Splicing up the Diagnosis: Using Targeted RNA Studies to Reclassify Exome Sequencing Variants

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BACKGROUND

- RNA analysis is valuable for providing evidence for variant classification
- Whole transcriptome (WTS) improves exome sequencing (ES) diagnostic yield
  - However, WTS is not yet feasible for all exome patients
- Targeted RNA studies provide many of the same benefits as WTS
- Careful selection of variants for RNA studies maximize the efficiency and efficacy

SCAN ME

AIM: Review ES cases between 2017-2021 with variant reclassification due to targeted RNA studies

METHODS

ES variant of uncertain significance (VUS) eligible for RNA studies **[Figure 1]**

Ambry Translational Genomics (ATG) Lab performs targeted RNA studies

Evidence generated used for variant classification

Reclassification report issued

RESULTS

- 9 cases were reclassified based on RNA evidence **[Table 1]**
- Prior to ES, all were undiagnosed after extensive genetic testing **[Figure 2]**
- Clinical indications reflect typical clinical ES cohort **[Figure 3]**

TABLE 1: RECLASSIFED VARIANTS

Case	Gene	Nucleotide change	Classification without RNA evidence	Classification with RNA evidence
1	*FOXP1*	c.1652+5G>C	VUS	P
2	*ANKRD11*	c.226G>A	VUS	P
3	*TNNT1*	c.32+5G>A	VUS	P
4	*ADNP*	c.201G>C	VUS	LP
5	*FMR1*	c.104+3_104+6delAAGT	VUS	P
6	*TRIP12*	c.4190+5G>A	VUS	P
7	*FOXP1*	c.1531-9_1534dup13	VUS	P
8	*PTEN*	c.210-12C>G	VUS	LP
9	*SPG11*	c.5866+5G>C	VUS	LP

FIGURE 1: ELIGIBILITY FOR RNA STUDIES

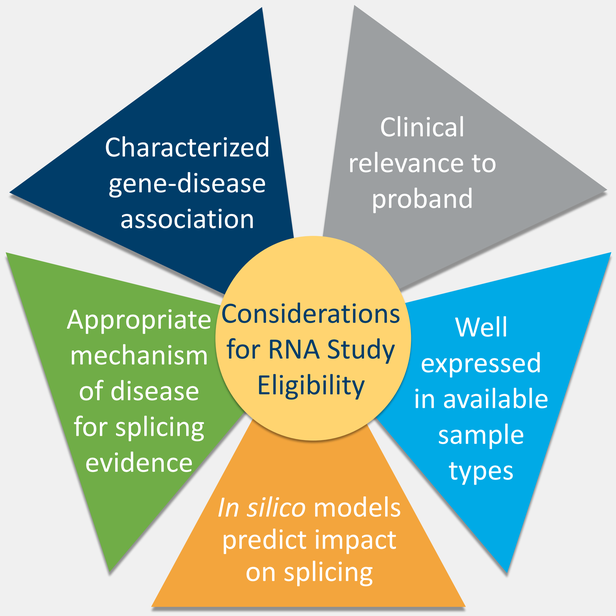


FIGURE 2: PREVIOUS GENETIC TESTING

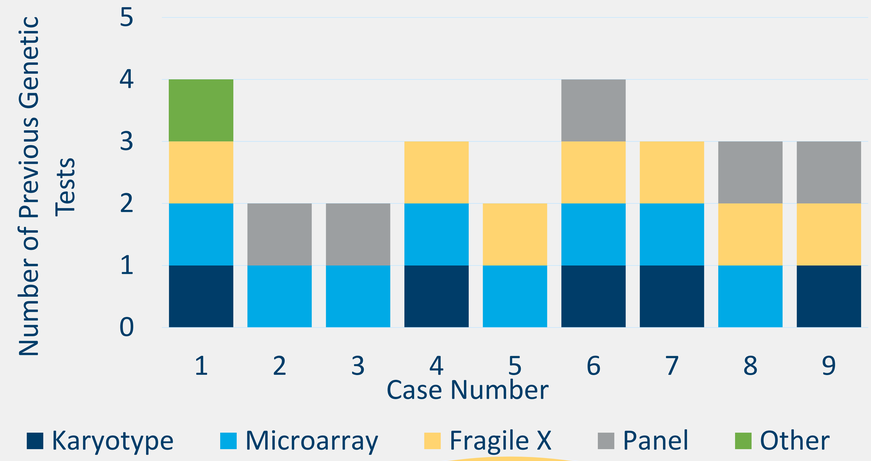
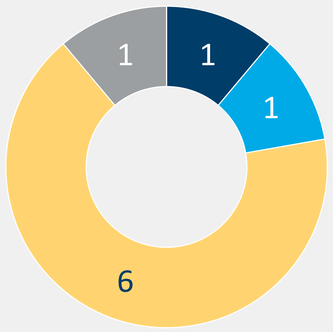


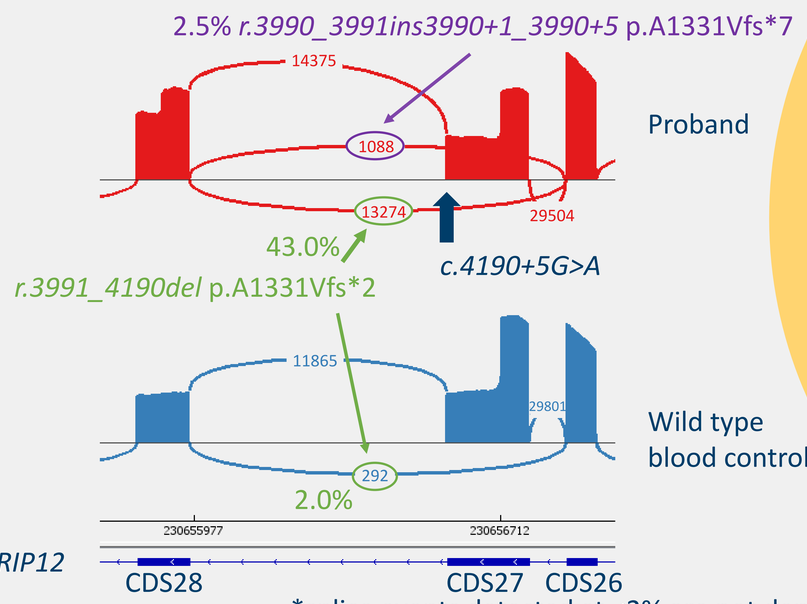
FIGURE 3: INDICATION FOR TESTING



CASE EXAMPLE

- Global developmental delay, Autism spectrum disorder, auditory hallucinations, and dysmorphic features
- Karyotype, CMA, and Fragile X normal
- Neurodevelopmental panel: *POLG* VUS which was found to be paternally inherited on ES
- ES revealed a VUS in *TRIP12* c.4190+5G>A
- RNA studies by the ATG lab resulted in a reclassification to pathogenic
  - Collective 45.5% abnormal splicing observed **[Figure 4]**

FIGURE 4: SPLICING FOR TRIP12 c.4190+5G>A



*splice events detected at <2% are not depicted for clarity

TAKE HOME POINTS

Targeted RNA studies can provide powerful evidence for VUS resolution

Clinical relevance to the proband, predicted splice effect, and mechanism of disease should be considered when selecting variants for RNA studies

Targeted RNA studies provide valuable insight for future transcriptome data