Genetic Testing in Neuromuscular Disorders

Understanding ordering and interpretation of genetic tests is paramount for clinical management.

By James P. Orengo, MD, PhD and David R. Murdock, MD

Neuromuscular disorders (NMDs) include pathology of muscle, neuromuscular junction, nerves, and neurons in the spinal cord, brainstem and cerebrum, making this a very heterogeneous category of neurologic disorders. Presentations also vary from as early as in utero to advanced age. A feature commonly shared amongst NMDs is genetic origin based on Mendelian gene mutations. In fact, the majority of neurologic disorders caused by genetic mutations belong to the subclass of NMDs. For the neuromuscular practitioner, a strong command of how to order appropriate genetic testing, as well as how to interpret the result is paramount for diagnosis and treatment of NMDs. In this article we discuss the types of genetic tests available (Table), how to make a rational selection, and how to interpret the results.

Effectiveness of Genetic Testing

Genetic testing options have grown significantly in the past few years. With the advancement of next generation sequencing and disease gene panels, the days of having to choose which individual gene to sequence are gone. There are mutations in over 500 genes known to be causative of NMD.1 Often the only way to differentiate disorders within subclasses of NMDs (eg, limb-girdle muscular dystrophies [LGMDs]), is via direct genetic mutation identification.2 Determining a precise molecular diagnosis is important because it provides a mode of inheritance, for example autosomal dominant versus recessive and can dictate prognosis, progression, and critical comorbidities for screening.3,4

The American Association of Neuromuscular and Electrodagnostic Medicine (AANEM) recognize the importance of genetic testing in NMDs and developed a position statement regarding its utility. The 2016 consensus guidelines state that genetic testing plays a vital role in the diagnosis and management of NMD because of cost effectiveness, disease management, quality of life, family planning, and participation in clinical trials.5

Molecular diagnosis is very cost effective if it supplants a diagnostic odyssey and avoids unnecessary empiric treatments. Frequently in NMDs, diagnosis can be elusive and requires extensive workup to gather enough information to formulate a reasonable diagnostic hypothesis. Testing might include numerous MRIs, electromyography, muscle and/or nerve biopsies, lumbar puncture, PET and/or CT scans, and extensive blood tests. Even after all these tests are obtained, a concrete diagnosis may not be achieved, and then there

<table>
<thead>
<tr>
<th>Technology</th>
<th>Sanger</th>
<th>Panel</th>
<th>Mitochondrial DNA</th>
<th>Whole-exome sequencing</th>
<th>Whole-genome sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technology</td>
<td>Chain termination</td>
<td>NGS</td>
<td>NGS</td>
<td>NGS</td>
<td>NGS</td>
</tr>
<tr>
<td>Genes tested</td>
<td>Single</td>
<td>Few-hundreds</td>
<td>37 (mitochondrial genome)</td>
<td>20,000 (coding only)</td>
<td>20,000 (coding and non-coding)</td>
</tr>
<tr>
<td>Cost</td>
<td>$$</td>
<td>$</td>
<td>$$</td>
<td>$$</td>
<td>$$$</td>
</tr>
<tr>
<td>Variants detected</td>
<td>SNV, InDel</td>
<td>SNV, InDel, CNV</td>
<td>SNV, InDel</td>
<td>SNV, InDel</td>
<td>SNV, InDel, CNV, Repeats</td>
</tr>
<tr>
<td>Potential VUS</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
</tr>
</tbody>
</table>

Abbreviations: CNV, copy number variation; InDel, small insertions and deletions; NGS, next-generation sequencing; Repeats, repeat expansions; SNV, single nucleotide variant; VUS, variant of uncertain significance.
is a trend to treat individuals with NMD empirically with expensive therapies that can have serious side effects, including high dose intravenous steroids, intravenous immunoglobulin (IVIG), chemotherapy, and plasma exchange. Genetic testing through selection of a rational gene panel may have a better chance of capturing a diagnosis in NMD, and therefore should be considered as a first-line option rather than a last-choice option when everything else comes back negative. Another strength of genetic testing in terms of cost effectiveness is that once a person is successfully diagnosed, if there are other family members with similar phenotypes, they will also be saved from the diagnostic odyssey and may simply need a single confirmatory genetic test to assign them a diagnosis.

