Neuromuscular Disease

Genetic Testing Recommendations

PROVINCIAL GENETICS PROGRAM
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Introduction

Ontario Health has been mandated by the Ministry of Health to *"implement genetic testing and develop a comprehensive provincial genetics program for all genetic services."* To fulfill this mandate, the Provincial Genetics Program (PGP) was launched in April 2021. The PGP and Provincial Genetics Advisory Committee (PGAC) identified Neurogenetics as a priority domain for development in Ontario, resulting in the formation of the Neurogenetics Expert Group. The role of the Expert Group is to develop evidence-based guidance for the provision of genetic testing and counselling services.

The Expert Group, in collaboration with health care professionals, laboratory scientists, administrators, and patient and family advisors, developed genetic testing recommendations for individuals with neuromuscular diseases in the province of Ontario.

Guidance Document Scope

This document includes recommendations for standardized, coordinated, and evidence-based genetic testing for patients and families with genetic neuromuscular diseases (NMDs), both in the pediatric and adult settings. This encompasses the following genetic disorders:

- Disorders of the peripheral nervous system (e.g., Charcot-Marie-Tooth disease [CMT])
- Anterior horn cell disease (e.g., spinal muscular atrophy [SMA]), except for amyotrophic lateral sclerosis (ALS)[a]
- Neuromuscular junction disorders (e.g., myasthenic syndromes)
- Muscle disease (e.g., myopathies, muscular dystrophies, and neuromuscular channelopathies)

This report offers recommendations aimed at improving healthcare for Ontarians with a suspected NMD. The recommendations are not focused on a single organization, but rather the Expert Group believes that collaboration between various branches of the Ministry of Health, coordination across the healthcare sector, and leadership from key players (hospitals, research organizations, industry, and patient advocacy groups) are essential to making a positive impact on the care of Ontarians living with NMDs. The proposed recommendations cater to the needs of the patient and provider communities and propose new or revised guidance to simplify genetic diagnostics for NMDs.

[a] Individuals with ALS follow a distinct care pathway from the other NMDs in this report. Genetic testing recommendations for ALS were kept out of scope for this document but will be developed separately.

Neuromuscular Disease

NMDs encompass the spectrum of diseases where the primary abnormality or lesion is in the peripheral nervous system (PNS)[1]. The unifying aspect of NMDs is abnormal muscle function and the resulting sequelae from it. This can include chronic signs and symptoms, most typically related to muscle weakness, such as abnormal or impaired ambulation, joint contractures, skeletal deformities (particularly scoliosis), altered sensory perception (in neuropathies), and respiratory and/or oromotor function failure. It also includes dynamic impairments, such as exercise intolerance, myalgia, rhabdomyolysis, and fatigable weakness. In total, genetic neuromuscular disorders are frequently associated with significant lifelong morbidities, which are often severely disabling, and, in many cases, associated with premature mortality[1].

This guidance document focuses on conditions where the suspected underlying primary cause could be genetic (i.e., genetic NMDs). Historically, the diagnostic process for NMDs has followed a tiered approach that begins with clinical recognition, followed by ancillary diagnostic studies including creatinine phosphokinase (CPK), screening metabolic laboratories, muscle biopsy, and electromyography (EMG)/nerve conduction study (NCS), and eventually is completed with genetic testing. However, the evolution in rapid, low-cost, comprehensive genetic testing has led to a revision of this process, with a "genetics first" approach now recommended by expert providers and supported by studies in the medical literature[2–4].

The advantages of uncovering the genetic basis of NMDs include: shortening the diagnostic odyssey, avoiding unnecessary screening tests and non-indicated therapies that can carry associated harm and additional costs, the opportunity for accurate disease prognostication and family planning, and accessing disease-modifying therapies indicated by diagnosis, including existing treatments and emerging therapies in clinical development[5,6]. Many inherited NMDs are multi-systemic, involving cardiac, respiratory, and other organ systems. By arriving at a molecular diagnosis, the clinician can arrange appropriate and potentially life-saving surveillance or referrals (e.g., to a cardiologist for investigation and consideration of an implantation of a defibrillator-pacemaker in a patient with limb-girdle muscular dystrophy [LGMD] or myotonic dystrophy type 1 [DM1])[6]. The evidence for disease-modifying treatment and management after diagnosis is an important factor that drives rapid, timely diagnostics. In other words, rapid diagnosis leads directly to improved clinical care.

Guiding Principles

Timely, accurate genetic diagnosis is critical for patients with NMD. While some new gene-disease associations are still likely to emerge, the genes responsible for the majority of NMDs are largely known with ~360 genes leading to approximately 50% diagnostic yield of genetic testing in this population[7–9]. As such, tests that encompass these genes represent an effective strategy for NMD genetic testing.

Five important variables dictate the optimal testing strategy, the first-line genetic tests, and essential/recommended non-genetic testing. These are:

1) Age of the patient
2) Age of onset of symptoms
3) Duration of symptoms
4) Localization within the neural axis
5) Family history

Patient age at the time of genetic testing is important for two reasons. Firstly, it impacts the accuracy of the history and physical exam for disease localization. Particularly in children under 24 months of age, it can be challenging to distinguish between disorders of the central nervous system (CNS) and PNS, and to differentiate within the PNS itself. Further, neurogenetic diseases affecting very young children can involve multiple aspects of the nervous system, while appearing to affect the PNS preferentially[10]. Secondly, the age of the patient also affects the likelihood of non-genetic conditions. Acquired NMDs are much more common when the disease onset is in later childhood and adulthood, and rare, but not impossible in children under 2 years old. In general, in pediatrics (0-18 years), most neuromuscular conditions have a genetic underpinning, while in adulthood, more careful consideration of non-genetic diseases is important in the diagnostic process[11].

Similarly, the **onset and duration of the disease** are important factors to consider[12]. Along with the individual's age, they are relevant to establish a differential diagnosis, and thus offer important instruction in terms of critical genetic causes that need to be considered. In general, disease onset close to birth and long history of symptoms (duration) may suggest a more likely genetic origin. Overall, given these variables, the specific recommendations for NMDs are presented by age group.

A thorough clinical examination and history can aid in identification of the specific sub type of NMD and in **localizing the disease within the neural axis**. Some clinical symptoms and signs point to peripheral nerve localization, such as distal muscle weakness, presence of hand and foot deformities (e.g., hammertoes, pes cavus), absent reflexes, and deficits in sensory modalities. Others may indicate a neuromuscular junction localization, including episodic weakness and involvement of the facial musculature. Finally, some may suggest localization to the muscle itself, including proximal weakness (with many muscular dystrophies), diffuse weakness including the face (congenital myopathies), and dynamic symptoms like myalgias and exercise intolerance. Localization may also be aided by ancillary non-genetic diagnostic tests like serum CPK, electrophysiology (i.e., EMG and NCS), imaging, and biopsy[13] (note these tests should not be required prior to genetic testing). Testing strategies need to encompass the broad differential of NMDs and the potential for uncertainty related to localization

within the peripheral neural axis, while also offering the potential for more refined and delineated testing. Given this, the recommendation is for a comprehensive neuromuscular panel, or genome-wide sequencing (GWS) in specific circumstances.

Finally, **family history** is an integral component of a comprehensive genetic assessment. Family history provides valuable information for diagnosis based on the pattern of inheritance in the family, particularly in conditions with variable expressivity[12]. In addition, if genetic testing has previously been completed on other affected family members, these results can help to determine if there is already a confirmed hereditary NMD in the family and subsequently direct genetic testing towards a known pathogenic or likely pathogenic variant in an NMD-associated gene. Family history of NMD increases the likelihood of a genetic cause and helps inform the testing strategy. For example, in DM1, which is caused by a trinucleotide repeat expansion on *DMPK* (>34 CTG repeats), de novo pathogenic expansions are rare and most individuals with DM1 inherited an expanded allele from a parent with an expanded range[14]. Family history may aid in identifying the risk of other family members, prompt cascade family testing, and diagnose mildly affected or asymptomatic individuals.

The overall prevalence of genetic neuromuscular disorders is not known, and the specific prevalence of subtypes of NMDs is also uncertain in many cases. The most common genetic NMDs include Duchenne muscular dystrophy (DMD), Charcot Marie Tooth disease (CMT), DM1, 5q related SMA, and Facioscapulohumeral muscular dystrophy (FSHD) (Table 1)[15]. As these conditions require specific, single gene testing, they must be considered alongside multigene panels and genome sequencing (GS). Variation in prevalence for some conditions (e.g., DM1) is well known due to founder effect variation, such as in NMD with high prevalence in individuals of French-Canadian ancestry.

Table 1. Prevalence of Common Neuromuscular Genetic Conditions

Syndromes	Prevalence
5q related spinal muscular atrophy	1 in 8,000
Becker muscular dystrophy	1 in 18,450
Charcot-Marie-Tooth disease	1 in 2,500
Congenital myasthenic syndrome	1 in 100,000
Congenital myopathy	1 in 26,000
Duchenne muscular dystrophy	1 in 5,000
Facioscapulohumeral dystrophy	1 in 10,000-25,000
Limb-girdle muscular dystrophy	1 in 14,500-123,000
Myotonic dystrophy type 1	1 in 20,000
Spinal and bulbar muscular atrophy	1 in 50,000 males
Spinal muscular atrophy	1 in 10,000

Ordering Physicians

Neurogenetic diseases are most commonly diagnosed and managed by neurologists and medical geneticists. To support timely, equitable access to genetic testing, physicians across specialties with relevant expertise should be able to order neurogenetic testing to eligible patients that are within their scope of practice, including genome-level sequencing. Non-genetics trained physicians are highly encouraged to utilize educational and training materials related to genetic testing prior to sending tests, and to work in collaboration with local medical genetics clinics to adopt innovative models of care delivery (e.g., mainstreaming)[16,17]. As part of implementation planning, resources will be identified, adapted, and/or developed by the PGP.

