RNAseq

Broadening the path towards a definitive diagnosis in Duchenne and Becker muscular dystrophy.

In Duchenne and Becker muscular dystrophy (DBMD), a definitive genetic diagnosis is critical for optimal patient care, disease management, family counseling and planning, and appropriate therapy selection. DNA sequencing tests to detect variants such as deletions, duplications and small point mutations in the *DMD* gene are valuable tools to get to that diagnosis. However, many patient cases remain unresolved after testing. A negative DNA sequencing result does not definitively rule out a genetic basis for or contribution to a patient's disease. Further, diagnostic DNA sequencing reports often include variants of uncertain significance (VUS), making it unclear if these variants are causative (or pathogenic).

RNAseq can help.

RNA sequencing (RNAseq), also known as transcriptome sequencing, can identify a broad range of potentially disease-causing variant types that are beyond current detection capabilities of DNA sequencing alone. These include variants such as splice-site, deep intronic and regulatory region (promoter and enhancer) mutations. And RNAseq goes a step further. This analysis can determine the effect identified variants have on gene function and expression in certain tissue types, elucidating the genetic mechanism causing a disease or disorder.

A valuable tool in the diagnosis of neuromuscular disorders.

A recent study performed RNAseq in a cohort of patients with undiagnosed neuromuscular disorders for whom DNA sequencing did not return a definitive genetic diagnosis. Following RNAseq analysis, a diagnosis was achieved in 35% of the cases. RNAseq was able to detect previously unidentified structural variations in disease associated genes in a small portion of these cases. In the remaining cases, RNAseq identified aberrant splicing which pinpointed numerous coding and non-coding variants responsible for a range of splice defects such as exon skipping, exon extension, and exonic and intronic splice gain[1]. Functional defects that drive disease-causing mechanisms such as these often result from variants which are undetectable or unable to be classified as pathogenic by DNA sequencing, leading to a potential "negative" DNA sequencing result or inconclusive results due to VUS identification and leaving the patient without a definitive diagnosis. The increased diagnostic yield in this cohort demonstrates the value of applying RNAseq to diagnosis of neuromuscular disorders.

RNAseq can:

- Improve diagnostic yield over DNA sequencing alone
- Reduce the potential for receiving inconclusive or "negative" test results
- Provide the functional evidence needed to classify a VUS as pathogenic or non-pathogenic
- Accurately characterize variants as disease-causing to inform patient care and therapy selection

1. Cummings BB, Marshall JL, Tukiainen T, et al. Improving genetic diagnosis in Mendelian disease with transcriptome sequencing. Sci Transl Med. 2017;9(386):eaal5209. doi:10.1126/scitranslmed.aal5209

RNAseq should be considered for patients with:

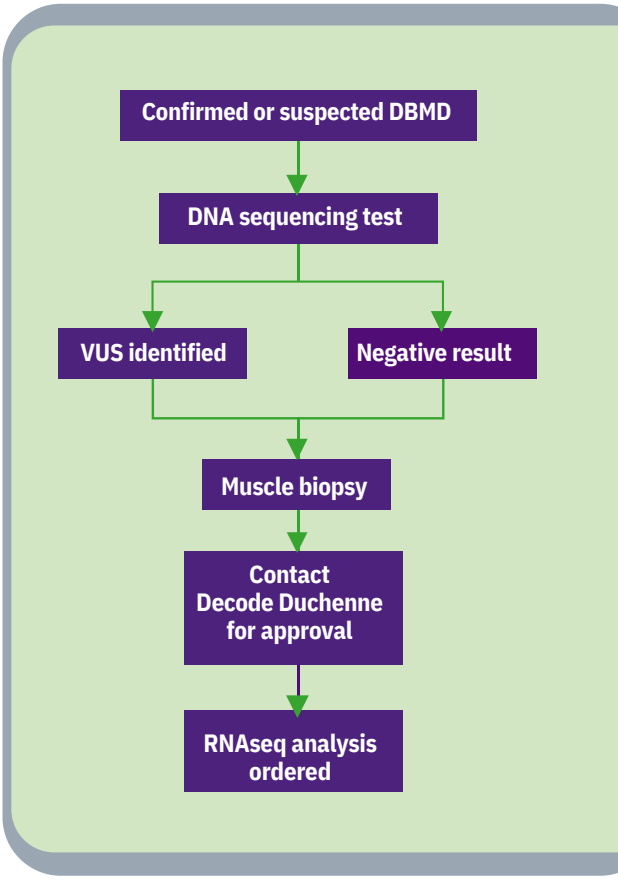
- A negative, no variant identified, or no finding result from *DMD* sequencing tests
- Variant(s) of uncertain significance (VUS) identified in *DMD*

How to access RNAseq testing

Contact Ann Martin at decode@parentprojectmd.org to determine if RNAseq is right for your patient.

If approved for RNAseq through the Decode Duchenne program, a test requisition and consent form will be provided, as well as shipping and handling instructions for submitting the muscle biopsy to PerkinElmer Genomics.

A copy of the patient's prior genetic test report(s) is required if testing was not performed by PerkinElmer Genomics.



Sample requirements for RNAseq

RNAseq requires disease-relevant tissue. In the case of DBMD, muscle biopsy tissue is required. The PerkinElmer Genomics RNAseq test is amenable to most types of muscle biopsy tissue including fresh (flash frozen preferred) and cultured muscle biopsy fibroblasts.

Upon testing approval from Decode Duchenne, please contact Ruby Liu, Sr. Laboratory Genetic Counselor at Ruby.Liu@perkinelmer.com for detailed sampled collection, submission & shipping instructions.

Turnaround Time (TAT)

TAT varies. PerkinElmer Genomics will provide more detail on expected TAT at sample submission.

Cost

RNAseq testing is offered at no cost to Decode Duchenne program patients.

Reporting & return of results

If prior genetic testing was performed by PerkinElmer Genomics, the original report will be amended to include RNAseq results. If genetic testing was performed at an outside laboratory, a new report for RNAseq will be generated.

This RNAseq testing and accompanying analysis & interpretation performed by PerkinElmer Genomics is for research use only.