

RNA Sequencing to Help Identify Rare Genetic Disorders

Lisa Salz, MS, CGC¹, Robert Rigobello, MS, CGC¹, Xiaonan Zhao, PhD, FACMG^{1,2}, Morgan Driver, PhD, MS, CGC¹, Sydney Lau, MS, CGC¹, Jason Chibuk, MS, CGC¹, Wilson Wu, PhD, FACMG^{1,2}, Hongzheng Dai, PhD, FACMG^{1,2}, Linyan Meng, PhD, FACMG^{1,2}, Fan Xia, PhD, FACMG^{1,2}, Christine Eng, MD^{1,2}

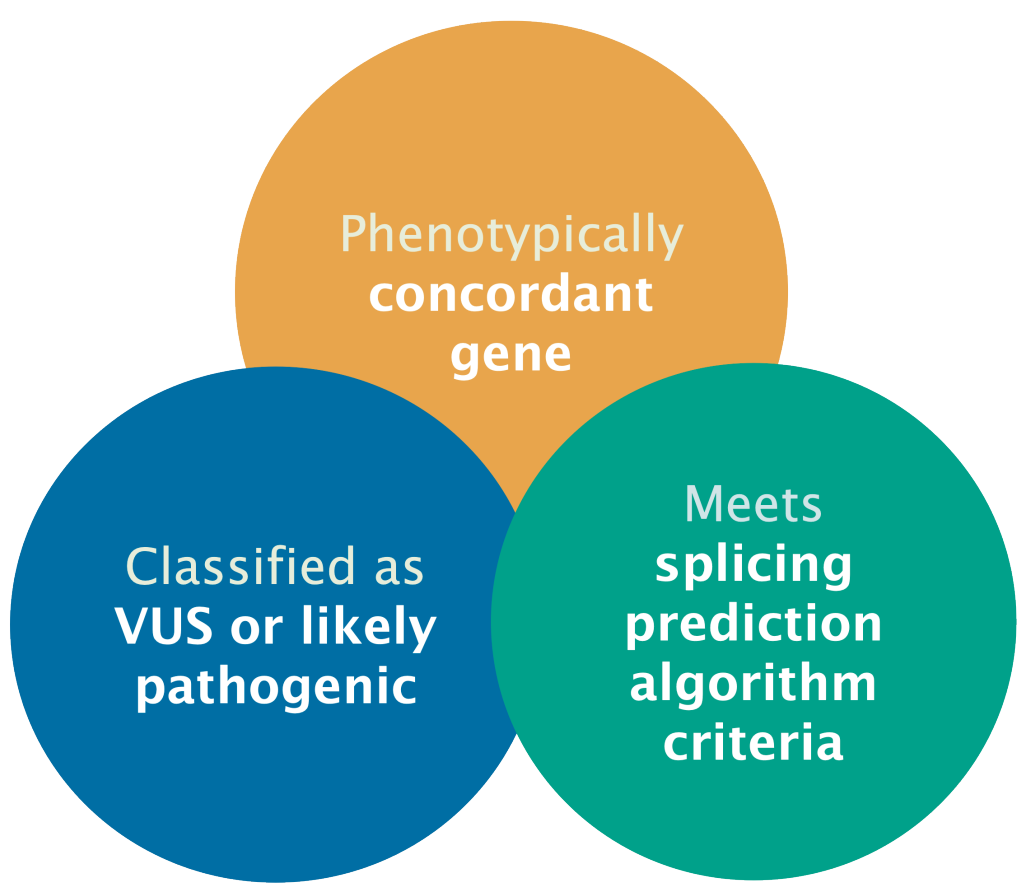
1) Baylor Genetics Laboratory, Houston, TX, USA.
2) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX, USA.

