noncoding intronic regions. Here, we describe the use of concurrent DNA and RNA genetic testing (DGT/RGT) to identify disease-causing intronic variants that would have yielded inconclusive or negative results with DNA-only genetic tests.

Methods: Patients were clinician-referred for concurrent DNA and RNA hereditary cancer panel testing between March 2019 and April 2020. Molecular results were retrospectively reviewed to assess the spectrum of clinically-actionable intronic variants beyond 5 base pairs on either side of the intron.

Results: The addition of RGT led to the detection and classification of 11 pathogenic (PV)/likely pathogenic intronic variants (VLP) deeper than +/-5 nucleotides, all of which had published management guidelines, impacting 15 different families. Five PV/VLP were detected in *BRCA1/2: BRCA1* c.5152+6T>G (two families), *BRCA1* c.5333-6T>G, *BRCA1* c.5407-25T>A (two families), *BRCA1* c.81-9C>G, and *BRCA2* c.8755-9T>A. For all five PV/VLP, members from at least one family had previously been identified to carry the variant at a different laboratory with a 'variant of unknown significance' classification, and the classification in ClinVar from other clinical labs was 'uncertain significance'. Four PV/VLP were detected in *ATM*: c.2466+1552G>C (three families), c.497-2661A>G, c.8152-18T>G, and c.8584+16A>G, all of which were absent from ClinVar. *APC* c.933+829A>G was detected in 3 generations of a classic familial adenomatous polyposis family and was absent from ClinVar. *MSH2* c.2459-12A>G was detected in a classic Lynch family that had previously remained without a molecular diagnosis despite extensive testing. This variant appeared in ClinVar with conflicting interpretations.

Conclusions: Without RNA evidence, detection and interpretability of intronic DNA variants is limited, as evidenced by absent, uncertain, or conflicting entries in ClinVar. Observation of recurrent deep-intronic variants within a limited set of individuals receiving concurrent DGT/RGT suggests that a considerable number of families with PV/VLPs may be missed with stand-alone DGT. Ongoing DGT/RGT will improve diagnosis of high-risk families and play a role in improving medical management and cancer risk reduction strategies for patients and their family members.