Inconclusive Test Results

Depending on the age and the subtype of neuromuscular disease, the yield of first-line genetic testing is approximately 50%[18] and can be as high as 80%[19] (for conditions like congenital myopathies)[20]. However, a significant number of cases remain unsolved after testing. The results may be inconclusive, with no identification of causative pathogenic or likely pathogenic (P/LP) variants or variants of uncertain significance (VUSs).

For a test where a diagnosis is not established, additional testing may be indicated at the discretion of the expert[21]. For example, GWS may be pursued for panel inconclusive individuals when there is sufficient justification for broader testing. This is also the case with panels that yield VUSs that are deemed to not be the cause of disease.

For tests where VUSs are identified, additional diagnostic studies may be indicated. Parental and familial testing may be recommended to add context to the VUS, as some VUSs can be re-classified based solely on such studies. Additional studies may also include muscle biopsy, muscle magnetic resonance imaging (MRI), electromyography, and RNA analysis.

Referral to Medical Genetics

Non-genetics physicians are encouraged to refer to medical genetics and/or genetic counselling for guidance when needed. This may include assistance with pre and/or post-test genetic counselling, prenatal counselling, consultation for challenging diagnostic cases, and aid with variants outside the scope of neuromuscular practice (e.g., secondary findings on GWS).

Genetic Testing Recommendations for Individuals with Suspected Neuromuscular Disease

Disease Presentation Between 0-24 Months

1) First line testing recommendation is GWS that encompasses the broad differential of neurogenetic syndromes and includes evaluation individual gene/exon-level copy number variants (CNVs). A trio-based approach to GWS testing (sequencing of patient and both biological parents) is recommended to optimize variant interpretation whenever possible.
2) Testing can be done concurrently and/or following CPK, *DMPK* repeat expansion testing for DM1, methylation status assessment for Prader-Willi syndrome (PWS)/Angelman syndrome (AS), and deletion/duplication analysis of *SMN1* and *SMN2* for SMA, when appropriate.
3) Testing can be pursued preceding or concurrently with cytogenetic analysis (e.g., chromosomal microarray analysis [CMA]).
4) In the rare situation where an acquired case is suspected, exclusion of acquired disease can precede genetic testing.
5) Familial variant testing, including prenatal diagnosis, should be offered to at-risk family members following genetic counselling based on the inheritance pattern of the condition.
6) A rapid turnaround time of ≤2 weeks should be strongly considered for critically-ill neonates to support treatment and care planning.

Genetic NMDs are a common cause of severe neonatal and early infant disease and are part of the broad differential for neonatal hypotonia and developmental delay[22]. Given the challenges of distinguishing between primary NMDs and broader neurogenetic syndromes, many of which may have involvement of the peripheral nervous system, and the accelerated need for timely diagnosis in this age group, GWS with detection of genome-wide CNVs is the recommended first-line genetic test (exome or genome sequencing). If there is a high(er) degree of diagnostic certainty, particularly if supported by ancillary test results, then a specialized NMD test or targeted panel may be ordered. The decision on whether to pursue sequential testing or opt for GWS upfront should be made based on the physician's judgment, taking into consideration factors such as the patient's clinical presentation, family history, and the likelihood of identifying the genetic cause. However, to expedite diagnosis and treatment planning, it is critical to avoid tiered testing in this age group whenever possible, making GWS the main recommendation in the majority of cases[10].

DM1 is the most prevalent neuromuscular disorder of infants[14]. As such, testing for DM1 should be pursued in most infants with suspected NMD, even in the absence of parental signs/symptoms of DM1, as several cases have been reported with asymptomatic parents. If the clinical examination is definitively not in-keeping with DM1, then DM1 testing need not be pursued. Similarly, trisomy 21 and PWS[23] are causes of neonatal hypotonia that require specialized testing[24]. Overall, cytogenetic analysis, DM1, PWS, and serum CPK are appropriate tests to be considered concurrently with GS based on the patient's phenotype.

SMA is also a common cause of neuromuscular weakness in individuals under 18 months of age. Currently, newborn screening (NBS) in Ontario includes SMA and identifies approximately 95% of all cases, missing SNVs[25]. If SMA is highly suspected, testing should be pursued that includes dedicated multiplex ligation probe amplification (MLPA) of the *SMN1* and *SMN2* genes.

Importantly, the yield of microarray in individuals between 0 to 24 months old with suspected NMD is low and should not be required prior to GWS or multigene panel testing. However, given the expanded differential diagnosis in this age, which includes hypotonia and the broader group of neurogenetic syndromes (where 10% of disease burden is due to multigene CNVs), an assessment of deletions and duplications is warranted and should be completed concurrently. In addition, certain non-genetic tests that historically have been pursued in this age group are documented to have essentially zero yield and thus should NOT be sent. In particular, these are the "metabolic screening" labs and include acylcarnitine profile, serum amino acids, and urine organic acids[26]. The exception would be if there is a high level of suspicion for an inborn error of metabolism.

Non-genetic disease is an extremely rare cause of NMDs in this age group. In infants with features of a neuromuscular junction disorder, transient myasthenia of the newborn should be considered based on thorough maternal history and consideration of direct testing[27]. In endemic areas, infantile botulism should be considered in the correct clinical context (acute to subacute onset of progressive weakness, constipation, reduced pupillary responses), and the appropriate diagnostic test pursued (EMG/NCS + anti-toxin studies). Guillain-Barre Syndrome (GBS) rarely can be seen in individuals <18 months and should be screened in infants and young children with a consistent presentation (acute to subacute onset of ascending weakness, absent reflexes)[15].

Disease Presentation Between 24 Months to 18 Years

1) First line genetic testing should be a multigene panel (Figure 1) that includes all the genes and variants with a proven association with NMDs that are on the differential diagnosis for the patient. The clinical laboratory should be able to accurately detect all clinically relevant nucleotide and copy number variants within each gene. If feasible, follow-up testing of parents or other blood relatives may be recommended for variant interpretation.
2) In atypical and/or complex presentations, the first-line testing can involve a more comprehensive multigene panel, GWS, and/or a set of tests that includes all gene variants with proven association with NMDs that are on the differential diagnosis for the patient.
3) Gene variants in NMDs that can only be detected by non-sequencing technologies should be available to order sequentially or concurrently with a multigene panel and/or GWS.
4) Upon consultation with the laboratory and based on a reasonable likelihood of clinical benefit to the patient, the ability to order expanded testing (additional gene panels or GWS) should be available to ordering clinicians if initial testing with a multigene panel does not explain the patient's clinical presentation and symptomatology.
5) Dystrophinopathies testing (deletion/duplication analysis plus sequencing), encompassing DMD, Becker muscular dystrophy (BMD), and other rare DMD-related disorders, should be considered as the first-line test (i.e., before other genetic testing) in males with CPK levels greater than 800. Otherwise, ancillary studies are not required prior to genetic testing, but, if available, can be used to guide the choice of testing.

6) In the rare situation where an acquired case is suspected, exclusion of acquired disease may precede genetic testing.
7) Familial variant testing, including prenatal diagnosis, should be offered to at-risk family members following genetic counselling based on the inheritance pattern of the condition.

The ability to distinguish primary NMDs from the broader group of neurogenetic syndromes greatly increases as children enter the ambulant age range. This ability continues to increase as children age and can more actively participate in the physical examination. Genetic causes continue to predominate in this age group, however, and genetic testing is typically the first line of diagnostic study after history, physical examination, and serum CPK measurement. As mentioned above, NMDs are subdivided based on the aspect of the peripheral nervous system they impact (e.g., neuropathy, neuromuscular junction, myopathy, muscular dystrophy). However, there can be an overlap in terms of presentation and symptomatology between these subdivided NMD entities. Therefore, the testing platform should include all neuromuscular-associated genes. Testing options should preserve the ability to send a more disease subtype-specific test that includes all genes for a given disease entity[28] (e.g., a panel of genes that encompass all causes of CMT). Subtype-specific testing can be considered when the diagnostic certainty for a subtype is high, especially when ancillary test results are available that support it[28] (such as abnormal nerve conduction velocity in a patient with suspected CMT). However, it is critical to avoid a lengthy tiered testing approach as this creates a significant diagnostic delay that can lead to delayed access to treatment and other benefits of diagnosis. Additionally, the evidence shows that this approach is ultimately not cost-effective[29].