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INTRODUCTION TO RNA SEQUENCING (RNA-SEQ)

- Variants of uncertain significance (VUS) present interpretation and management challenges for healthcare providers and patients
- RNA sequencing (RNA-seq) provides functional evidence for variants expected to impact splicing and gene expression identified by traditional sequencing approaches, allowing for reclassification of VUS
- RNA-seq, which has been used in hereditary cancer testing to increase diagnostic yield, has become available in the rare disease setting through genome sequencing (GS) and exome sequencing (ES)
- Here, clinical cases resolved by RNA-seq performed at a clinical diagnostic laboratory will be discussed

METHODS AND CRITERIA FOR RNA-SEQ



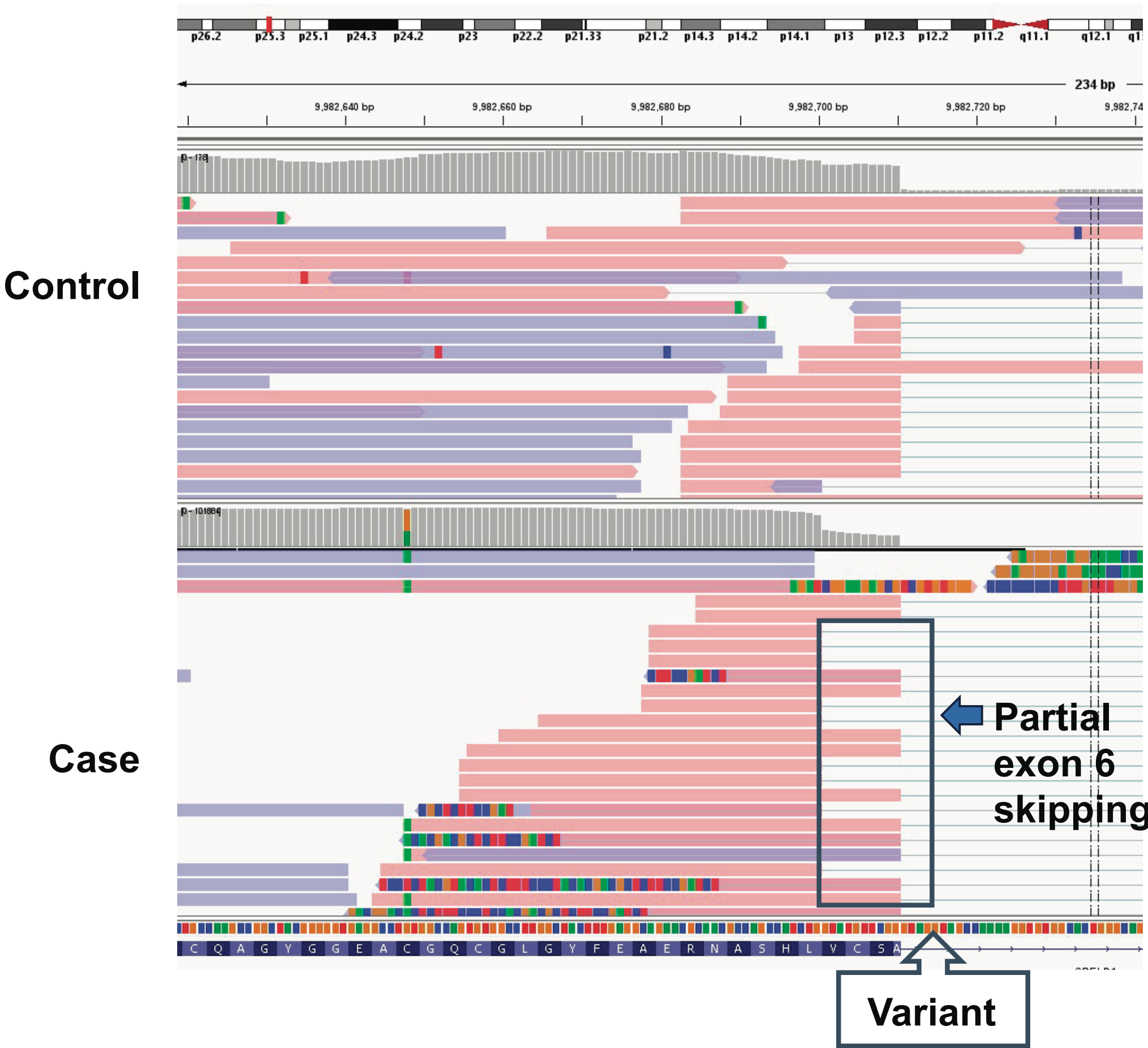
- DNA and RNA extracted from peripheral EDTA blood for clinical ES/GS cases with additional consent obtained for reflex RNA-seq
- Inclusion criteria: variants classified as VUS or likely pathogenic, associated with patient phenotype, and predicted to affect splicing using SpliceAI prediction algorithm and reviewed in Alamut Visual Plus
- Reflex RNA-seq performed on NovaSeq; splicing events detected via manual IGV review using Sashimi plots
- Variant reclassification based on RNA-seq functional evidence using internal guidelines and ACMG criteria

