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

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BACKGROUND

- RNA studies have proven to be an important tool in clarifying the pathogenicity of variants that may affect splicing
- Some clinical laboratories offering RNA studies to clarify the pathogenicity of variants identified by DNA testing.
- RNA studies are increasingly important to clarify the pathogenicity of unique variants identified on exome and genome sequencing

OBJECTIVES: Assess the outcomes of targeted RNA studies between January 2018 and May 2025 for exome and neurology panels at one commercial lab

RESULTS

- RNA studies were performed for 50 unique variants in 44 genes
  - *FOXP1, IFT140, ITGB2, LZTR1, RMND1,* and *SLC20A2* had more than one variant tested
- Most variants were intronic, but missense, nonsense, and silent variants also benefitted from RNA studies **[Figure 2]**
- 90% of variants (n=45) started as VUS and RNA studies resulted in a reclassification of 52% (n=26) **[Figure 3]**
  - 88% of reclassifications resulted in P/LP finding, increasing diagnostic yield
  - 56% relative decrease in VUS from 45 to 20
- More external cases (n=33) compared to internal cases (n=17**) [Figure 4]**
  - Influx of external cases were seen in 2024 when an exome-paired RNA studies offering was launched
- There were no significant differences in VUS reclassification rates between internal and externally referred cases (p=0.7575 for VUS upgrades and p=1.0 for VUS downgrades)

Figure 2: Variant Types Analyzed by RNA Studies

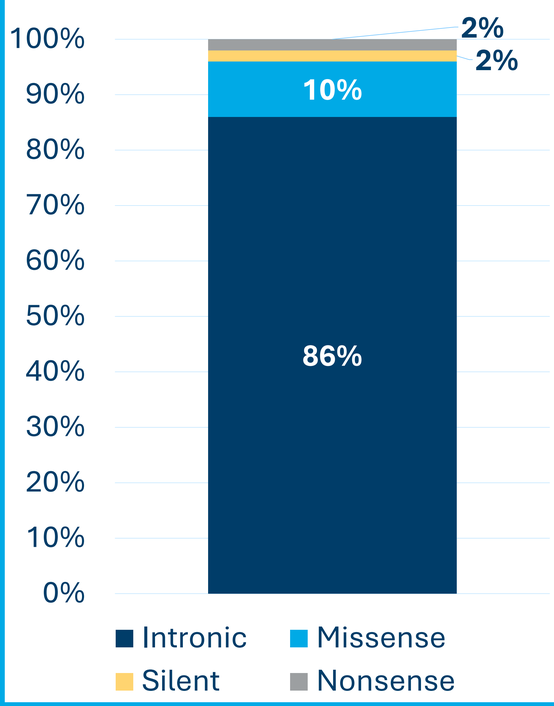
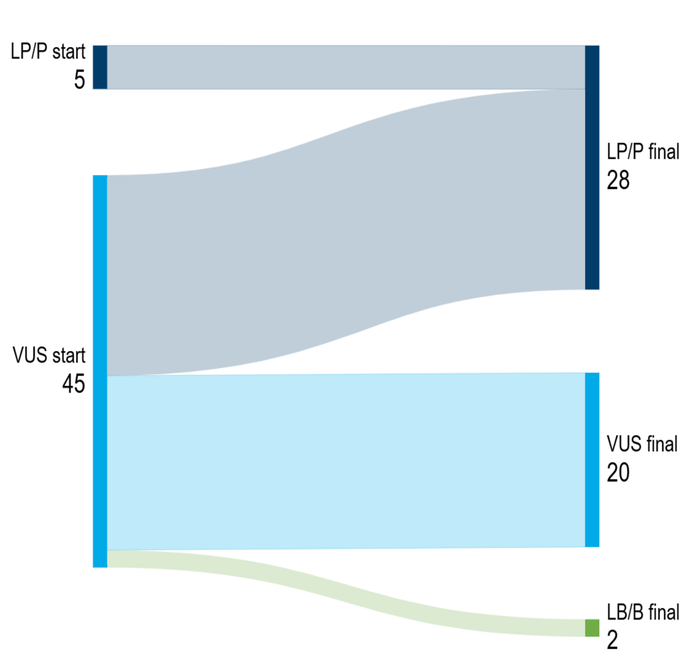


Figure 3: RNA Studies Impact on Variant Classification



METHODS

- Variants were either identified through testing at our laboratory (prospective) or by request following testing at external laboratories (retrospective) **[Figure 1]**
- Outcomes analyzed by origin of cases, frequency over time, and the diagnostic impact of RNA studies