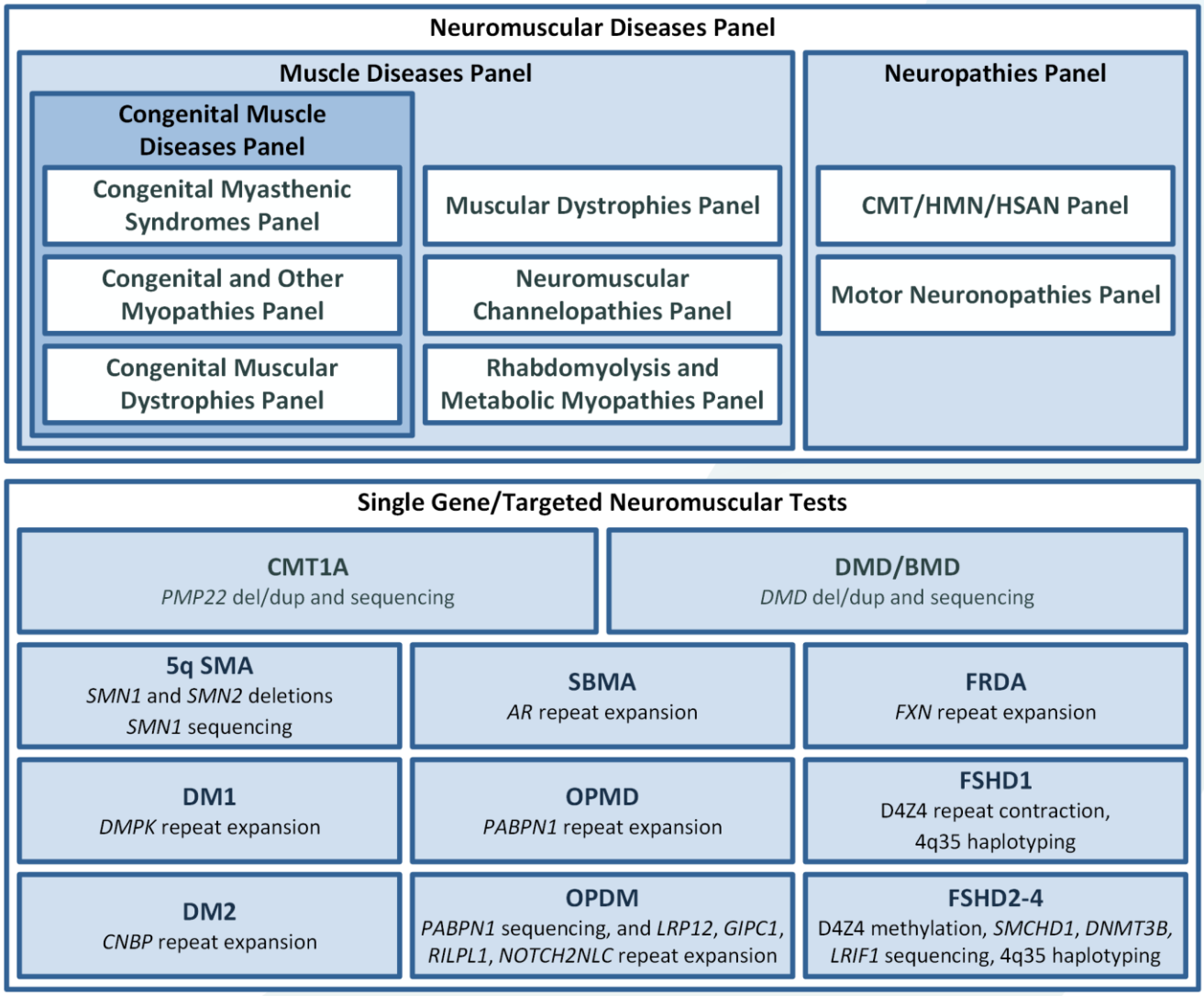
As with children 0-24 months, DM1 and SMA are also important considerations in the 24-month to 18-year age group. When there is a clinical suspicion of SMA, standard 5q SMA testing and the neuronopathies panel are indicated for individuals born prior to the implementation of NBS for SMA in Ontario (January 2020). In addition to DM1 and SMA, the dystrophinopathies are one of the most common neuromuscular diseases and warrant consideration in boys with CPK levels greater than 800. FSHD is another condition that typically presents in the teenage years though symptom onset can begin earlier in childhood[30].

For this age range, acquired/non-genetic NMDs warrant more careful and frequent consideration. Acute/subacute causes of weakness include GBS and myositis. Subacute/chronic causes include chronic inflammatory demyelinating polyradiculopathy (CIDP), myositis (particularly dermatomyositis), and myasthenia gravis (MG). For these conditions, onset is essentially always after the first 6-12 months of life and more typically starts in older children. Testing for these conditions is indicated in the appropriate clinical setting (e.g., a child over the age of 12 months with fluctuating weakness, particularly involving the eyelids or extraocular muscles, should be evaluated for MG by acetylcholine receptor (AChR) antibodies and repetitive stimulation nerve conduction studies).

Testing for CMT, SMA, DM1, DM2, DMD, oculopharyngeal muscular dystrophy (OPMD), FSHD1, and FSHD2 should all be available as separate “stand-alone” tests, as an accurate molecular diagnosis requires non-sequencing technologies alone, or in combination with sequencing. Sequencing platforms have limitations in detecting certain genetic variations, such as certain deletions and duplications, repeat expansions or contractions, or methylation changes. These limitations may be overcome using alternative techniques or as sequencing technologies improve.

For instance, for FSHD, repeat contraction array testing is performed in the chromosome 4 and 10 D4Z4 region with haplotyping[30]. Genes causing and mimicking FSHD, SMA, DMD, and DM1 may also be tested in a multigene panel. New technologies such as optical genome mapping for testing diseases like FSHD can continue to be incorporated as and when they become available[31]. Ancillary studies are not required prior to genetic testing, but if obtained can be used to guide the choice of testing. Non-genetic testing to exclude acquired causes should be obtained in the appropriate setting such as nerve conduction velocities for CIDP/GBS, repeated nerve stimulation study, and AChR antibodies for MG, muscle imaging for myositis).

Figure 1. Comprehensive Neuromuscular Disease Genetic Testing



CMT1A, Charcot-Marie-Tooth disease (CMT) type 1A; Del/dup, deletion/duplication analysis; DM1, myotonic dystrophy type 1; DM2, myotonic dystrophy type 2; DMD/BMB, Duchenne muscular dystrophy and Becker muscular dystrophy; FRDA, Friedrich ataxia; FSHD1, facioscapulohumeral muscular dystrophy (FSHD) type 1; FSHD2, FSHD type 2; HMN, hereditary motor neuropathy; HSAN, hereditary sensory and autonomic neuropathy; OPDM, oculopharyngodistal myopathy; OPMB, oculopharyngeal muscular dystrophy; SBMA, spinal and bulbar muscular atrophy; SMA, spinal muscular atrophy.

Adult and Late-Onset Presentation

1) First-line genetic testing should include single gene and/or multigene panels (Figure 1) that best fit the patient's phenotype. The clinical laboratory should be able to accurately detect all clinically relevant CNVs at the individual gene/exon level. If an acquired cause for NMD is suspected, it should be ruled out prior to genetic testing. If feasible, follow-up testing of parents or other blood relatives may be recommended for variant interpretation.
2) In atypical and/or complex presentations, the first-line testing can involve a more comprehensive multigene panel, GWS, and/or a set of tests that includes all gene variants with a proven association with NMDs that are on the differential diagnosis for the patient.
3) Gene variants in NMDs that can only be detected by non-sequencing technologies should be available to order sequentially or concurrently with a multigene panel and/or GWS.
4) Upon consultation with the laboratory and based on a reasonable likelihood of clinical benefit to the patient, the ability to order expanded testing (additional gene panels or GWS) should be available to ordering clinicians if initial testing with a multigene panel does not explain the patient's clinical presentation and symptomatology.
5) Familial variant testing, including prenatal diagnosis, should be offered to at-risk family members following genetic counselling based on the inheritance pattern of the condition.

Acquired NMDs may mirror the signs and symptoms traditionally considered to be associated with genetic NMDs. Importantly, acquired NMDs have a higher prevalence in adults than the pediatric population and often have disease-modifying treatments, and thus need to be considered and evaluated with appropriate testing (e.g., NCS/EMG, appropriate autoimmune testing, muscle biopsy where needed) prior to genetic testing[12]. Conversely, it is increasingly recognized that genetic NMDs can present and/or come to clinical attention in adulthood and may represent a substantial fraction of patients in adult NMD clinics, with up to 39% diagnostic yield of genetic testing[32].

