Clinical Utility of Transcriptome RNA-Sequencing in Diagnosing Rare Genetic Disorders: Initial Experiences

Mei Ling Chong[1,2], Jefferson Sinson[1], Nhi Ho[1,3], Patricia A Ward[1], Jill A Rosenfeld[1], Kristina Macakova[1], Gladys E Zapata[1], Becky Maywald[1], Chunjing Qu[2], Lindsay Burrage[1,3], Seema Lalani[1,3], Matthew T. Wheeler[4], Devon Bonner[5], Rodrigo Mendez[4], Jonathan A Bernstein[5], Carrie Bailey[6], Dave Viskochil[6], Undiagnosed Diseases Network, Christine M Eng[1,2], Brendan Lee[1,3], Pengfei Liu[1,2]

1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA
2) Baylor Genetics, Houston, TX, USA
3) Texas Children's Hospital, Houston, Texas, USA
4) Department of Medicine - Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA, USA
5) Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA
6) Department of Pediatrics, University of Utah, Salt Lake City, UT, USA

4027T

INTRODUCTION

- Transcriptome RNA-sequencing (TxRNA-seq) has emerged as a powerful complementary tool to genome and exome sequencing for diagnosing patients with rare genetic disorders.
- Here, we present our initial experience with validated TxRNA-seq and highlight its utility in making molecular diagnoses.

RESULTS

TxRNA-seq provided a positive diagnostic result in 11 of 45 cases (24%). Among these, GS reports showed either indeterminate findings with uncertain variant pathogenicity or partial phenotype–gene/variant overlap (n=7), poor SpliceAI predictions for potentially disease-causing variants (n=1), or negative results (n=3). TxRNA-seq further revealed abnormal splicing in 6 cases, absence of nonsense-mediated decay in 2 cases, altered gene expression associated with disease in 2 cases, and downstream signature changes in 1 case. Collectively, we leveraged TxRNA-seq to uncover disease-causing genes and their variants through the following transcriptome-based strategies:

CASE	GENE	ABNORMALITY	TRANSCRIPT	RNA ABERRATION	DNA VARIANT
1	*RASA1*	Splicing	NM_002890.3	r.[2487_2488ins214,2488_2560del]	c.2488-214T>G
2	*CARS2*	Splicing	NM_024537.4	r.466_571del	c.466G>T
3	*PRIM1*	Splicing	NM_000946.2	r.1145_1243del	c.1243+4A>G
4	*NOTCH2*	Splicing	NM_024408.4	r.1681_1682ins1682-181_1682-1	c.1682-1G>A
5	*SGCE*	Splicing	NM_003919.3	r.391_463del Low expression of *SGCE*	c.463+5G>A
6	*MRPL11*	Splicing	NM_016050.5	r.123_124ins124-259_124-1	c.124-115G>A
7	*SATB1*	Expression	NM_002971.6	Absence of NMD	c.431dup (p.Y144*)
8	*NLRP12*	Expression	NM_144687.4	Absence of NMD	Exon 5 deletion
9	*GNB4*	Expression	NM_021629.4	Low expression of *GNB4*	c.232A>T (p.Lys78Ter)
10	*ATAD3 gene cluster*	Expression	NM_001039211.3 NM_031921.6 NM_001170535.3	High expression of *ATAD3* gene cluster	*ATAD3* duplication
11	*RNU12*	Transcriptome signature	NR_029422.2	r.89a>g; r.77u>a	n.89A>G; n.77T>A

1. Expression evidence prioritized disease-causing genes and refined clinical phenotype assessment

Patient: 9-year-old male with progressive neurologic features, developmental delay, autism spectrum disorder, celiac disease, failure to thrive, short stature, and right hydronephrosis.

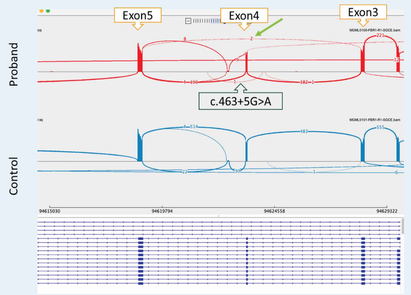
- Duo GS: Indeterminate findings, *ATP6V0A1*: c.2161C>A (p.Q721K), predicted deleterious in-silico
- Clinical assessment did not support autosomal dominant developmental and epileptic encephalopathy 104 (OMIM#619970) due to *ATP6V0A1*
- TxRNA-seq revealed significantly reduced *SGCE* expression with exon 4 skipping caused by c.463+5G>A
- Clinical re-evaluation identified additional phenotype, myoclonus with intermittent jerking of the left lower extremity
- The patient's phenotype is consistent with myoclonic dystonia-11 (OMIM#159900) caused by *SGCE*

