

Title: The Missing Piece: Targeted RNA Studies Reduce Uncertainty and Increase Diagnostic Yield

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Abstract: RNA studies have proven to be an important tool in clarifying the pathogenicity of variants that may affect splicing. Recently, clinical laboratories have started offering RNA studies using various methods, including RNA sequencing paired with DNA and targeted RNA analysis to clarify the pathogenicity of variants identified by DNA testing. As clinical use of exome and genome sequencing becomes more prevalent, targeted RNA studies will become increasingly important to clarify the pathogenicity of unique variants.

Here, we reviewed the outcome of targeted RNA studies between January 2018 and May 2025 for exome and neurology panels. Criteria for RNA studies include variants in characterized genes with clinical relevance to the proband, predicted impact on splicing by in silico models, sufficient gene expression in blood, and an established loss-of-function mechanism of disease. Variants were either identified through testing at our laboratory or by request from clinicians to aid in resolving the classification of variants identified at external laboratories. We examined the origin of cases, frequency over time, and the diagnostic impact of these RNA studies. We compared groups using Fisher's exact test.

During the study period, RNA studies were performed for 50 unique variants in 44 genes. Genes with more than one variant included FOXP1, IFT140, ITGB2, LZTR1, RMND1, and SLC20A2. 86% (n=43) of variants were intronic, 10% (n=5) were missense, 2% (n=1) nonsense, and 2% (n=1) silent. The vast majority of variants (90%; n=45) started as VUS, 8% (n=4) were likely pathogenic (LP), and 2% (n=1) were pathogenic (P).

RNA studies resulted in a variant reclassification 52% (n=26) of the time, and 88% (n=23/26) of variant reclassifications resulted in a new diagnosis. Overall, the diagnostic rate in this cohort increased from 10% (n=5) to 56% (n=28). There was a 56% relative decrease in VUS from 45 to 20, with RNA data most often supporting a VUS upgrade to LP or P. In two instances, RNA studies resulted in a VUS downgrade to likely benign (LB).

The cohort was made up of more external cases needing clarification (n=33) compared to internal cases (n=17), and a large influx was seen in 2024 when a clinical product for RNA studies following exome sequencing was launched. There were no significant differences in VUS reclassification between internally detected variants and externally referred cases (p=0.7575 for VUS upgrades and p=1.0 for VUS downgrades), suggesting RNA studies are just as useful on a proactive setting compared to selection of previously-tested variants.

These findings highlight the role of RNA studies in clarifying the pathogenicity of variants, resulting in a reclassification for 52% of variants. RNA studies reduced the number of VUS and increased the diagnostic yield. The availability of RNA studies to clarify VUS on exome and genome sequencing at the time of testing will further increase the clinical utility of these tests.