

Expanding the clinical reach of RNA sequencing: Evaluating testing outcomes of concurrent germline DNA and RNA genetic testing in a cohort of 43,000 individuals undergoing hereditary cancer testing.

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Importance: Precision medicine has become mainstreamed in health care, and its utility is especially evident in the field of oncology. Personalized surveillance, prophylaxis, and cancer treatment options for individuals with hereditary cancer predisposition are informed by results of germline genetic testing. Improvements to genomic technology, such as the availability of RNA sequencing, may increase identification of individuals eligible for personalized interventions by improving the accuracy and yield of germline testing.

Objective: Assess the cumulative impact of paired DNA and RNA testing on detection of disease-causing germline genetic variants and resolution of variants of uncertain significance (VUS) and the resulting clinical implications.

Design, setting, and participants: Paired DNA and RNA sequencing was performed on 43,524 individuals undergoing germline testing for hereditary cancer indication at a single diagnostic laboratory from March 2019 through April 2020. Demographics, clinical data, and test results were curated as samples were received and changes to variant classification were assessed over time.

Main Outcomes and Measures: We assessed the overall results by variant type, the effect of RNA evidence on variant classification, and the corresponding impact on cancer risk management. Increase in diagnostic yield, decrease in VUS rate, and difference in positive and negative predictive values were also evaluated.

Results: Variant classification was impacted in 549 individuals. Medically significant upgrades were made in 97 individuals, including 70 individuals who had a variant reclassified from VUS to Pathogenic/Likely Pathogenic (P/LP) and 27 individuals who had a novel deep intronic P/LP variant that would not have been detected using DNA sequencing alone. This corresponded to eligibility for increased surveillance in 14.2% (n=78) and for surgical options in 5.8% (n=32) of RNA impacted individuals. We found that 17.1% (93 of 545) of P/LP splicing variants were dependent on RNA evidence for classification and 71.1% (312 of 439) of existing splicing VUS were resolved by RNA evidence. The evidence generated during this one-year study period was then applied to individuals with DNA-only testing and have led to reclassifications in 7,602 individuals.

Conclusions and Relevance: The ability to perform RNA sequencing concurrently with DNA sequencing represents an important advancement in germline genetic testing by improving detection of novel variants and classification of existing variants.