RNA and Reclassification: Assessing RNA Data in Rare Disease

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BACKGROUND

- **Exome sequencing** (ES) has a higher diagnostic yield when paired with complementary methods, like RNA analysis which helps interpret the functional impact of splicing variants.
- **Interpretation of RNA data** is complex and requires expertise to appropriately weigh this evidence as part of overall variant classification.

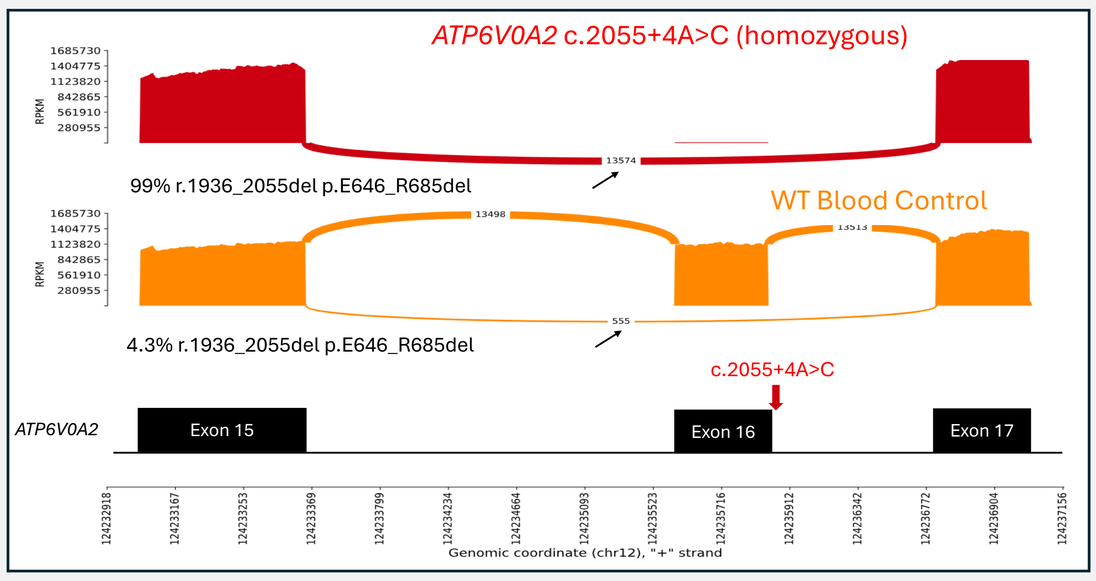
AIM: Compare two cases of aberrant splicing to examine how RNA analysis impacts variant classification in rare disease.

METHODS & RESULTS

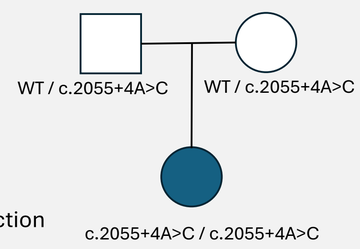
1. ES identified variants with predicted splice impacts for two patients with rare syndromic neurodevelopmental disorders **[Table 1]**.
2. Targeted RT-PCRseq was performed on whole blood **[Figure 1]**.
3. RNA data evaluation was applied to variant classification **[Figure 2]** leading to reclassification in Case 1 and no change in Case 2 **[Table 1]**.

FIGURE 1: RNA STUDIES RESULTS

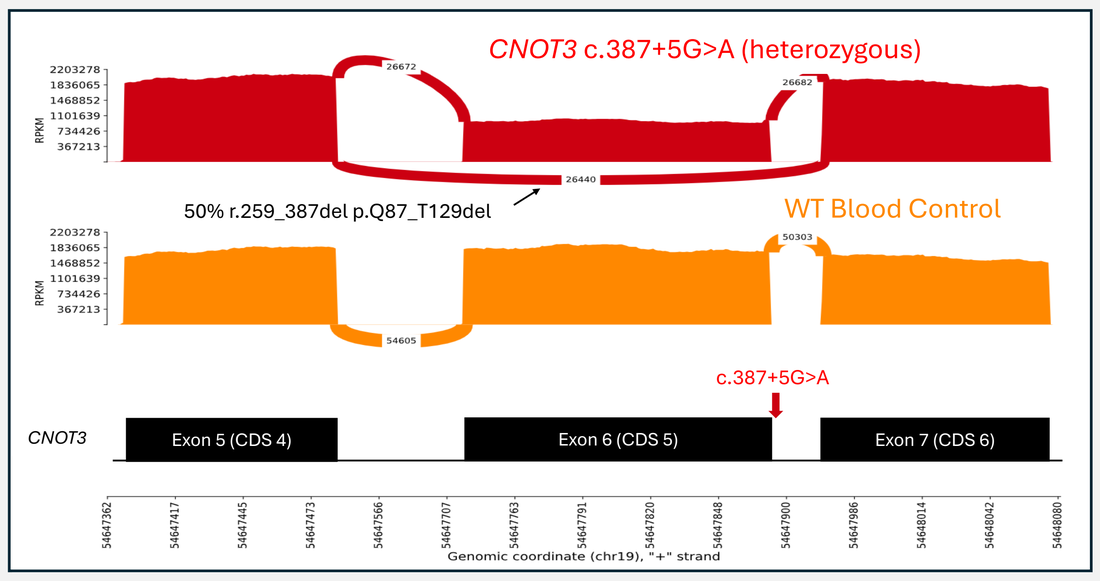
Case 1



5-year-old female with:
- Dysmorphic features
- Global developmental delay
- Hypotonia
- Febrile seizures
- Poor vision
- Tenting of skin
- Lissencephaly & sensory integration dysfunction