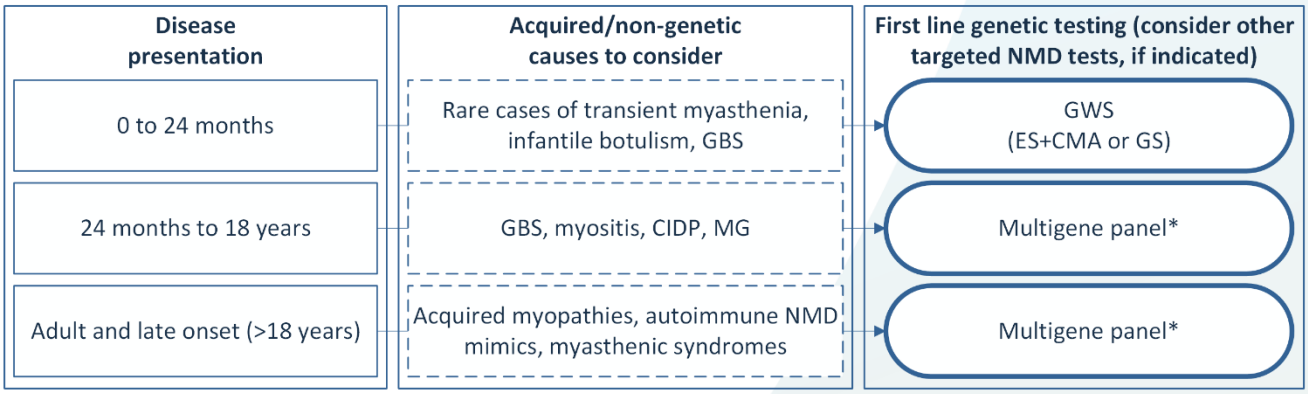
For adults with NMD, non-sequencing single gene tests are also required for accurate molecular diagnosis. Given that there are over 300 genes identified for NMDs and their significant phenotypic and genotypic overlap, if the initial single gene testing is inconclusive, or if the phenotype does not match any of the single gene disorders, the majority of adult-onset NMDs require multigene testing strategies[21]. For example, patients with phenotypic presentations such as distal motor neuropathies, hereditary sensory autonomic neuropathies, or LGMD will likely require multigene panel testing as their first-line genetic study unless family history suggests a specific gene to be responsible or else their presentation is in keeping with one of the genetic causes that require single testing (in which case, single gene testing would be done first, with panel testing pursued upon an inconclusive result).

It is important for clinicians to consider appropriate testing to assess for genetic NMD autoimmune mimics for myopathies (e.g., myositis-specific antibodies, anti-mitochondrial antibody testing), myasthenic syndromes (e.g., AChR, MUSK, LRP4 with repetitive NCS or single fibre EMG studies), or neuropathy-related antibodies (e.g., anti-MAG testing). Muscle biopsy and muscle MRI may provide evidence to antibody-negative acquired myopathies. Otherwise, ancillary studies are not required prior to genetic testing, but, if available, can be used to guide the choice of testing.

Clinical Guide to the Use of Neuromuscular Disease Panels

When choosing the most appropriate test for a patient and/or family, the test should encompass the genes associated with the likely differential diagnoses and be sufficiently comprehensive to include the NMDs with important clinical overlap (Figure 2). The choice of panels should be driven by clinical judgement, informed by the patient phenotype, family history, and diagnostic certainty. The information presented below could assist in test selection and establishing eligibility.

Figure 2. Summary of the Genetic Testing Strategy for Individuals with Suspected Neuromuscular Disease



* GWS could be considered for atypical and/or complex presentations, or after inconclusive multigene panel results for highly suspicious cases. CIDP, chronic inflammatory demyelinating polyneuropathy; CMA, chromosomal microarray analysis; ES, exome sequencing; GBS, Guillain-Barré syndrome; GS, genome sequencing; GWS, genome-wide sequencing; MG, myasthenia gravis; NMD, neuromuscular disease

Considerations for Genetic Testing of Neuromuscular Disease

Familial Variant Testing

- Familial variant testing, including prenatal diagnosis, should be offered to at-risk family members following genetic counselling based on the inheritance pattern of the condition.

Genome-wide Sequencing

- Disease presentation occurs between 0-24 months.
- Cases with an uninformative multigene panel if high suspicion of a genetic diagnosis remains.
- Testing should be done concurrently with other non-sequencing molecular investigations when appropriate.
- A trio-based approach is recommended.

Neuromuscular Disease Panels

MUSCLE DISEASES PANEL

- Genetic muscle diseases encompass all conditions affecting primarily the neuromuscular junction and/or the muscle itself.
- Muscle weakness is the main sign and symptom, and typically manifests as impairment in muscle function, including altered gait, delayed motor milestones, difficulties with stairs, upper limb disability, and cranial muscle challenges such as reduced facial movements, difficulty chewing, and swallowing[1].

CONGENITAL MUSCLE DISEASES PANEL

- Congenital muscle diseases are conditions that present within the first 18 months of life.
- The primary manifestations are hypotonia and weakness, and the main consequences are delayed motor development, respiratory failure, and feeding difficulties.
- The three main subtypes of congenital muscle disease are congenital myasthenic syndrome (CMS), congenital myopathy (CM), and congenital muscular dystrophy (CMD), plus congenital myotonic dystrophy.
- While the conditions may potentially be distinguished by exam features and ancillary testing results (CPK, brain MRI, and muscle biopsy), the clinical features are sufficiently overlapping that testing that encompasses all three subtypes is most typically required and optimal for achieving a rapid and accurate diagnosis.

CONGENITAL MYASTHENIC SYNDROMES PANEL

- CMSs are disorders where the primary genetic defect is in a gene that encodes a component of the neuromuscular junction, including the presynaptic and postsynaptic junctions and the intra-junctional cleft[33].
- Onset of symptoms is typically at or around the time of birth, though symptoms can emerge at later ages as well.
- The classic presentation is one of fluctuating weakness and muscle fatigability, though often symptoms can appear quite static. Often the facial musculature is involved, though there are cases where signs and symptoms are exclusively limb/girdle[33].
- Repetitive stimulation nerve conduction studies can be helpful to suggest a CMS, with the classic result being an electrodecrement. However, it is important to note that some patients with CMS will have normal nerve conduction velocities, and some patients with other forms of muscle disease will have abnormal repetitive stimulation.
- Key conditions on the differential diagnosis include congenital myopathies, myotonic dystrophy type I, and acquired disorders of the neuromuscular junction (such as MG).

- Several therapeutic interventions are available for many CMS subtypes, and data exist suggesting different first-line therapies (and drugs that are contra-indicated) for different types.

CONGENITAL AND OTHER MYOPATHIES PANEL

- CMs are disorders where the causative genetic defect is in a gene that encodes a protein with primary role(s) in muscle structure and function. Gene products can be loosely divided into sarcomeric (thin and thick filament) proteins, excitation-contraction coupling proteins, and components of the membrane trafficking apparatus[34].
- CMs classically present weakness (often diffuse) and hypotonia in the neonatal period, though they may come to clinical attention at any age group. They often involve facial musculature (with the classic myopathic facial appearance), including impaired eye movements in a subset of individuals. The neonatal presentation can also include multiple joint contractures (arthrogryposis multiplex congenita) and a paucity of movement (fetal akinesia syndrome) [34].
- Historically, CMs were defined, named, and diagnosed based on features seen on muscle biopsy, with nomenclature such as centronuclear myopathy, core myopathy (central core disease and minicore myopathy), nemaline myopathy, and congenital fiber type disproportion. Important conditions on the differential diagnosis include myotonic dystrophy type I, CMSs, and congenital muscular dystrophies.

CONGENITAL MUSCULAR DYSTROPHIES PANEL

- CMDs are disorders of the extracellular matrix and the sarcolemma membrane. The major subtypes are *LAMA2*-related CMD, *LMNA*-related CMD, *COL6*-related CMD, and the dystroglycanopathies.
- Onset is within the first 18 months of life and includes hypotonia and extremity weakness. Facial musculature is typically spared (except for some mild lower facial weakness in some instances)[35].
- The *COL6* subtype includes the combination of joint hypermobility and joint contractures (including congenital hip dysplasia or dislocation) as well. Dystroglycanopathies may have significant CNS manifestations, including seizures and cognitive developmental delay[2].
- CPK in all subtypes is typically elevated (>800), though it can be normal with *COL6*-related myopathies. Muscle biopsy shows dystrophic changes and immunostaining on biopsies can enumerate specific subtypes.
- The differential diagnosis includes congenital myopathies, DM1, and SMA.

MUSCULAR DYSTROPHIES PANEL

- Muscular dystrophies are a heterogeneous group of genetic disorders historically defined by the combination of extremity muscle weakness, elevated serum CPK, and dystrophic changes on muscle biopsy (inflammation, degeneration, regeneration, fibro-fatty replacement). Importantly, patients with muscular dystrophy may present without one or multiple of these features[35].

- Muscular dystrophies can be approximately grouped based on age of onset and pattern of weakness and have historically been named accordingly (CMD, LGMD, Emery-Dreifuss muscular dystrophy (also called scapulo-peroneal), dystrophinopathy (DMD and BMD), OPMD, FSHD, and myotonic dystrophy[35].
- Some muscular dystrophy subtypes have features that enable distinction and identification. These include dystrophinopathies (high CPK, limb-girdle weakness in boys), FSHD (early involvement of the face and scapular musculature), Emery–Dreifuss muscular dystrophy (EDMD) (triad of scapuloperoneal weakness, early-onset joint contractures, and cardiac arrhythmias), DM1 (see above), and OPMD.
- Some require specialized genetic testing and/or should be considered for testing outside of a larger panel. Commonly, however, these conditions are difficult to accurately distinguish and thus necessitate an encompassing genetic test that includes all potential muscular dystrophy genes[35].