GS: Indeterminate

INDETERMINATE FINDINGS

DISEASE	INHERITANCE PATTERN	GENE / VARIANT	VARIANT TYPE	GENOTYPE	INHERITED FROM	VARIANT CLASSIFICATION
Developmental And Epileptic Encephalopathy 104	Autosomal Dominant	ATP6V0A1: c.2161C>A, p.Q721K	Sequence Variant	Heterozygous	Mother Is Negative.	Variant Of Uncertain Significance

TxRNA-seq:



METHODS

- Under the Undiagnosed Diseases Network, we prospectively enrolled 45 patients with diverse and previously undiagnosed clinical presentations, representing multiple specialties.
- Our goal was to evaluate the diagnostic utility and clinical outcomes following TxRNA-seq.
- Patients with available genome sequencing (GS) data were included in this study, with 25 fibroblast and 20 blood specimens subjected to TxRNA-seq.
- We first analyzed TxRNA-seq data for expression outliers, splicing events, and transcriptome signatures. Genes with suspicious findings that aligned with patient phenotypes were then prioritized, and the corresponding DNA variants were further investigated using GS.

2. Identified novel disease-candidate genes by integrating DNA variants, expression, and splicing evidence

Patient: 25-year-old female with parental consanguinity presenting with an undiagnosed neurodegenerative and ataxic disorder, highly suspicious for Leigh syndrome.

- Negative whole mitochondrial sequencing, ETC analysis, and targeted mitochondrial mutational testing
- Negative GS
- TxRNA-seq revealed *MRPL11* as the lowest expressed gene
- *MRPL11* NM_016050.5:c.124-115G>A led to intron 6 retention and was not present in public databases
- *MRPL11* is a nuclear gene encodes a 39S subunit component of the mitochondrial ribosome

3. Confirmed the effect of disease-causing variants using transcriptome signatures

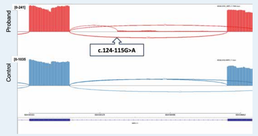
Patient: 3-year-old female with global developmental delay, progressive cerebellar ataxia, congenital borderline microcephaly, moderate cerebellar atrophy, mild pontine atrophy, hypotonia, oropharyngeal dysphagia, and dysmorphic features.

- Indeterminate GS findings: compound heterozygous variants identified in *RNU12*
- No abnormal expression or splicing detected in noncoding RNA, *RNU12*
- Transcriptome analysis showed increased splicing outliers, predominantly affected minor intron–containing genes. This pattern is characteristic of minor spliceosome pathway defects (PMID: 36056453).
- TxRNA-seq confirmed a global abnormal splicing signature of minor intron, serving as a functional assay for this case.

GS: Negative

NEGATIVE FINDINGS

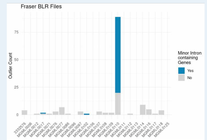
TxRNA-seq:



GS: Indeterminate

DISEASE	INHERITANCE PATTERN	GENE / VARIANT	VARIANT TYPE	GENOTYPE	INHERITED FROM	VARIANT CLASSIFICATION
COADS Syndrome	Autosomal Recessive	RNU12:NR_029422.2:n.89A>G	Sequence Variant	Heterozygous	Mother: Sanger Sequencing	Variant Of Uncertain Significance
COADS Syndrome	Autosomal Recessive	RNU12:NR_029422.2:n.77T>A	Sequence Variant	Heterozygous	The Anonymous Sperm Donor: Sanger Sequencing	Variant Of Uncertain Significance

TxRNA-seq: Global abnormal splicing signature



CONCLUSIONS

The advantages of TxRNA-seq in clinical diagnostics were demonstrated through direct transcript-level assessment, uncovering pathogenic mechanisms that DNA-based methods often fail to detect. Our initial clinical experience underscores the application of RNA-seq in improving diagnostic yield and refining molecular interpretations in complex rare disease cases.

Presented at the American Society of Human Genetics (ASHG) Annual Meeting, Oct 14-18, Boston, MA, USA.