MNG LABORATORIES
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RNA Sequencing:
Case Studies & Clinical Relevance

Next-generation sequencing has evolved into a powerful diagnostic tool helping thousands get answers to the most challenging disease diagnoses. Even with today's newest equipment, many diagnostic reports may include one or more VUS that are hard to interpret as being potentially pathogenic or uninvolved.

RNA sequencing can help re-classify a VUS, both in coding and non-coding regions, as a disease-causing variant. Additionally, this analysis can detect gene functionality and determine expressivity in an individual or specific tissue types.

RNA Sequencing Can Improve Diagnostic Outcomes

Detection of Transcripton and Gene Expression Levels

- Detects up or down regulation of transcript and gene expression levels
- Detects changes in the relative abundance of transcript copies with tissue-specific effects

Detection of Residual Gene Functionality

- Confirms any effect of intronic or coding sequence mutations on the process of splicing
- Identifies effects on gene functionality due to deletions of exons

Verification of VUS Effects

- Confirms functional effects and potential pathogenicity of a reported VUS

Tissues Validated for RNA Sequencing

Blood | Fibroblast Cell Lines | Muscle Tissue | Brain Tissue

Case 1: Becker Muscular Dystrophy, *DMD*

Clinical Information: Nine year-old male patient presenting with muscle weakness and suspected Becker Muscular Dystrophy.

Previous Testing: Serum CK testing was high, ~13,000. Immunohistochemistry analysis of muscle showed increased myofiber size variation and a broad range of atrophic and hypertrophic fibers. Sequencing analysis and microarray deletion/duplication testing were negative.

Family History: Maternal uncle diagnosed with Becker Muscular Dystrophy.

Gene-specific RNA sequencing: Targeted analysis of the *DMD* gene in a muscle tissue sample.

Outcome: We identified decreased expression of the *DMD* gene due to a duplication in the second intron that effects processing or stability of transcript levels.

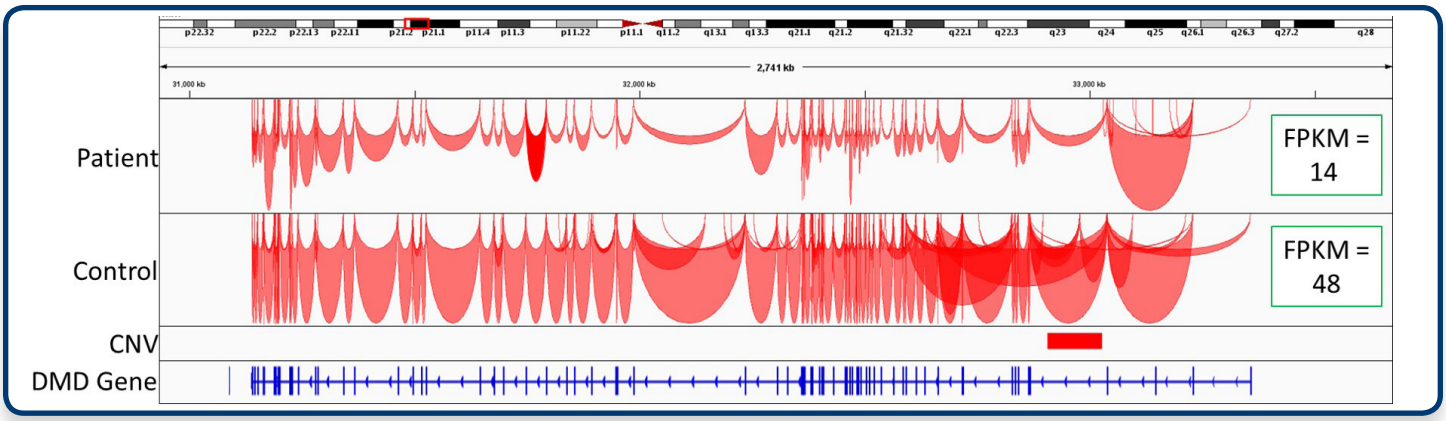

Figure 1: Decreased RNA transcript expression levels are seen in the patient sample compared to the control due to an identified intragenic duplication (red box).

Case 2: Muscular Dystrophy, *DMD*

Clinical Information: Thirteen year-old male patient presenting with muscle weakness, suspected to have Duchenne muscular dystrophy.

