Concurrent DNA and RNA genetic testing identifies more patients with Lynch syndrome than DNA testing alone

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BACKGROUND: An increasing number of patients have been tested for Lynch syndrome in recent years due to the inclusion of the mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*) on most hereditary cancer panels. While this has increased the number of Lynch syndrome diagnoses, particularly among patients with uncharacteristic phenotypes, it has also increased the number of patients with variants of unknown significance (VUS) in these genes. Additionally, a portion of patients with suspected Lynch syndrome remain without a molecular diagnosis due to intrinsic limitations in the detection range of standard deoxyribonucleic acid (DNA)-based testing. We assessed the ability of a novel genetic testing approach involving simultaneous DNA and ribonucleic acid (RNA) analysis to increase pathogenic variant detection and decrease the burden of VUS in MMR genes in a multigene panel setting.

METHODS: Patients were referred for concurrent RNA sequencing alongside DNA hereditary cancer panel testing by ordering clinicians from 17 collaborating medical centers across the United States. Test results were evaluated for patients whose testing included all four MMR genes (n=2425). Alternative splicing events identified in patient RNA samples were compared to healthy controls to determine significance.

RESULTS: The addition of RNA sequencing led to a 14% relative increase in the identification of clinically-actionable variants (pathogenic/likely pathogenic) in MMR genes (increased from 21 to 24). These included an individual with a novel pathogenic *MSH2* variant occurring outside the standard analytical range of DNA genetic testing and two unrelated individuals with a *PMS2* VUS reclassified as likely pathogenic based on the splicing data provided by RNA sequencing. In addition, two *MSH2* VUS, each observed in multiple unrelated individuals, were reclassified as benign/likely benign. Altogether, these RNA-related reclassifications resulted in a 5% relative decrease in inconclusive MMR gene results (decreased from 146 to 139). In addition, reclassification reports were issued to 24 previously-tested patients who also carried these variants.

CONCLUSION: Concurrent RNA and DNA genetic testing increases the clinical impact of Lynch syndrome testing through increasing the diagnostic yield while simultaneously decreasing the number of MMR gene VUS.