The second point the ANNEM raises for the utility of genetic testing in NMD is related to disease management. Many NMDs are multisystemic, and in some cases, other affected organs can lead to serious yet preventable comorbidities, (eg, LGMD type 1B, where sudden cardiac death from arrhythmias can be prevented with defibrillator placement.) In other cases molecular diagnosis may prompt targeted screening that can affect quality of life (eg, myotonic dystrophy with diabetes and early cataract screening). In some cases, molecular diagnosis can change therapeutic decisions (eg, use of acetazolamide in calcium-channelopathy associated with ataxia or antisense oligonucleotides in spinal muscular atrophy [SMA]).

An underappreciated, yet important aspect of genetic testing utility in NMD is the impact on psychologic well-being. Typically, there are long delays in diagnosis of rare genetic disease, 5 to 7 years on average. Studies show that individuals being tested and their family members report significant emotional distress and lower health-related quality of life during that period. Reaching a diagnosis more quickly with genetic testing can make a sizable difference in quality of life. Learning an inheritance pattern for a disease is critical for genetic counseling and family planning. Spouse carrier testing, in vitro fertilization coupled with pre-implantation diagnosis and selection, postnatal screening, and screening of yet unaffected family members may all be options to consider with a genetic diagnosis. Ascertaining a precise genetic diagnosis may also allow for enrollment in clinical trials or disease registries. For example, a diagnosis of amyotrophic lateral sclerosis (ALS) caused by C9orf72 hexanucleotide repeat expansion will be a necessary criterion for enrollment into a clinical trial for antisense oligonucleotide therapy—an emerging and highly successful strategy for other NMDs.

**Types of Genetic Tests**

There are several forms of genetic tests for the practitioner to choose from and the decision can be daunting. The first consideration is whether to take a hypothesis-driven or shotgun approach. Hypothesis-driven testing assumes the practitioner has a reasonable idea or hypothesis of which NMD is affecting an individual, based on presentation and neuromuscular examination findings, and is using a genetic test to interrogate that hypothesis. A shotgun approach, in contrast, assumes the practitioner believes the NMD to have genetic origin without knowing the precise gene or panel of genes affected and, therefore, wishes to screen as many possibilities at once to find an answer.

We can classify the various forms of genetic tests into 7 broad categories: single gene tests, disease panels, nucleotide repeat expansion testing, mitochondrial DNA sequencing, whole exome sequencing, copy number variation, and whole genome sequencing.

**Hypothesis-Driven Genetic Testing**

Individual gene tests were the first commercial genetic testing to come onto the market and sequence an individual gene using Sanger sequencing, which involves reading a contiguous piece of DNA 1 base at a time, known as chain termination. Individual gene testing remains the standard for accuracy; however, a major limitation is the number of base pairs that can be read at a time. Large genes, introns, and other noncoding regions are often partially read or not read at all. Single gene tests often focus on mutation hotspots within a gene, or areas in the gene known from the literature to be frequently mutated in disease. This essentially eliminates discovery of novel mutation sites. Single gene tests are useful when the practitioner is highly confident of a diagnosis and is using genetic testing for confirmation (eg, a boy with suspected Duchenne's muscular dystrophy).

In contrast to Sanger sequencing, next generation sequencing (NGS) utilizes massively parallel sequencing to generate millions of short reads (100-200 base pairs each) at once, which are then aligned to a reference sequence. This method allows for sequencing tens to thousands of genes at once in a far more cost- and time-effective manner. Additionally, NGS allows improved sequencing depth and coverage of gene, improving discovery power by identifying novel gene variants not previously associated with a disease. With many advantages over Sanger sequencing, NGS technology has become the predominant genetic testing technology and is the basis for the most common form of genetic testing for NMD, gene panels. These tests consist of multiple genes sequenced at the same time with NGS that are not necessarily related to each other functionally or by location within the genome but rather are grouped together based on producing the same phenotype when mutated. Panels provide high coverage for detection of single nucleotide variants, small insertions and deletions, and larger copy number variants (eg, exon deletions). An example of when it is most appropriate to order a disease gene panel test is in the workup for poten-
tial familial ALS, for which there are now more than 20 genes identified as causative when mutated.\textsuperscript{18}