NEUROMUSCULAR CHANNELOPATHIES PANEL

- Channelopathies are a heterogenous group of disorders caused by dysfunctional ion channels present on the cell or organellar membranes. Channelopathies can be caused by either genetic or acquired factors.
- Considering the distribution of ion channels in the body, channelopathies encompass a wide variety of diseases, including epilepsy, ataxia, and movement disorders. In the neuromuscular system, channelopathies typically present with dynamic/episodic signs and symptoms such as muscle stiffness, myalgias, myotonia, and periodic paralysis.
- Named conditions of the muscle associated with mutations in genes that encode ion channels include myotonia congenita, paramyotonia congenita, hyperkalemic periodic paralysis, hypokalemic periodic paralysis, Andersen-Tawil syndrome, potassium-aggravated myotonia, and malignant hyperthermia.
- Mutations in some channel genes can also cause non-dynamic conditions, and in particular, several channel genes are also associated with congenital myopathies (examples include *SCN4A*, *RYR1*, and *CACNA1S*). Also, some non-ion channel disorders may include signs or symptoms typically associated with channelopathies (e.g., myotonic dystrophy)[36].
- There are some specific therapies that can alleviate symptoms that are genotype and phenotype specific.

RHABDOMYOLYSIS AND METABOLIC MYOPATHIES PANEL

- Rhabdomyolysis is defined as a medical condition associated with the rapid breakdown of muscle. It is associated with muscle pain and weakness, elevated serum CPK levels, and the potential for secondary end-organ damage[37].
- Individuals experiencing a single episode of rhabdomyolysis most typically have precipitating causes including infection, toxin/drug exposure, and excessive exercise.

- Recurrent rhabdomyolysis, conversely, often is due to an underlying genetic predisposition that manifests symptoms upon exposure to provoking factors. Causes include mutations associated with a variety of other muscle conditions, including channelopathies, myopathies, and muscular dystrophies. A leading cause of recurrent rhabdomyolysis is metabolic myopathy[37].
- Metabolic myopathies are the conditions that encompass dysfunction in the energy metabolism pathways in the muscle (i.e., fatty acid metabolism, glycogen metabolism, and mitochondrial oxidative phosphorylation). In addition to recurrent rhabdomyolysis, other signs/symptoms include myalgias, exercise intolerance, muscle cramping, and persistent weakness in some cases.

NEUROPATHIES PANEL

- Genetic neuropathies are defined as conditions that primarily impact the peripheral nerve, including motor and sensory neurons and the Schwann cells that enwrap them.
- Overarching signs include weakness (when motor neurons are impacted) and sensory changes (when sensory neurons are impacted).
- In genetic neuropathies, sensory changes are predominantly reflected as reduced or loss sensation, though pain and dysesthesias can also occur[38]

CHARCOT-MARIE-TOOTH DISEASE, HEREDITARY MOTOR NEUROPATHY, AND HEREDITARY SENSORY AND AUTONOMIC NEUROPATHY PANEL

- CMT, or hereditary motor and sensory polyneuropathy, is the most common subtype of neuropathy and one of the most overall common neuromuscular disorders.
- The condition is primarily defined by length-dependent weakness and sensory changes. Accompanying signs include distal muscle atrophy, pes cavus, hammer toes, and hand muscle wasting.
- There is tremendous genetic heterogeneity, though mutations in 4 genes account for nearly 90% of all genetically resolved cases (*PMP2*, *MPZ*, *GJB1*, *MFN2*), with 50% associated with *PMP22* duplication[39].
- Note that there are 4 subtypes of CMT that are based on inheritance patterns and part of the nerve that is primarily abnormal: CMT1 (dominant, demyelinating), CMT2 (dominant, axonal), CMT3 (severe early onset), CMT4 (recessive, usually demyelinating). For CMT1 and CMT2, there are often extensive family trees of affected members, though there can also be de novo presentations, often in severe cases.
- Hereditary motor neuropathy (HMN) is when only the peripheral motor nerves are impacted (and thus sensory function is normal), whereas hereditary sensory and autonomic neuropathy (HSAN) is when only peripheral sensory and autonomic nerves are involved.
- There is considerable genetic overlap between HMN or HSAN and CMT.
- Nerve conduction studies have an important role in suggesting the diagnosis of CMT/HMN/HSAN and also for orienting as to which type of neuropathy is present.

- Careful consideration should be given to late-onset neuropathies, such as Friedrich ataxia, as it may mimic CMT[40].

NEURONOPATHIES PANEL (NON-5Q SPINAL MUSCULAR ATROPHY)

- Neuronopathies are a subset of neuropathy where the primary defect is death/destruction of the neuron. They can be primarily sensory (i.e., impacting the peripheral sensory neuron/dorsal root ganglia) or motor (i.e., impacting the anterior horn cell). Of note, most sensory neuronopathies are non-genetic in etiology[41].
- The classic neuronopathy is 5q SMA. The expanded neuronopathy group includes mainly other forms of SMA (e.g., distal SMA, SMA with respiratory distress, X-linked SMA, SMA with lower extremity predominance) and the overlapping category of distal motor neuropathy.
- Clinical features include muscle weakness (which is often progressive), fasciculations, muscle atrophy/wasting, and contracture formation[41].
- There is considerable genetic overlap between non-5q motor neuropathies and Charcot-Marie-Tooth disease.

Single Gene and Other Specialized Neuromuscular Tests

CHARCOT-MARIE-TOOTH DISEASE TYPE 1A

- CMT1A is the most common form of CMT. It is inherited in an autosomal dominant manner[42].
- Distal muscle weakness, atrophy, and sensory loss may progress slowly. It is often associated with pes cavus and bilateral foot drop.
- The most common cause of CMT1A is the duplication of the region that contains *PMP22*, accounting for 50% of all CMT cases, but pathogenic deletions and SNVs have also been identified[42].

DYSTROPHINOPATHIES

- Dystrophinopathies describe any condition caused by mutations in the dystrophin (*DMD*) gene. The most common presentations are Duchenne muscular dystrophy (onset after the first six months of life but before age 4 years with limb-girdle weakness and high CPK) and BMD (onset after age 5 years with limb-girdle weakness and/or dynamic muscle symptoms such as myalgias and rhabdomyolysis)[43,44].
- DMD/BMD typically affects boys and men, though females may manifest a range of symptoms. DMD is the most common childhood muscular dystrophy and thus should be considered first in boys with appropriate clinical history.

5Q SPINAL MUSCULAR ATROPHY

- 5q SMA is a common recessive NMD in individuals <18 months of age[45].

- In July 2020, NBS in Ontario included 5q SMA and is expected to identify approximately 95% of all cases[25].
- If 5q SMA is highly suspected, testing should be pursued that includes dedicated deletion/duplication and sequencing analysis of the *SMN1* and *SMN2* genes.

SPINAL AND BULBAR MUSCULAR ATROPHY

- SBMA is a gradually progressive muscle weakness disorder associated with mild androgen insensitivity.
- SBMA is inherited in an X-linked manner. The age of onset is between the 2nd and 5th decade.
- Genetic testing for SBMA involves the identification of an expansion of a CAG trinucleotide repeat (>35 CAGs) in exon 1 of the *AR* gene by molecular genetic testing.
- Disorders in the differential diagnosis of SBMA include adult-onset SMA, CMT, and ALS[46].

FRIEDRICH ATAXIA

- FRDA is an autosomal recessive genetic disorder that causes progressive ataxia, muscle weakness, sensory loss. Individuals with FRDA are likely to present cardiomyopathy (~66%), diabetes mellitus (up to 30%), and 25% with atypical presentation (i.e., later onset and retained tendon reflexes). It is caused by biallelic pathogenic variants in *FXN*, which reduce the production of frataxin, a protein involved in iron metabolism and mitochondrial function[47].
- The diagnosis is established by detecting biallelic pathogenic variants in *FXN*, usually an abnormally expanded GAA repeat in intron 12[48].

MYOTONIC DYSTROPHY TYPE 1 AND TYPE 2

- Myotonic dystrophy is a repeat expansion multi-system disorder with two subtypes, DM1 and DM2. Myotonia is a unifying clinical feature.
- DM1 (due to CTG expansion in the 3'UTR of the *DMPK* gene) is far more common and is the only subtype that presents in childhood. DM1 is notable for the following constellation of features: temporal wasting and "myopathic" facies, myotonia, distal weakness, and cardiac features. Patients also experience progressive cognitive issues and may manifest cataracts and insulin resistance[49].
- DM2 (caused by CCTG repeat expansion in the CNBP gene) shares features of myotonia and cataracts, but typically presents proximal greater than distal weakness and more prominent myalgias.
- DM1, unlike DM2, exhibits prominent genetic anticipation, and strong correlation between repeat size and age of onset and severity. Only DM1 presents with a congenital form, which is characterized by hypotonia, weakness, myopathic facies, and feeding difficulties at/around the time of birth[50].

- Due to the nature of the mutations that cause DM1 and DM2, specialized genetic testing that detects repeat expansions is required[50].

OCULOPHARYNGEAL MUSCULAR DYSTROPHY

- OPMD is a condition that affects the muscles of the eyelids and pharynx, causing drooping eyelids (ptosis) and difficulty swallowing (dysphagia). As the disease progresses, other symptoms may develop, including limited upward gaze, weakness and atrophy of the tongue, difficulty chewing, a wet voice, weakness in facial and axial muscles, and weakness in the proximal limb-girdle muscles, particularly in the lower limbs[51].
- Molecular genetic testing for OPMD involves looking for a GCN trinucleotide repeat expansion in the *PABPN1* gene. There is also a cryptic mutation that can be missed with just repeat testing, and thus gene sequencing of the region is the optimal test.
- The mean age of onset for ptosis and dysphagia is 48 and 50 years old respectively. However, individuals with longer GCN expansions or who are compound heterozygous or homozygous for the GCN expansion may experience an earlier onset of symptoms (before 30 years of age) [51].