Figure 1: Prospective v. Retrospective RNA Studies Workflows

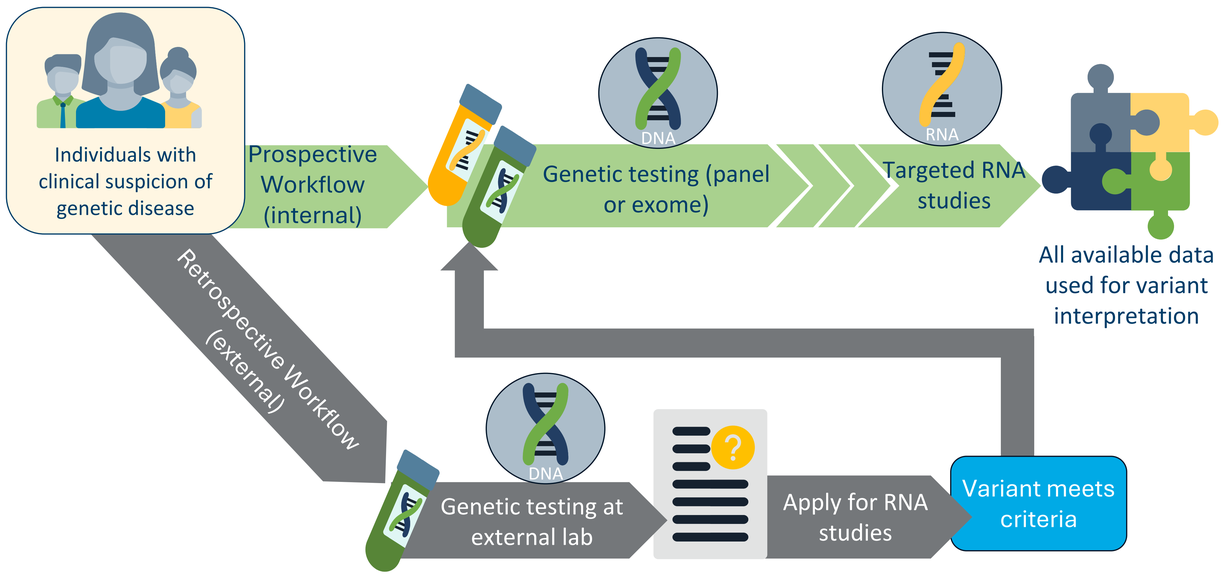
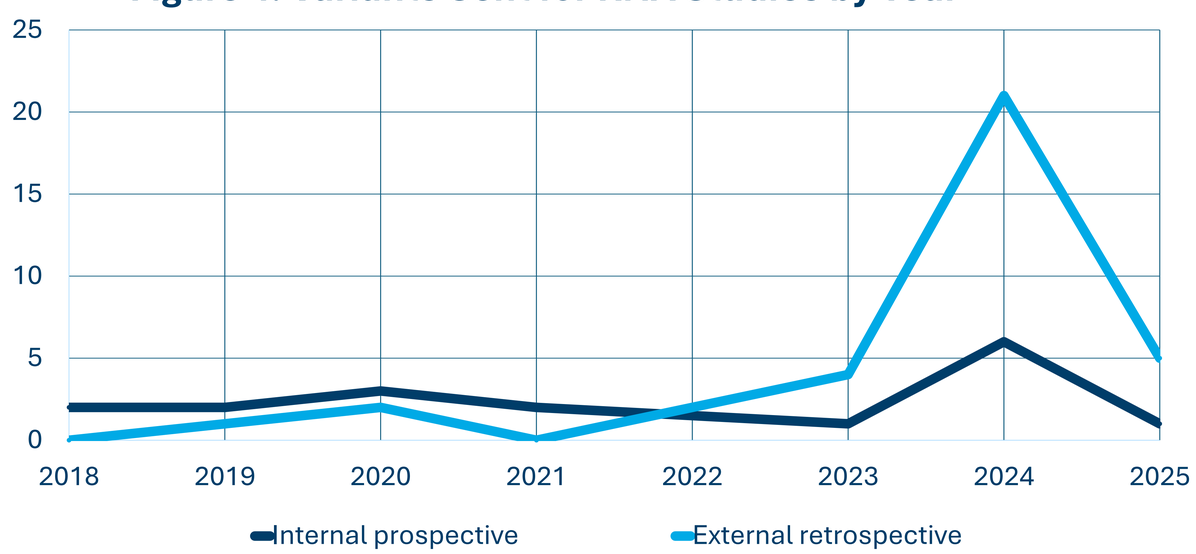


Figure 4: Variants Sent for RNA Studies by Year



TAKE HOME POINTS

- RNA studies reduce the number of VUS and increase the diagnostic yield
- Targeted RNA studies are in demand and are a practical way to generate the evidence required for ES VUS resolution
- Variety of variant types benefit from RNA studies
- Proactive approaches to RNA studies streamline variant reclassification