Despite being the most commonly used form of genetic testing for NMD, gene panels do have several limitations. First a panel is only as good as the genes included in the panel. Delays occur from when a novel mutation is established as causative and when genetic testing laboratories add it to their available panels, making panels a constantly evolving product that will change year to year. Because of the way genes are captured for sequencing and short read lengths, NGS-based panels have difficulty detecting tandem repeats found in repeat expansion diseases. A prime example of such a disease is the GGGGCC hexanucleotide repeat that when expanded to greater than 300 repeats in C9ORF72 causes familial ALS. The current standard for detecting these repeats is a polymerase chain reaction (PCR) and southern blot-based assay, which can be expensive and laborious to perform. Using the earlier example of familial ALS, although a disease gene panel will survey all known point mutations at once, it will, critically, miss the most common familial ALS mutation, the C9ORF72 repeat expansion.\textsuperscript{18} This highlights how gene panels are only as good as what they look for and underscores the utility of reflexing to repeat expansion testing when indicated. There are other examples, such as myotonic dystrophy, in which the phenotype is very characteristic and repeat expansion testing should be the first-line genetic test ordered to confirm the diagnosis.

There is a separate and independent pool of DNA that resides in the mitochondria, referred to as the mitochondrial genome. Diseases involving mitochondrial health represent an important segment of NMD (eg, myoclonus epilepsy with ragged red fibers [MERRF] caused by mutations in MT-TK).\textsuperscript{19} If a diagnosis is suspected to be rooted in mitochondrial dysfunction, sequencing mitochondrial DNA must be considered.

**Shotgun Genetic Testing**

There are cases where no reasonable hypothesis about which gene is causing an NMD can be made. Perhaps disease presentation does not fit a characteristic pattern. Perhaps a reasonable hypothesis was generated but could not be validated, and nonetheless the practitioner is convinced the underlying basis of disease is genetic. In these cases, we switch our approach from a hypothesis driven one to a shotgun approach. Instead of using NGS to examine a rationally assembled panel of disease genes, it can be used to sequence all known genes at once.

The most common form of genetic testing taking a shotgun approach is whole exome sequencing (WES). The entire genome consists of approximately 3 billion base pairs; however, only 1.5% of those bases located that are located coding regions, known as exons, are read to make proteins. In WES all exons are sequenced at the same time, however, noncoding regions, mitochondrial DNA, and repeat expansions are not detected. Although WES is up to 10 times more expensive than disease gene panels, it has the major advantage of a shotgun approach, speeding up the time to diagnosis. If WES comes back negative and the practitioner is convinced there may be a genetic basis of disease it may be important to reflex testing to surveying for large chromosomal aberrations with karyotype or chromosomal microarray (CMA). These methods can identify large deletions, duplications or translocations that cannot be detected with WES. Although not many NMDs are caused by chromosomal aberrations, this still remains an important add-on test to complete genetic testing.

Currently, WES is the best clinical choice for shotgun genetic testing. Advances in technology leading to deeper sequencing, at reduced costs, are making way for an emerging option, whole genome sequencing (WGS). At the time of this review, WGS is not a reasonable option clinically. We discuss it because this will most likely change, and WGS may supplant all other genetic testing. There are already examples of NMDs diagnosed with WGS.\textsuperscript{20,21} Sequencing the entire genome—all 3 billion bases—WGS includes mitochondrial DNA and identifies single nucleotide variants, small insertions and deletions, copy number variants, and even structural variations (eg, repeat expansions).\textsuperscript{22} The major roadblocks to use of WGS today are costs and interpretation. The costs are more than 5 times that of WES, and WGS is not typically covered by insurance companies. Interpretation is beyond the scope of the practitioner because of the volume of data to review and put in context regarding whether a mutation is pathogenic or incidental. The higher level of interpretation for WGS keeps it in the research domain at present. As more individuals’ genomes are sequenced, and we gain greater insight into which mutations are pathogenic, algorithms will be developed to quickly interpret WGS data and couple it with phenotype data imputed by the practitioner to make a diagnosis.

**Interpreting Genetic Test Results**

Interpretation of genetic variants is based on guidelines from the American College of Medical Genetics and Genomics (ACMG) in 2015, which standardize the criteria used to classify a variant (eg, as pathogenic, a variant of uncertain significance [VUS], or benign).\textsuperscript{23} Interpretation is based on factors that include population frequency, variant type (eg, loss of function), location in gene, and functional data. It is important to note that unlike pathogenic or likely pathogenic variants, clinical decision-making should not be made based on a VUS. A freely accessible ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) is an excellent (Continued on page 41)
resource to share interpretations of variants identified in laboratories, making it much easier now to see how others classify variants.


James P. Orengo, MD, PhD
Department of Neurology
Baylor College of Medicine
Houston, TX

David R. Murdock, MD
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, TX

Disclosures
JPO and DRM report no disclosures.