RNA-SEQ CLINICAL CASE EXAMPLES

Case 1: 2-YEAR-OLD MALE

- Admitted to the PICU for status epilepticus
- Developmental delay, hypotonia, prolonged QTc interval also noted
- Unremarkable family history
- Uninformative CMA, seizure panel, congenital hypotonia panel, and Prader-Willi syndrome testing at outside laboratories

- Rapid trio GS result - *CRELD1* c.575G>A, p.C192Y (pathogenic, maternal) and c.637+3A>T (VUS, paternal)
- Biallelic variants in *CRELD1* are associated with Jeffries-Lakhani Neurodevelopmental Syndrome (JELANS)
- RNA-seq result - the paternal VUS causes a premature stop codon and partial skipping of exon 6, leading to reclassification as likely pathogenic and JELANS diagnosis
- Fewer than 20 patients with JELANS have been described in the literature
- This patient's phenotype matches many of the features observed in other patients, including early-onset seizures and hypotonia and global developmental delays
- Some patients also have been found to have cardiac arrhythmias



CLINICAL OUTCOMES

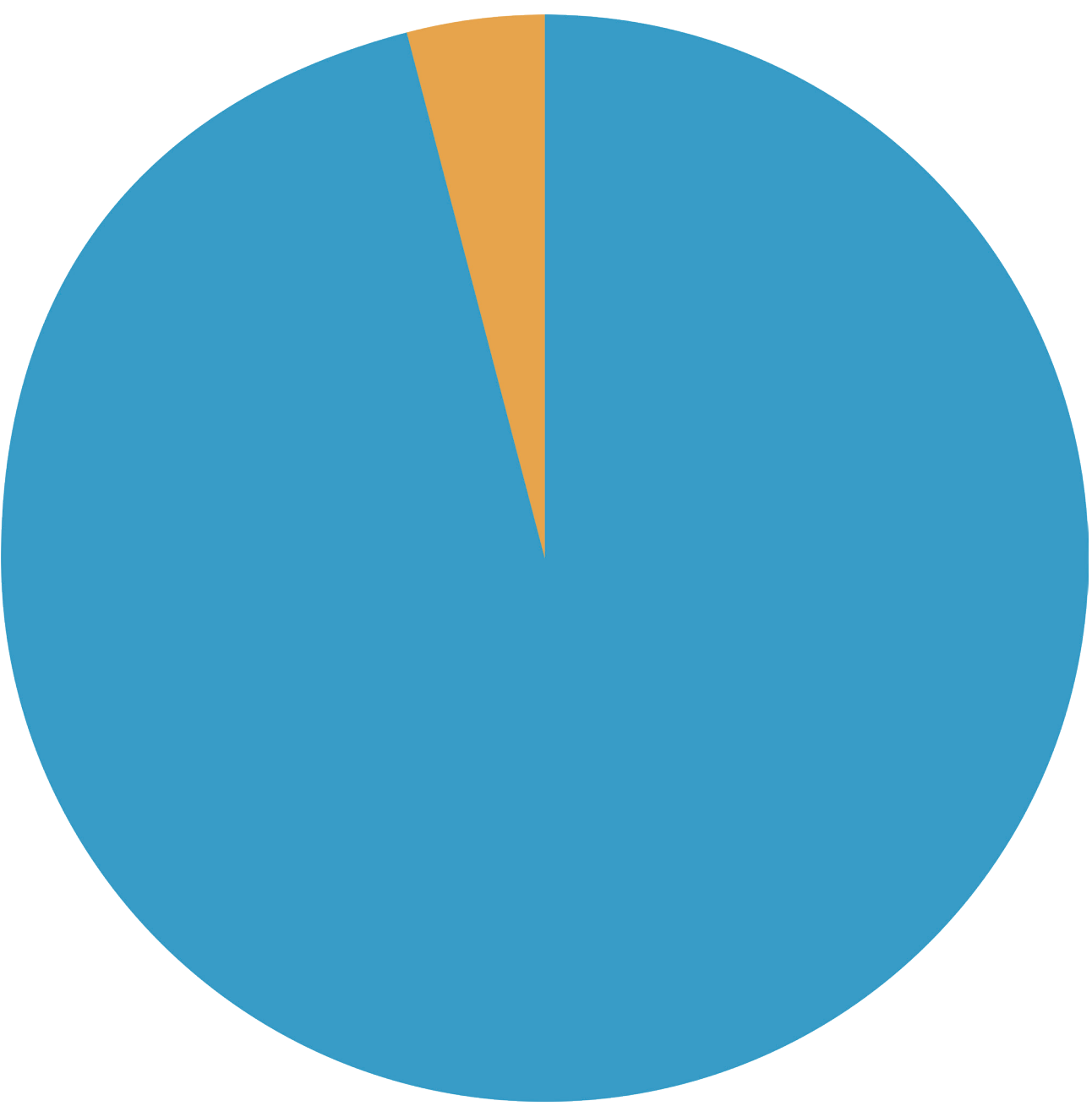
- **RNA-seq prompted additional patient phenotyping given rarity of the condition**
- **This diagnosis also confirms that QTc prolonging agents should be avoided**

CONCLUSIONS

- The addition of reflex RNA-seq to ES/GS enables robust detection of splicing variants in blood, allowing for reclassification of these variants using functional evidence.
- RNA-seq has demonstrably impacted clinical management of patients with reclassified variants, leading to management and surveillance changes, clinical trial eligibility, and other benefits.