OCULOPHARYNGODISTAL MYOPATHY

- Oculopharyngodistal myopathy (OPDM) is a rare adolescent or adult-onset hereditary muscle condition that affects the eyes and throat, as well as the muscles of the face, lower legs, and arms, resulting in drooping eyelids (ptosis), slurred speech (dysarthria), paralysis or weakness of the eye muscles (ophthalmoplegia), and muscle weakness in the distal limbs[52].
- OPDM is recognized as a distinct disease from OPMD, though both share overlapping clinical features.
- There are 4 known types of OPDM. Genetically, the disease-causing genes for OPDM1, 2, 3, and 4 are CGG repeats in the 5'-untranslated region of *LRP12*, *GIPC1*, *NOTCH2NLC*, and *RILPL1*, respectively[53].
- When OPDM is suspected, testing should include *PAPBN1*, which remains the most common cause in this group, and the four OPDM genes in cases where *PAPBN1* is inconclusive.

FACIOSCAPULOHUMERAL DYSTROPHY

- FSHD is a clinical entity featuring scapular winging and facial muscle involvement at presentation. The scapular weakness may be asymmetric[54]. FSHD is genetically heterogeneous. Diagnosis requires specialized testing.
- FSHD1 accounts for approximately 90% of all cases of FSHD and it involved contraction of a *D4Z4* repeat on a permissive chromosome 4q35 haplotype.
- FSHD2, 3, and 4 are also rarely caused by a heterozygous single gene mutation in either *SMCHD1*, *DNMT3B*, and *LRIF1* and a permissive chromosome associated with *D4Z4* hypomethylation[54].

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Neurogenetics Expert Group

Members

Dr. James Dowling (Chair), Neurologist, The Hospital for Sick Children
Kimberly Amburgey, Genetic Counsellor, The Hospital for Sick Children
Dr. Tugce B. Balci, Medical Geneticist, London Health Sciences Centre
Lauren Brady, Genetic Counsellor, Hamilton Health Sciences
Dr. Susan Crocker, Clinical Laboratory Director, Kingston Health Sciences Centre
Dr. Soghra Jougheh Doust, Medical Geneticist, Peterborough Regional Health Centre
Dr. David Alexandre Dyment, Medical Geneticist, Children's Hospital of Eastern Ontario
Dr. Renato P. Munhoz, Neurologist, Toronto Western Hospital
Dr. James Stavropoulos, Clinical Laboratory Director, The Hospital for Sick Children
Dr. Mark Tarnopolsky, Neuromuscular and Neurometabolic Clinician, McMaster Children's Hospital
Dr. Jodi Warman-Chardon, Neurologist, The Ottawa Hospital

Ontario Health

Dr. Raymond Kim, Provincial Head, PGP
Kathleen Bell, Manager, PGP
Luis G. Peña, Senior Specialist, PGP
Kaitlyn Lemay, Analyst, PGP
Angela Du, Senior Specialist, PGP
Jerome Nguyen, Coordinator, PGP
Dr. Wilson Yu, Team Lead, PGP

Appendices

Appendix A: Neuromuscular Disease Genetic Testing Panels Summary

The Expert Group recommends GS or ES + CNV analysis (a genome-first approach) for infants from 0 to 24 months old with a clinical suspicion of neuromuscular disease. From 24 months until 18 years, the recommendation is to order multigene panels based on the clinical findings and clinical judgement of the subtype of NMD. For anyone above the age of 18 years, the suggestion is to order panels with sufficient clinical findings after ruling out the acquired cause of NMD.

Some of the genetic defects underlying certain inherited muscle diseases, such as FSHD and OPMD, are not detectable by next-generation sequencing. For this reason, the expert group has advised that these tests and sequencing-based broad multigene panels (Figure 1), which contain comprehensive genes associated with NMDs to be developed in the province. Sequencing and non-sequencing tests could be ordered sequentially or concurrently based on the differential diagnosis and results from ancillary testing.

The panels were curated to include relevant genes associated with inherited NMDs. Panels should encompass genes associated with the likely differential diagnoses and be sufficiently comprehensive to include NMDs with substantial clinical overlap. The panels' structure and design should allow flexibility to the ordering clinician to select a more targeted or broad panel depending on the diagnostic certainty. The choice of panels should be driven by clinical judgement, informed by the patient phenotype, and dictated by disease certainty.

The panels should capture the coding regions and flanking intron/exon boundaries and identify relevant copy number variants (CNVs) of all genes. Select relevant intronic variants should be included for the genes listed in the panel.

The Expert Group followed an evidence-based framework for each panel to achieve consensus on which genes to include on NMD panels. A review of the technical specifications should be completed prior to the implementation of the panels in Ontario. ClinGen, Genomic England PanelApp, and/or GeneTable of Neuromuscular Disorders curations were not available for all the disease entities included in the molecular panels.

Evidence Framework for Gene Inclusion

The following constitutes the list of resources and evidence thresholds for inclusion:

- **Clinical Genome Resource (ClinGen):** Genes curated as Moderate, Strong, or Definitive for gene-disease validity in ClinGen.
- **Genomics England PanelApp:** Genes identified as Green using the Genomics England PanelApp and nominated by the Expert Group member(s).
- **GeneTable of Neuromuscular Disorders:** NMD-specific genetic information public database.
- **Expert Consensus:** Genes for which there is supportive evidence in the literature and vetted by the Expert Group members.

Table A1. Gene Contents for Neuromuscular Disease Genetic Testing Panels

Genetic Test	Genes
Neuromuscular Diseases Panel **(440 genes)**	*AARS1, ABCA1, ABHD12, ABHD5, ACAD9, ACADL, ACADM, ACADVL, ACTA1, ACTN2, ACVR1, ADSS1, AGL, AGRN, AGTPBP1, AHNAK2, AIFM1, ALDOA, ALG14, ALG2, AMPD1, ANO5, APTX, ARHGEF10, ARSA, ASAH1, ASCC1, ASCC3, ATL1, ATL3, ATM, ATP1A1, ATP1A2, ATP2A1, ATP5F1D, ATP7A, B3GALNT2, B4GALNT1, B4GAT1, BAG3, BCKDHB, BICD2, BIN1, BSCL2, BVES, C1QBP, CACNA1A, CACNA1H, CACNA1S, CADM3, CAPN3, CASQ1, CAV3, CAVIN1, CCDC78, CCT5, CD59, CFAP276, CFL2, CHAT, CHCHD10, CHD8, CHKB, CHRNA1, CHRNB1, CHRND, CHRNE, CHRNG, CLCN1, CLN3, CLTCL1, CNTN1, CNTNAP1, COA7, COL12A1, COL13A1, COL6A1, COL6A2, COL6A3, COLQ, COQ4, COQ8A, COX6A1, CPOX, CPT1A, CPT2, CRPPA, CRYAB, CTDP1, CYP27A1, DAG1, DARS2, DCAF8, DCTN1, DEGS1, DES, DGAT2, DGUOK, DHTKD1, DMD, DNAJB2, DNAJB4, DNAJB6, DNM2, DNMT1, DNMT3B, DOK7, DOLK, DPAGT1, DPM1, DPM2, DPM3, DST, DUX4, DYNC1H1, DYSF, ECEL1, EGR2, ELP1, EMD, ENO3, EPG5, ERCC6, ERCC8, ETFA, ETFB, ETFDH, EXOSC3, FAH, FBLN5, FBXO38, FDX2, FGD4, FHL1, FIG4, FKBP14, FKRP, FKTN, FLAD1, FLNC, FLVCR1, FXN, FXR1, GAA, GALC, GAN, GARS1, GATM, GBA2, GBE1, GBF1, GDAP1, GFPT1, GGPS1, GIPC1, GJB1, GJB3, GJC2, GLA, GMPPB, GNB4, GNE, GOLGA2, GOSR2, GYG1, GYS1, HACD1, HADHA, HADHB, HARS1, HINT1, HK1, HMBS, HNRNPA1, HNRNPA2B1, HNRNPDL, HOXD10, HRAS, HSPB1, HSPB3, HSPB8, HYCC1, IARS2, IGHMBP2, INF2, INPP5K, ISCU, ITGA7, ITPR3, JAG1, JAG2, KARS1, KBTBD13, KCNA1, KCNA2, KCNE3, KCNJ18, KCNJ2, KIF1A, KIF1B, KIF5A, KLHL40, KLHL41, KY, LAMA2, LAMA5, LAMB2, LAMP2, LARGE1, LDB3, LDHA, LIMS2, LITAF, LMNA, LMOD3, LPIN1, LRIF1, LRP12, LRP4, LRSAM1, LYST, MAP3K20, MARS1, MB, MCM3AP, MCOLN1, MEGF10, MFN2, MGME1, MICU1, MLIP, MMACHC, MME, MORC2, MPDU1, MPV17, MPZ, MSTN, MSTO1, MT-ATP6, MT-CO1, MT-CO2, MTM1, MTMR2, MTRFR, MT-RNR1, MT-TL1, MTTP, MUSK, MYBPC1, MYBPC3, MYH2, MYH3, MYH7, MYL1, MYL2, MYMK, MYO18B, MYO9A, MYO9B, MYOT, MYPN, NAGA, NAGLU, NARS1, NDRG1, NEB, NEFH, NEFL, NGF, NHERF1, NMNAT2, NOTCH2NLC, NTRK1, OBSCN, OPA1, OPA3, ORAI1, PABPN1, PAX7, PCK2, PDHA1, PDK3, PDSS1, PDSS2, PEX10, PEX7, PFKM, PGAM2, PGK1, PGM1, PHKA1, PHKB, PHYH, PIEZO2, PLEC, PLEKHG5, PMM2, PMP2, PMP22, PNKP, PNPLA2, PNPLA8, POGLUT1, POLG, POLG2, POLR3A, POLR3B, POMGNT1, POMGNT2, POMK, POMT1, POMT2, POPDC3, PPOX, PRDM12, PREPL, PRKAG2, PRNP, PRPS1, PRX, PTPN11, PURA, PUS1, PYGM, PYROXD1, RAB7A, RAPSN, RBCK1, REEP1, RETREG1, RFC1, RILPL1, RNASEH1, RPH3A, RRM2B, RXYLT1, RYR1, RYR3, SACS, SBF1, SBF2, SCN10A, SCN11A, SCN4A, SCN9A, SCO2, SELENON, SEPTIN9, SETX, SGCA, SGCB, SGCD, SGCG, SGPL1, SH3TC2, SIGMAR1, SIL1, SLC12A3, SLC12A6, SLC16A1, SLC18A3, SLC22A5, SLC25A1, SLC25A19, SLC25A20, SLC25A3, SLC25A32, SLC25A4, SLC25A42, SLC25A46, SLC52A2, SLC52A3, SLC5A7, SMCHD1, SMN1, SNAP25, SORD, SOX10, SPAST, SPEG, SPG11, SPTBN4, SPTLC1, SPTLC2, STAC3, STIM1, SUCLA2, SURF1, SVIL, SYNE1, SYNE2, SYT2, TAFAZZIN, TANGO2, TCAP, TFG, TK2, TMEM43, TNNC2, TNNI2, TNNT1, TNNT3, TNPO3, TOR1AIP1, TPM2, TPM3, TRAPPC11, TRIM2, TRIM32, TRIM54, TRIM63, TRIP4, TRMT5, TRPA1, TRPV4, TSFM, TTN, TTPA, TTR, TUBB3, TYMP, UBA1, UNC13A, UNC45B, VAMP1, VAPB, VCP, VMA21, VPS13A, VRK1, VWA1, WARS1, WNK1, XK, XPA, YARS1, YARS2, ZFHX2, ZFYVE26*