Case 2



26-year-old male with:
- Intellectual disability
- Global developmental delay
- Autism
- Behavioral abnormalities
- Short stature
- GI issues
- Dysmorphic features

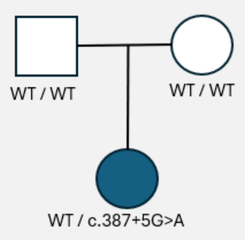
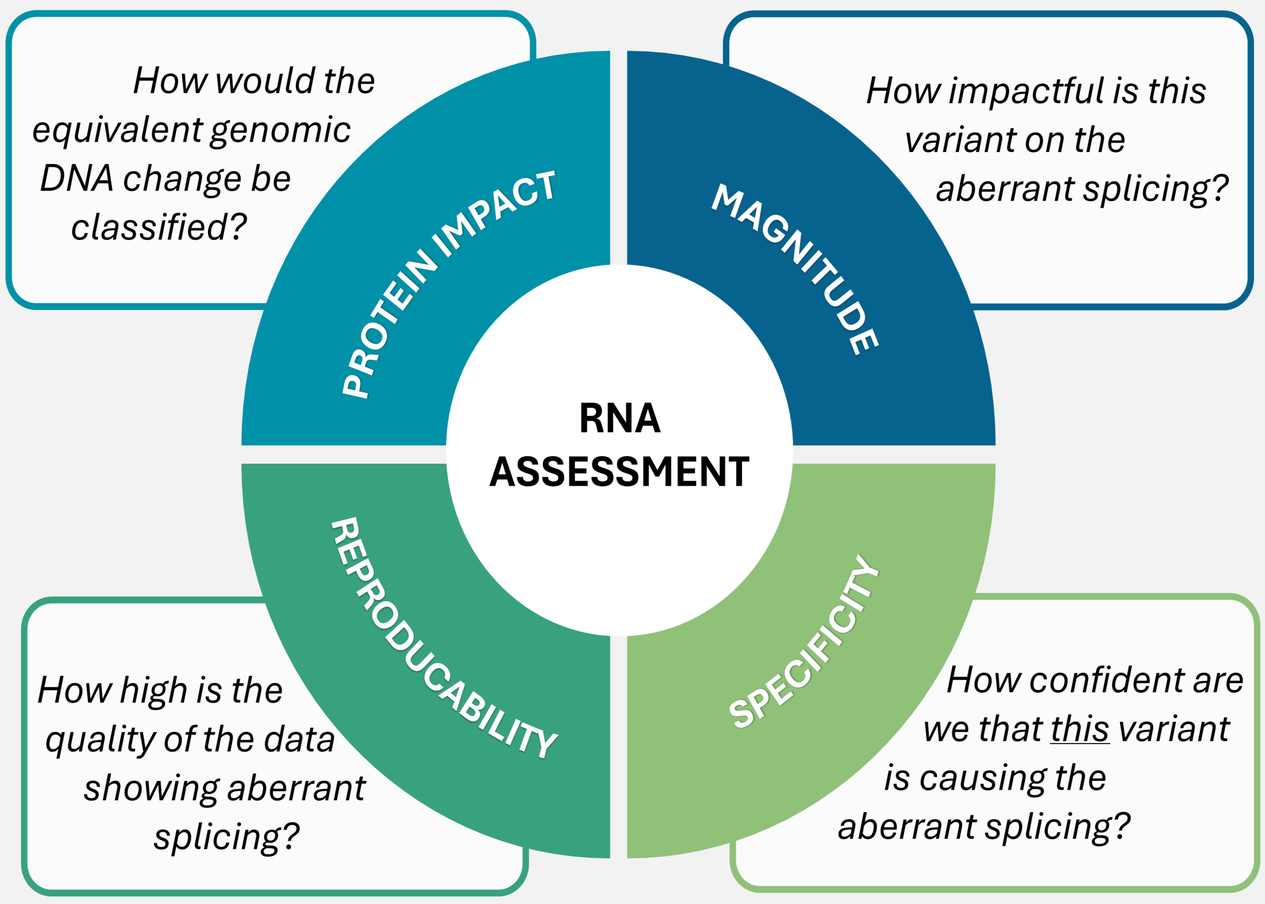


TABLE 1: APPLYING RNA EVALUATION TO VARIANT CLASSIFICATION

Case	1	2
Gene (c.)	*ATP6V0A2* (c.2055+4A>C)	*CNOT3* (c.387+5G>A)
Zygosity	Homozygous	Heterozygous (*de novo*)
Condition	AR cutis laxa, type IIA	Syndromic ID disorder
Magnitude	High PSI (99.03%)	High PSI (49.82%)
Specificity	Absent in controls; SpliceAI = 0.53 donor loss	Absent in controls; SpliceAI = 0.70 donor loss
Reproducibility	One assay only	One assay only
Protein Impact	Germline deletion of exon 16 is pathogenic	Effect of exon 5 skipping is uncertain
RNA Impact	VUS → LP	VUS → VUS

FIGURE 2: RNA DATA EVALUATION FACTORS



TAKE HOME POINTS

- Confirmation of **aberrant splicing** is only one facet of evaluating RNA studies.
- Consideration of the **splicing impact on the protein** is essential for accurate variant classification.
- Adding supportive RNA evidence enhances the potential **for future variant reclassification**.
- RNA analysis combined with ES offers potential to resolve VUS and **increase diagnostic yield**.