Previous Testing: Immunohistochemistry analysis of a muscle biopsy revealed the absence of dystrophin. Deletion/duplication analysis of the *DMD* gene identified a deletion of exon 44.

Family History: Negative

Gene-specific RNA sequencing: Targeted analysis of the *DMD* gene in a muscle tissue sample was used to confirm the functional affects of the exonic deletion.

Outcome: We identified altered splicing due to a deletion of exon 44 in the *DMD* gene.

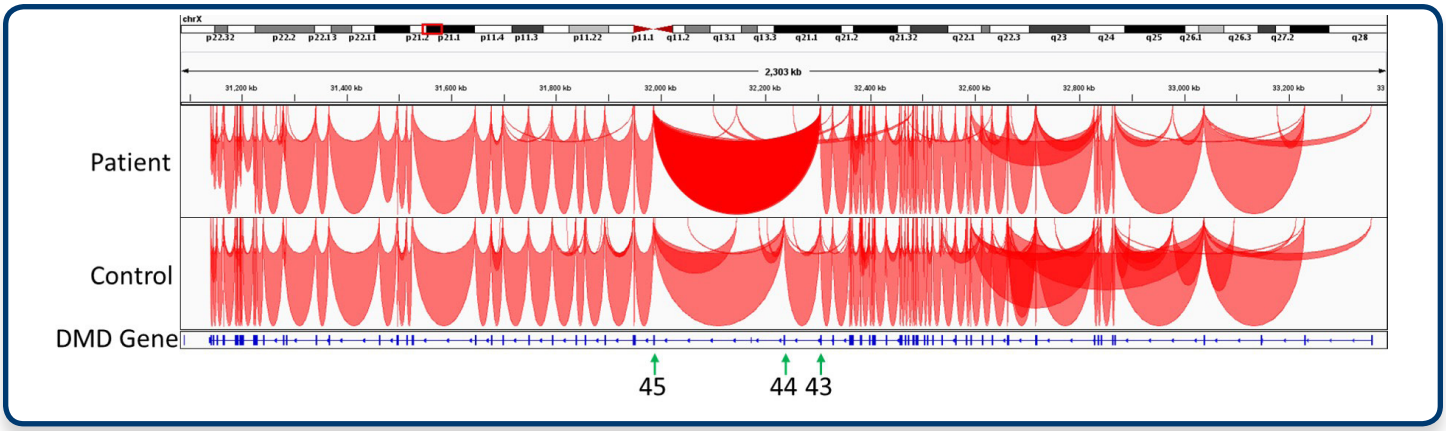

Figure 2: RNA sequencing shows skipping of exon 44 in the patient sequence, creating an abnormal splicing from exon 43 to 45, and to a smaller degree, other downstream exons. The affected exons are labeled with green arrows and numbers.

Case 3: Prader-Willi Syndrome

Clinical Information: Seventeen year-old male patient presenting with clinical symptoms suggestive of Prader-Willi Syndrome.

Previous Testing: Methylation analysis showed altered methylation with implied loss of paternal expression at chromosome 15q11.2.

Family History: Negative

Gene-specific RNA sequencing: Targeted analysis of the imprinted locus in a blood sample was tested to confirm loss of expression consistent with deletion of an unmethylated paternally inherited allele.

Outcome: No RNA expression was detected in the Prader-Willi locus in the patient sample, consistent with loss of the non-methylated allele.

