Title: RNA-guided Clarity: The Potential for Resolving Variant Uncertainty in Clinical Exome Sequencing

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Despite the potential of exome sequencing (ES) as a primary diagnostic tool, over half of the patients tested remain without a definitive diagnosis. While RNA studies have increased detection rates and variant interpretation in oncology, their use in broader rare disease contexts is limited due to concerns about gene expression in whole blood. This study aims to quantify the impact of proactive RNA analysis in ES by identifying the percentage of reported variants suitable for RNA analysis.

We retrospectively analyzed all variants reported on clinical ES cases from March 14, 2019, to December 31, 2023. Variants were categorized by alteration type and classification at the time of the initial exome report. Variants were evaluated to see how many met the following RNA studies criteria at our laboratory: There is sufficient phenotype overlap with reported patient phenotype, the gene has at least moderate gene-disease validity (GDV), the reported variant is expected to be spliceogenic (intronic, synonymous, or missense with a spliceAI score >0.2), the gene has sufficient expression in blood (≥0.5 TPM in whole blood per Genotype-Tissue Expression (GTEx) Portal) and the mechanism of disease for the gene-disease relationship is loss of function.

Out of 4607 cases reported in the study period, the diagnostic yield was 19.5% (900/4607), and 16.9% of cases (779/4607) received an uncertain report due to one or more Variants of Uncertain Significance (VUS). 37% (1692/4607) had at least one clinically relevant variant reported for a total of 2032 variants, and of these 1993 variants were in genes with at least moderate GDV. 11.2% (228/2032) were putative splicing variants, including 150 intronic variants (7.4%; range +/-1 to +78) and 78 exonic variants (3.8%; spliceAI score of >0.2). Of the 228 putative splicing variants, 61.4% (n=140) were classified as likely pathogenic or pathogenic (LP/P), and 38.6% (n=88) were VUS. 57.5% (131/228) of putative splicing variants met our laboratory's criteria for RNA analysis. Of all VUS reported in this cohort, 3.5% (37/1049) met RNA studies criteria.

Variants with predicted splicing impacts are a significant portion of VUS in clinical exome cohorts. This data indicates RNA is well-expressed in blood for most genes evaluated, suggesting RNA studies could clarify 3.5% of reported VUS. Integrating RNA analysis with ES is a viable method to enhance diagnostic accuracy, marking a major advancement in genetic diagnostics and opening new avenues for patient benefit.