Diagnostic Utility of RNA Sequencing for Reclassification of Rare Disease Variants by Exome and Genome Sequencing

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BACKGROUND

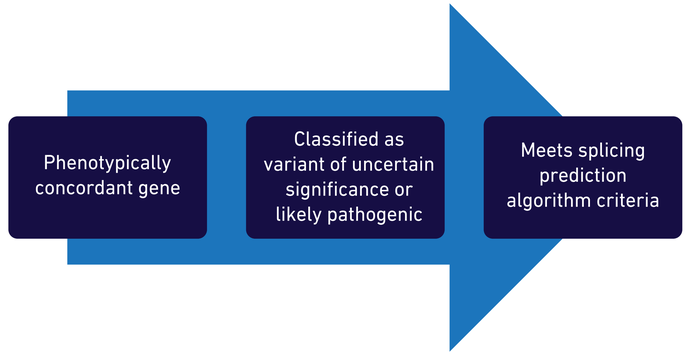
- Variants of uncertain significance (VUS) present significant challenges in interpretation and medical management for healthcare providers and patients.[1]
- RNA sequencing (RNAseq), which has been used in hereditary cancer testing to aid in variant interpretation, has been increasingly used to provide functional evidence in genome sequencing (GS) and exome sequencing (ES).
- For variants expected to impact splicing and gene expression, RNAseq may allow for reclassification and greater diagnostic clarity.[2]
- Here, we describe our diagnostic laboratory's experience with leveraging clinically-validated RNAseq using EDTA blood to clarify the significance of variants obtained by GS/ES.

METHODS

Study Design: Retrospective review of GS/ES and RNAseq results.

Inclusion Criteria:

- Recent consecutive GS/ES cases with clinical RNAseq opt-in and where an eligible variant associated with the patient's phenotype was detected.
- Variant reclassification was based on RNAseq functional evidence using internal guidelines and ACMG criteria.

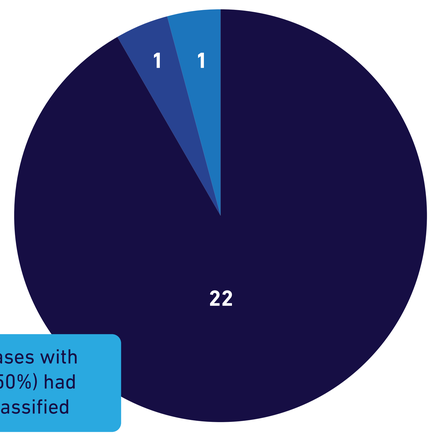


RESULTS

3594 consecutive GS/ES cases with RNAseq opt-in → 139 (3.9%) cases had at least one RNAseq eligible variant reported

Quick Facts about Eligible Variants

- Included 129 VUS, 17 likely pathogenic (LP), and one pathogenic (P)
- Most eligible variants were captured by GS (118/139)
- Included 51 unique deep intronic variants
- Eight cases with two unique eligible variants
- Ten cases homozygous for an eligible variant



Among 48 completed cases with an eligible variant, 24 (50%) had variants that were reclassified

If all cases in our cohort had completed RNAseq, approximately 1.8% of all cases would be expected to be reclassified

RNAseq eligible variants were identified in genes associated with a broad spectrum of diseases and different inheritance patterns. The genes below had eligible variants identified in two or more cases.

Gene	Cardiac	Neuromuscular	Neurology	Skeletal	Renal	Metabolic	Head & Neck
TTN	×	×					
COL4A3					×		×
DMD	×	×	×				
CHD5			×				×
KDM5C			×	×			
GLDC		×	×			×	

Among reclassified cases, 23 unique genes were identified. Reviewing GTEx Analysis v10 values for whole blood samples, 10 (43.5%) of these genes have median transcripts per million (TPM) <1.0.[3]

CONCLUSION

- Clinically validated RNAseq provides functional evidence to allow for more accurate variant classification in a significant number of GS/ES cases, **enhancing diagnostic yield**.
- Eligible variants were identified in a **variety of genes** associated with a wide spectrum of diseases and across inheritance patterns.
- Over a **third of eligible cases had noncoding variants** that would likely be missed by ES.
- Almost half of reclassified variants were within genes with **whole blood TPMs lower than 1.0**.
- The utility of **RNAseq to establish rare disease diagnoses** supports inclusion of this and other multiomic approaches for clinical GS and ES.

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References:

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2) Truty, R., Ouyang, K., Rojahn, S., Garcia, S., Colavin, A., Hamilngton, B., Freivogel, M., Nussbaum, R. L., Nykamp, K., & Aradhya, S. (2021). Spectrum of splicing variants in disease genes and the ability of RNA analysis to reduce uncertainty in clinical interpretation. *American journal of human genetics*, *108*(4), 696–708. https://doi.org/10.1016/j.ajhg.2021.03.006.

3) GTEx Portal Open Analysis Datasets [accessed 9/30/25]