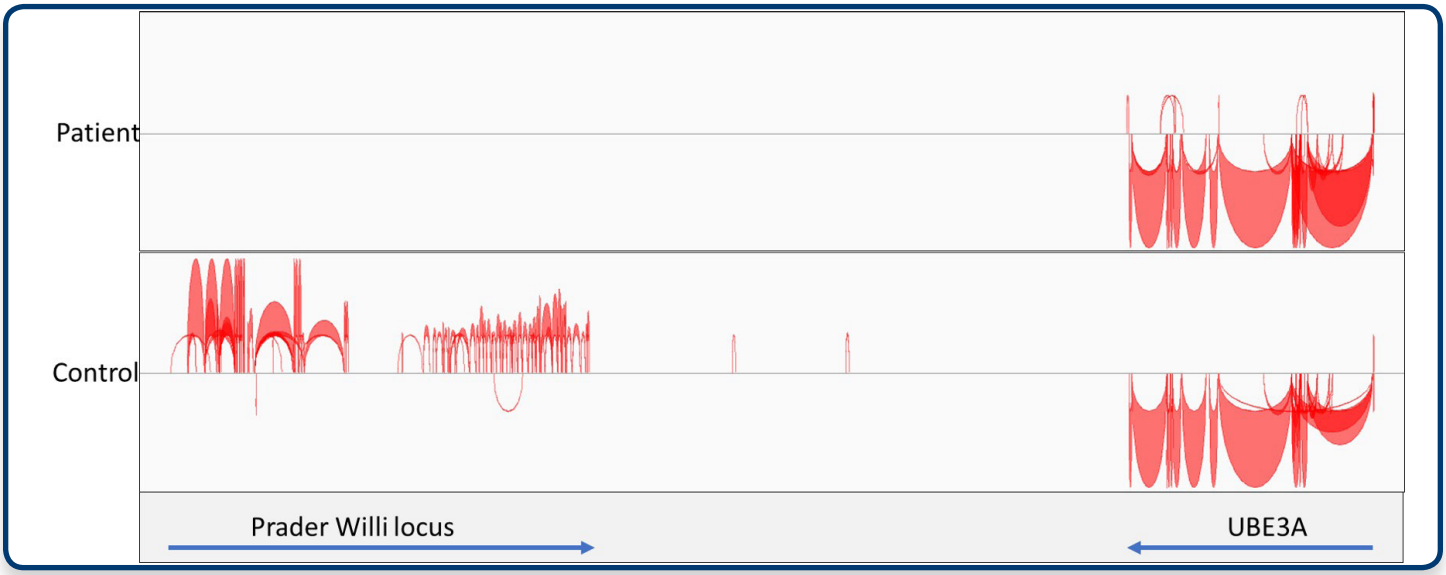

Figure 3: Analysis of the patient sample shows no RNA expression in the 15q11.2 region, consistent with previous testing results. Normal expression is seen in the control sample.

Case 4: Spinal Muscular Atrophy

Clinical Information: One year old female patient with a clinical diagnosis of Spinal Muscular Atrophy.

Previous Testing: None/Unknown

Family History: Negative

Gene-specific RNA sequencing: Targeted analysis of the *SMN1/SMN2* genes in blood was tested.

Outcome: We identified skipping of exon 7 in the *SMN1* transcript.

