Approaches to genetic diagnosis in neuromuscular conditions in the era of next generation sequencing

The diagnosis of neuromuscular disorders traditionally involved clinical and neurophysiological assessment, pathological evaluation of muscle and/or nerve biopsy and sequential testing of individual genes. Next generation sequencing (NGS) has revolutionised the diagnostic paradigm in genetic disorders, with the capability to capture and sequence genes, the entire exome (1% of the protein coding genome) or the entire genome. While this may overcome the diagnostic odyssey of sequential single gene testing and reduce the need for biopsies, it is important for clinicians to be aware of the challenges and limitations of NGS. It is difficult to detect structural DNA differences with whole exome sequencing (WES), including copy number variants such as intragenic deletions or duplications due to variation in depth of coverage, or the number of times a nucleotide is sequenced. While balanced translocations and inversions can be identified through WES, triplet repeats and other pathogenic variants within regions of repetitive DNA sequence are not accurately identifiable with current available technology. The detection of mitochondrial mutations is, in particular, well suited to whole genome sequencing (WGS) due to high depth of coverage for the mitochondrial genome, which facilitates the detection of heteroplasmy. Many of these principles apply to inherited neuromuscular disorders. Examples of pathogenic variants that cannot be identified accurately by WES but that could be identified by WGS include intragenic deletions and duplications in dystrophin, which account for up to 70% of pathogenic variants in Duchenne (figure 1A) and Becker muscular dystrophy and deletions of exon 7 and 8 in SMN1 in spinal muscular atrophy (figure 1B). Current NGS technology does not accurately resolve DNA expansions such as trinucleotide CTG repeats in DMPK in myotonic dystrophy type 1, CCTG repeats in intron 1 of ZNF9 in myotonic dystrophy type 2 (figure 1C), D4Z4 repeats in the subtelomeric region of chromosome 4 in facioscapulohumeral muscular dystrophy (figure 1D), PMP22 duplications in Charcot Marie Tooth Disease type 1A (figure 1E) and trinucleotide CAG repeat expansions in the androgen receptor gene in Kennedy disease (figure 1F). In each of these cases, the phenotype is very unique and testing of individual genes directed by phenotype should be undertaken as the first line of molecular analysis.

Determining the pathogenicity of variants identified by NGS is also a complex task, requiring analysis by experienced molecular pathologists and clinical genomicists, using bioinformatic tools appropriate for clinical interpretation. This requires detailed clinical phenotype characterisation, appropriate segregation of family members, and assessing the molecular and epidemiological properties of the variant. Variants at loci conserved across species, which are rare in controls and which alter protein structure and function, are more likely to be disease causing. Genetic counselling is critical prior to genomic testing, to enable the patient/carers to understand the test procedure, benefits and limitations, and potential consequences of test results. Taken together, the current diagnostic yield of NGS is 30–50%.

Figure 1 Characteristics of six common neuromuscular conditions that cannot be identified by NGS. (A) Calf hypertrophy in Duchenne muscular dystrophy. (B) Floppy infant seen in spinal muscular atrophy. (C) Long, thin face with hollow temples and drooping eyelids seen in myotonic dystrophy. (D) Facial muscle weakness and prominent scapulae with wasting seen in facioscapulohumeral dystrophy. (E) Pes cavus in Charcot-Marie-Tooth 1A. (F) Tongue atrophy seen in Kennedy’s Disease. NGS, next generation sequencing.
The diagnostic strategy for neuromuscular conditions fundamentally relies on clinician assessment, and NGS should be guided by this to provide diagnoses in a timely and cost effective manner.

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