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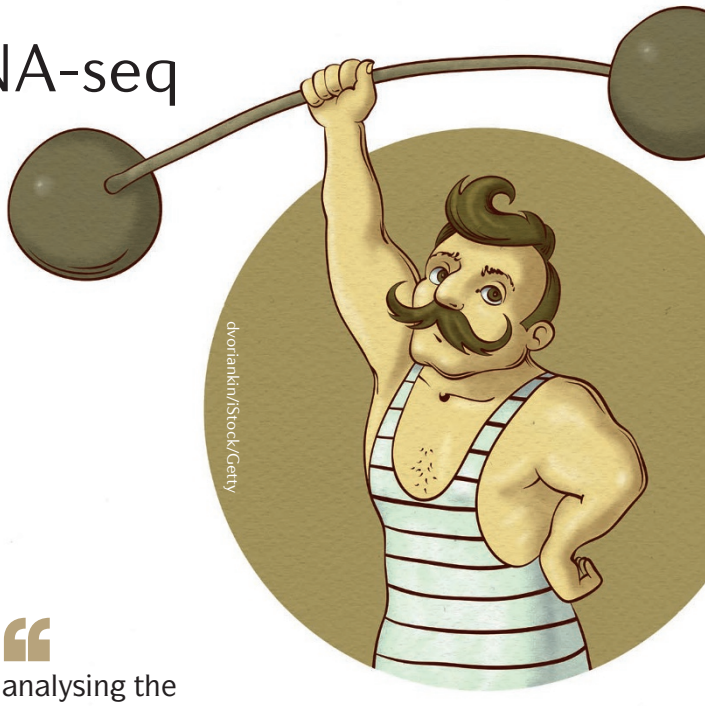
GENETIC TESTING

The diagnostic power of RNA-seq



Whole-exome sequencing (WES) and whole-genome sequencing (WGS) can only identify rare Mendelian genetic diseases in up to 50% of cases; our ability to interpret the functional and clinical importance of the genetic variants they identify is limited. Here, Cummings *et al.* show that analysing the transcriptome of diseased muscle tissue, using RNA sequencing (RNA-seq), aids in the diagnosis of genetic primary muscle disorders.

The authors sourced RNA from the muscle tissue of 63 patients with suspected monogenic muscle disorders; 13 of these patients harboured genetic variants known to affect transcription and 50 patients were undiagnosed following genetic analysis. They then performed RNA-seq using the protocol employed by the Genotype-Tissue Expression (GTEx) Consortium project and looked for transcript-level changes that were unique to patients as compared to 184 skeletal muscle RNA-seq control samples from the GTEx project. Using this approach, RNA-seq could identify the splice aberrations caused by the genetic variants known to cause disease in the 13 diagnosed patients, which validated this approach that subsequently enabled novel diagnoses.

Indeed, RNA-seq achieved a diagnosis rate of 35% and provided a diagnosis for 17 of the patients who were undiagnosed by genetic analysis. Coding and non-coding pathogenic variants resulted in a range of splicing defects, such as exon skipping, exon extension and exonic and intronic splice gain, which were not detected at the DNA level. Thus, for several Mendelian muscle disorders, RNA-seq provided a diagnosis and/or mechanistic insight. For example, RNA-seq revealed an additional exon extension event in the *NEB* gene in a patient with nemaline myopathy that was not picked up by WES, as well as a pathogenic missense variant in the *TTN* gene and synonymous variants in the *RYR1* (causing exonic splice gain) and *POMGNT1* (disrupting splice enhancer motifs) genes (the aberrant expression of these genes is associated with fetal akinesia, congenital fibre-type disproportion and α-dystroglycanopathy, respectively). Non-coding pathogenic variants, which were identified in eight patients, included an intronic variant in *DMD* (the gene that is mutated in Duchenne muscular dystrophy) that created a pseudo exon in three patients that was not detected by WGS or WES alone. RNA-seq also revealed three structural variants of *DMD*, which were confirmed by WGS.

Finally, by assessing the transcriptome of these patients, the authors identified a new genetic subtype of severe collagen VI-related dystrophy; RNA-seq identified an intron inclusion event in *COL6A1* that resulted in the inclusion of 24 amino acids in a region of collagen VI, the disruption of which causes collagen disorders. This intron inclusion was also detected in 27 additional patients in a cohort of patients with collagen VI-like dystrophy that had not been genetically diagnosed.

> “analysing the transcriptome of diseased muscle tissue … aids in the diagnosis of genetic primary muscle disorders”

The authors conclude that RNA-seq “can provide a substantial increase in diagnosis rate in patients for whom exome or whole-genome analysis has not yielded a molecular diagnosis”.

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ORIGINAL ARTICLE Cummings, B. B. *et al.* Improving genetic diagnosis in Mendelian disease with transcriptome sequencing. *Sci. Transl Med.* **9**, eaal5209 (2017)
FURTHER READING Byron, S. A. *et al.* Translating RNA sequencing into clinical diagnostics: opportunities and challenges. *Nat. Rev. Genet.* **17**, 257–271 (2016)