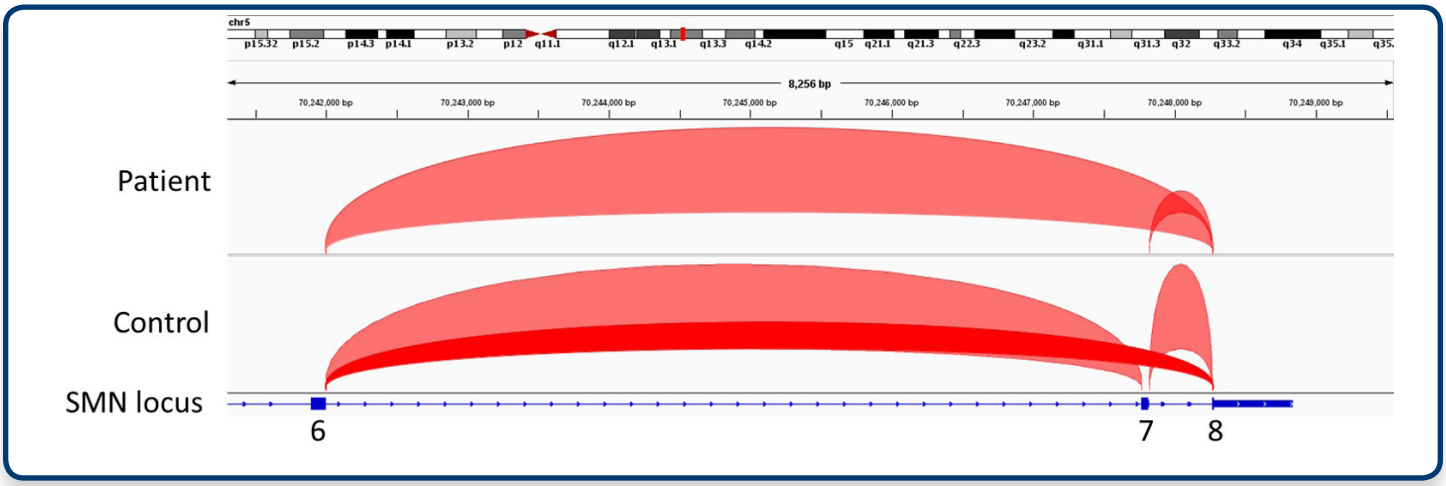

Figure 4: The Control sample (bottom) shows the expected splicing pattern of exon 6 with exon 7 and exon 7 with exon 8, characteristic of *SMN1*. The Control sample also shows the presence of transcripts with exon 6 to exon 8 splicing, characteristic of the *SMN2* gene. The Patient sample (top) shows a loss of the full length *SMN1* transcript, specifically exon 6 to exon 7 splicing, consistent with the clinical presentation.

Case 5: Epilepsy and Developmental Delay

Clinical Information: Six year-old male diagnosed with generalized epilepsy and developmental delay.

Previous Testing: Sequencing analysis of epilepsy related genes detected a variant in the *SYNGAP1* gene that had not been previously described (c.3583-9C>A; Chr6:33414343) that was predicted to be pathogenic.

Family History: Negative

Gene-specific RNA sequencing: Targeted analysis of *SYNGAP1* in a blood sample was performed.

Outcome: We identified interference of the variant with correct splicing resulting in an out of frame transcript (Val1195Alafs*27), as well as accumulation of unspliced hnRNA species carrying the variant. Based on these results the variant was reclassified as pathogenic.

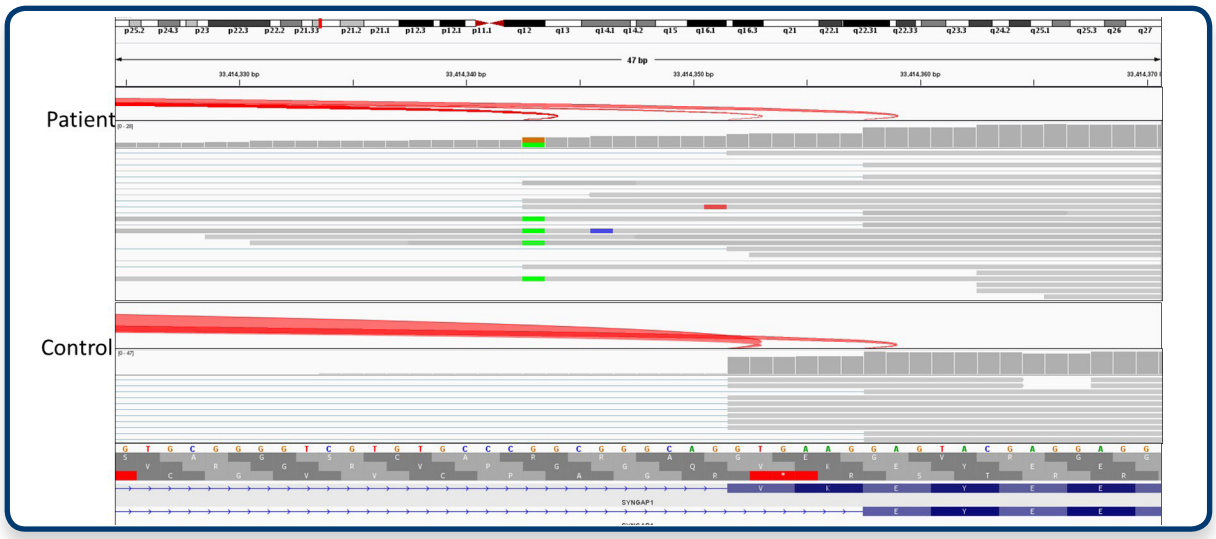

Figure 5: The figure shows a zoomed in view of exon 17 and the proceeding intron for *SYNGAP1*. The green boxes highlight the location of the reported variant in the Patient sample (top). This mutation creates an abnormal splice site and causes accumulation of unspliced transcripts enriched for the mutant allele.