Genetic Test	Genes
Muscle Diseases Panel **(261 genes)**	*ABHD5, ACAD9, ACADL, ACADM, ACADVL, ACTA1, ACTN2, ACVR1, ADSS1, AGL, AGRN, ALDOA, ALG14, ALG2, AMPD1, ANO5, ASCC3, ATP1A2, ATP2A1, ATP5F1D, B3GALNT2, B4GAT1, BAG3, BICD2, BIN1, BVES, C1QBP, CACNA1A, CACNA1H, CACNA1S, CAPN3, CASQ1, CAV3, CAVIN1, CCDC78, CFL2, CHAT, CHD8, CHKB, CHRNA1, CHRNB1, CHRND, CHRNE, CHRNG, CLCN1, CLN3, CNTN1, COL12A1, COL13A1, COL6A1, COL6A2, COL6A3, COLQ, COQ4, COQ8A, CPT1A, CPT2, CRPPA, CRYAB, DAG1, DES, DGUOK, DMD, DNAJB4, DNAJB6, DNM2, DNMT3B, DOK7, DOLK, DPAGT1, DPM1, DPM2, DPM3, DUX4, DYSF, ECEL1, EMD, ENO3, EPG5, ETFA, ETFB, ETFDH, FDX2, FHL1, FKBP14, FKRP, FKTN, FLAD1, FLNC, FXR1, GAA, GATM, GBE1, GFPT1, GGPS1, GIPC1, GMPPB, GNE, GOLGA2, GOSR2, GYG1, GYS1, HACD1, HADHA, HADHB, HNRNPA1, HNRNPA2B1, HNRNPDL, HRAS, IGHMBP2, INPP5K, ISCU, ITGA7, JAG2, KBTBD13, KCNA1, KCNE3, KCNJ18, KCNJ2, KLHL40, KLHL41, KY, LAMA2, LAMA5, LAMB2, LAMP2, LARGE1, LDB3, LDHA, LIMS2, LMNA, LMOD3, LPIN1, LRIF1, LRP12, LRP4, MAP3K20, MB, MCOLN1, MEGF10, MGME1, MICU1, MLIP, MPDU1, MSTN, MSTO1, MT-CO1, MT-CO2, MTM1, MUSK, MYBPC1, MYBPC3, MYH2, MYH3, MYH7, MYL1, MYL2, MYMK, MYO18B, MYO9A, MYOT, MYPN, NEB, NOTCH2NLC, OBSCN, ORAI1, PABPN1, PAX7, PDSS1, PDSS2, PFKM, PGAM2, PGK1, PGM1, PHKA1, PHKB, PIEZO2, PLEC, PNPLA2, PNPLA8, POGLUT1, POLG, POLG2, POMGNT1, POMGNT2, POMK, POMT1, POMT2, POPDC3, PREPL, PRKAG2, PURA, PUS1, PYGM, PYROXD1, RAPSN, RBCK1, RILPL1, RNASEH1, RPH3A, RRM2B, RXYLT1, RYR1, RYR3, SCN4A, SELENON, SGCA, SGCB, SGCD, SGCG, SIL1, SLC12A3, SLC16A1, SLC18A3, SLC22A5, SLC25A1, SLC25A20, SLC25A3, SLC25A32, SLC25A4, SLC25A42, SLC5A7, SMCHD1, SNAP25, SPEG, SPTBN4, STAC3, STIM1, SUCLA2, SVIL, SYNE1, SYNE2, SYT2, TAFAZZIN, TANGO2, TCAP, TK2, TMEM43, TNNC2, TNNI2, TNNT1, TNNT3, TNPO3, TOR1AIP1, TPM2, TPM3, TRAPPC11, TRIM32, TRIM54, TRIM63, TRIP4, TRMT5, TSFM, TTN, TYMP, UNC13A, UNC45B, VAMP1, VCP, VMA21, YARS2*
Congenital Muscle Diseases Panel **(166 genes)**	*ACTA1, ACTN2, ACVR1, ADSS1, AGRN, ALG14, ALG2, ASCC3, ATP2A1, B3GALNT2, B4GAT1, BAG3, BICD2, BIN1, CACNA1H, CACNA1S, CASQ1, CAV3, CCDC78, CFL2, CHAT, CHD8, CHKB, CHRNA1, CHRNB1, CHRND, CHRNE, CHRNG, CLN3, CNTN1, COL12A1, COL13A1, COL6A1, COL6A2, COL6A3, COLQ, CRPPA, CRYAB, DAG1, DES, DMD, DNAJB4, DNM2, DOK7, DOLK, DPAGT1, DPM1, DPM2, ECEL1, EPG5, FHL1, FKBP14, FKRP, FKTN, FLNC, FXR1, GAA, GATM, GFPT1, GIPC1, GMPPB, GOLGA2, GOSR2, HACD1, HNRNPA1, HNRNPA2B1, HRAS, IGHMBP2, INPP5K, ISCU, ITGA7, KBTBD13, KLHL40, KLHL41, KY, LAMA2, LAMA5, LAMB2, LAMP2, LARGE1, LDB3, LMNA, LMOD3, LRP12, LRP4, MAP3K20, MB, MCOLN1, MEGF10, MICU1, MPDU1, MSTN, MSTO1, MTM1, MUSK, MYBPC1, MYBPC3, MYH2, MYH3, MYH7, MYL1, MYL2, MYMK, MYO18B, MYO9A, MYOT, MYPN, NEB, NOTCH2NLC, PABPN1, PAX7, PIEZO2, PLEC, POMGNT1, POMGNT2, POMK, POMT1, POMT2, PREPL, PURA, PYROXD1, RAPSN, RILPL1, RPH3A, RXYLT1, RYR1, RYR3, SCN4A, SELENON, SGCA, SGCB, SGCD, SGCG, SIL1, SLC18A3, SLC25A1, SLC25A4, SLC5A7, SNAP25, SPEG, SPTBN4, STAC3, STIM1, SVIL, SYNE1, SYT2, TCAP, TNNC2, TNNI2, TNNT1, TNNT3, TNPO3, TOR1AIP1, TPM2, TPM3, TRAPPC11, TRIM32, TRIM54, TRIM63, TRIP4, TTN, UNC13A, UNC45B, VAMP1, VCP, VMA21*
Congenital Myasthenic Syndromes Panel **(36 genes)**	*AGRN, ALG14, ALG2, CHAT, CHD8, CHRNA1, CHRNB1, CHRND, CHRNE, CHRNG, COL13A1, COLQ, DOK7, DPAGT1, GFPT1, GMPPB, LAMA5, LAMB2, LRP4, MUSK, MYO9A, PLEC, PREPL, PURA, RAPSN, RPH3A, RYR1, SCN4A, SLC18A3, SLC25A1, SLC5A7, SNAP25, SYT2, TOR1AIP1, UNC13A, VAMP1*