RNA-SEQ IN AGGREGATE

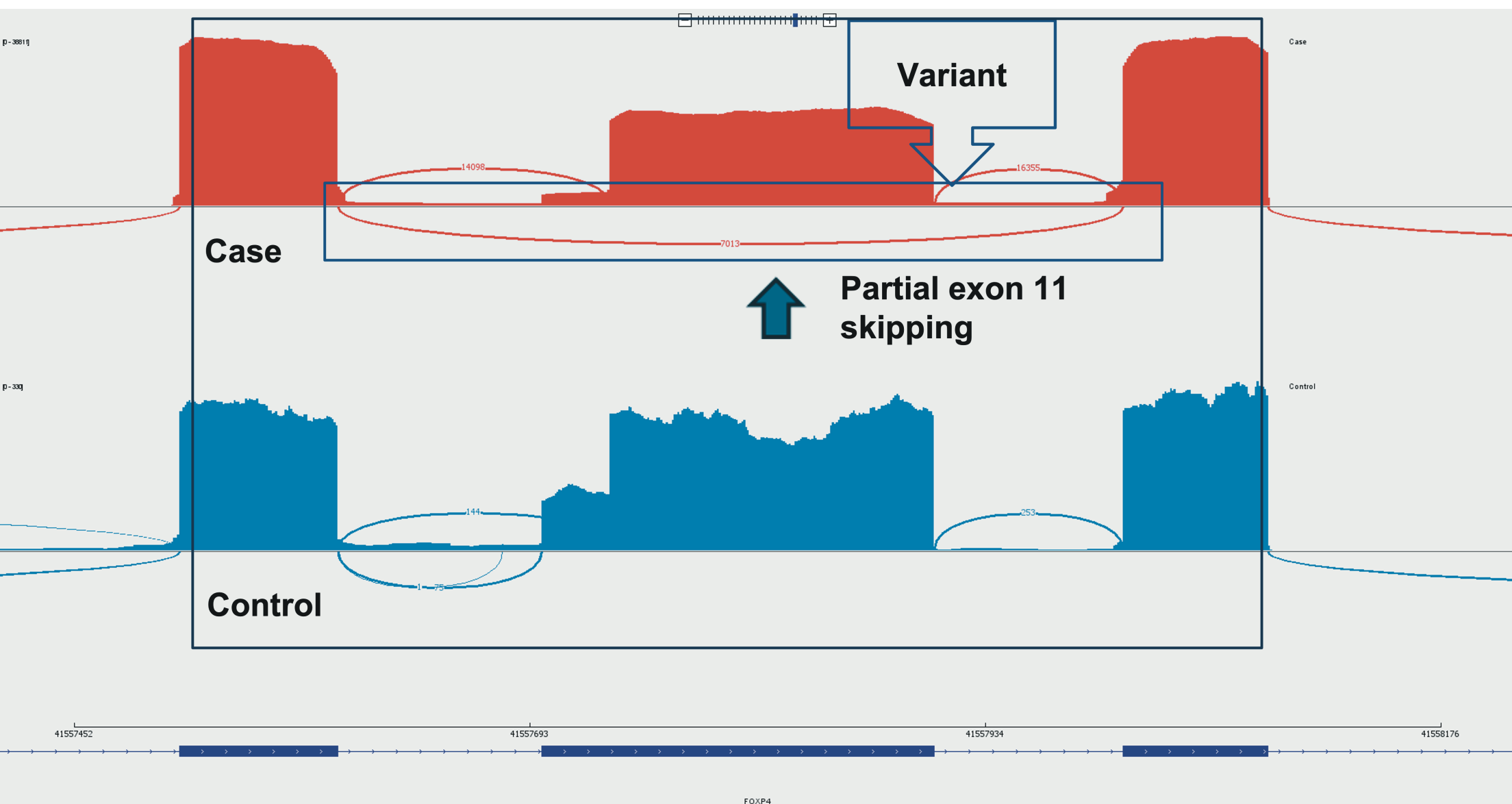
- From a retrospective review of ~3,200 consecutive ES and GS cases, 131 (4.1%) cases had at least one RNA-seq eligible variant identified
- Of cases with completed RNA-seq, 42.2% had a variant reclassification
- Overall, we would expect a 1.6% increase in overall diagnostic yield for ES and GS when RNA-seq is completed on all eligible cases
- This increase in yield aligns closely with another large cohort, where 1.7% of patients predicted to have variant reclassification across 9 clinical specialties¹



Case 2: 5-YEAR-OLD MALE

- Delayed speech and language development, motor delay, autistic behavior, hypotonia, EEG abnormality
- Abnormal carnitine level, inflammation of the large intestine
- Multiyear diagnostic odyssey with negative CMA and NGS panel testing from outside laboratories

- Trio GS result - *FOXP4* c.1357+2T>G (VUS, maternal)
- Heterozygous variants in *FOXP4* have been associated with a developmental disorder with language delay and congenital abnormalities
- RNA-seq result – partial skipping of exon 11 led to reclassification of the variant as likely pathogenic
- Several probands with an inherited *FOXP4* variant identified in the literature, with evidence for incomplete penetrance/variable expressivity as parents often had a milder or subclinical phenotype
- After testing completed the patient's mother was found to have had a degree of developmental and learning delay in childhood which had resolved



CLINICAL OUTCOMES

- **RNA-seq allowed for resolution of the patient's year-long diagnostic odyssey**
- **Antisense oligonucleotide therapy clinical trial was explored**