Genetic Test	Genes
Congenital and Other Myopathies Panel (99 genes)	*ACTA1, ACTN2, ACVR1, ADSS1, ASCC3, ATP2A1, BAG3, BICD2, BIN1, CACNA1H, CACNA1S, CASQ1, CAV3, CCDC78, CFL2, CHKB, CLN3, CNTN1, COL12A1, CRYAB, DES, DNAJB4, DNM2, DOK7, ECEL1, EPG5, FHL1, FKBP14, FLNC, FXR1, GATM, GIPC1, HACD1, HNRNPA1, HNRNPA2B1, HRAS, IGHMBP2, ISCU, KBTBD13, KLHL40, KLHL41, KY, LAMA2, LAMP2, LDB3, LMNA, LMOD3, LRP12, MAP3K20, MB, MCOLN1, MEGF10, MICU1, MSTN, MTM1, MYBPC1, MYBPC3, MYH2, MYH3, MYH7, MYL1, MYL2, MYMK, MYO18B, MYOT, MYPN, NEB, NOTCH2NLC, PABPN1, PAX7, PIEZO2, PLEC, PYROXD1, RILPL1, RYR1, RYR3, SCN4A, SELENON, SLC25A4, SPEG, SPTBN4, STAC3, STIM1, SVIL, TNNC2, TNNI2, TNNT1, TNNT3, TNPO3, TPM2, TPM3, TRIM32, TRIM54, TRIM63, TRIP4, TTN, UNC45B, VCP, VMA21*
Congenital Muscular Dystrophies Panel (48 genes)	*ACTA1, B3GALNT2, B4GAT1, CHKB, COL12A1, COL6A1, COL6A2, COL6A3, CRPPA, DAG1, DMD, DNM2, DOLK, DPM1, DPM2, FHL1, FKRP, FKTN, GAA, GMPPB, GOLGA2, GOSR2, INPP5K, ITGA7, LAMA2, LARGE1, LMNA, MICU1, MPDU1, MSTO1, PLEC, POMGNT1, POMGNT2, POMK, POMT1, POMT2, RXYLT1, RYR1, SELENON, SGCA, SGCB, SGCD, SGCG, SIL1, SYNE1, TCAP, TRAPPC11, TRIP4*
Muscular Dystrophies Panel (80 genes)	*ACADVL, ANO5, ATP2A1, BAG3, BVES, CAPN3, CAV3, CAVIN1, COL6A1, COL6A2, COL6A3, CPT2, CRPPA, CRYAB, DAG1, DES, DMD, DNAJB6, DNMT3B, DOK7, DPM3, DUX4, DYSF, EMD, FHL1, FKRP, FKTN, FLNC, GAA, GGPS1, GMPPB, GNE, GOSR2, HNRNPDL, JAG2, KBTBD13, LAMA2, LAMP2, LARGE1, LIMS2, LMNA, LPIN1, LRIF1, MTM1, MYH7, MYOT, ORAI1, PFKM, PHKA1, PLEC, POGLUT1, POMGNT1, POMGNT2, POMK, POMT1, POMT2, POPDC3, PYGM, PYROXD1, RYR1, SELENON, SGCA, SGCB, SGCD, SGCG, SMCHD1, STIM1, SYNE1, SYNE2, TAFAZZIN, TCAP, TK2, TMEM43, TNPO3, TOR1AIP1, TRAPPC11, TRIM32, TTN, VCP, VMA21*
Neuromuscular Channelopathies Panel (10 genes)	*ATP1A2, CACNA1A, CACNA1S, CLCN1, KCNA1, KCNE3, KCNJ18, KCNJ2, SCN4A, SLC12A3*
Rhabdomyolysis and Metabolic Myopathies Panel (106 genes)	*ABHD5, ACAD9, ACADL, ACADM, ACADVL, AGL, ALDOA, AMPD1, ANO5, ATP2A1, ATP5F1D, C1QBP, CACNA1S, CAPN3, CASQ1, CAV3, CHKB, COQ4, COQ8A, CPT1A, CPT2, CRPPA, DAG1, DGUOK, DMD, DNAJB6, DYSF, EMD, ENO3, ETFA, ETFB, ETFDH, FDX2, FHL1, FKRP, FKTN, FLAD1, GAA, GATM, GBE1, GMPPB, GYG1, GYS1, HADHA, HADHB, ISCU, ITGA7, LAMA2, LAMP2, LARGE1, LDHA, LPIN1, MGME1, MLIP, MT-CO1, MT-CO2, OBSCN, PDSS1, PDSS2, PFKM, PGAM2, PGK1, PGM1, PHKA1, PHKB, PNPLA2, PNPLA8, POLG, POLG2, POMGNT1, POMGNT2, POMK, POMT1, POMT2, PRKAG2, PUS1, PYGM, RBCK1, RNASEH1, RRM2B, RYR1, SCN4A, SGCA, SGCB, SGCD, SGCG, SIL1, SLC16A1, SLC22A5, SLC25A20, SLC25A3, SLC25A32, SLC25A4, SLC25A42, STAC3, SUCLA2, TAFAZZIN, TANGO2, TCAP, TK2, TNPO3, TRIM32, TRMT5, TSFM, TYMP, YARS2*

Genetic Test	Genes
Neuropathies Panel **(197 genes)**	*AARS1, ABCA1, ABHD12, AGTPBP1, AHNAK2, AIFM1, APTX, ARHGEF10, ARSA, ASAH1, ASCC1, ATL1, ATL3, ATM, ATP1A1, ATP7A, B4GALNT1, BAG3, BCKDHB, BICD2, BSCL2, CADM3, CCT5, CD59, CFAP276, CHCHD10, CLTCL1, CNTNAP1, COA7, COX6A1, CPOX, CTDP1, CYP27A1, DARS2, DCAF8, DCTN1, DEGS1, DGAT2, DHTKD1, DNAJB2, DNM2, DNMT1, DST, DYNC1H1, EGR2, ELP1, ERCC6, ERCC8, EXOSC3, FAH, FBLN5, FBXO38, FGD4, FIG4, FLVCR1, FXN, GALC, GAN, GARS1, GBA2, GBF1, GDAP1, GJB1, GJB3, GJC2, GLA, GNB4, HADHA, HADHB, HARS1, HINT1, HK1, HMBS, HOXD10, HSPB1, HSPB3, HSPB8, HYCC1, IARS2, IGHMBP2, INF2, ITPR3, JAG1, KARS1, KCNA2, KIF1A, KIF1B, KIF5A, LAMP2, LDB3, LITAF, LMNA, LRSAM1, LYST, MARS1, MCM3AP, MEGF10, MFN2, MMACHC, MME, MORC2, MPV17, MPZ, MT-ATP6, MTMR2, MTRFR, MT-RNR1, MT-TL1, MTTP, MYO9B, NAGA, NAGLU, NARS1, NDRG1, NEFH, NEFL, NGF, NHERF1, NMNAT2, NOTCH2NLC, NTRK1, OPA1, OPA3, PCK2, PDHA1, PDK3, PEX10, PEX7, PHYH, PLEKHG5, PMM2, PMP2, PMP22, PNKP, POLG, POLR3A, POLR3B, PPOX, PRDM12, PRNP, PRPS1, PRX, PTPN11, RAB7A, REEP1, RETREG1, RFC1, SACS, SBF1, SBF2, SCN10A, SCN11A, SCN9A, SCO2, SEPTIN9, SETX, SGPL1, SH3TC2, SIGMAR1, SLC12A6, SLC25A19, SLC25A46, SLC52A2, SLC52A3, SLC5A7, SMN1, SORD, SOX10, SPAST, SPG11, SPTBN4, SPTLC1, SPTLC2, SURF1, SYT2, TFG, TRIM2, TRIP4, TRPA1, TRPV4, TTPA, TTR, TUBB3, TYMP, UBA1, VAPB, VCP, VPS13A, VRK1, VWA1, WARS1, WNK1, XK, XPA, YARS1, ZFHX2, ZFYVE26*
CMT/HMN/HSAN Panel **(192 genes)**	*AARS1, ABCA1, ABHD12, AGTPBP1, AHNAK2, AIFM1, APTX, ARHGEF10, ARSA, ATL1, ATL3, ATM, ATP1A1, ATP7A, B4GALNT1, BAG3, BCKDHB, BICD2, BSCL2, CADM3, CCT5, CD59, CFAP276, CHCHD10, CLTCL1, CNTNAP1, COA7, COX6A1, CPOX, CTDP1, CYP27A1, DARS2, DCAF8, DCTN1, DEGS1, DGAT2, DHTKD1, DNAJB2, DNM2, DNMT1, DST, DYNC1H1, EGR2, ELP1, ERCC6, ERCC8, FAH, FBLN5, FBXO38, FGD4, FIG4, FLVCR1, FXN, GALC, GAN, GARS1, GBA2, GBF1, GDAP1, GJB1, GJB3, GJC2, GLA, GNB4, HADHA, HADHB, HARS1, HINT1, HK1, HMBS, HOXD10, HSPB1, HSPB3, HSPB8, HYCC1, IARS2, IGHMBP2, INF2, ITPR3, JAG1, KARS1, KCNA2, KIF1A, KIF1B, KIF5A, LAMP2, LDB3, LITAF, LMNA, LRSAM1, LYST, MARS1, MCM3AP, MEGF10, MFN2, MMACHC, MME, MORC2, MPV17, MPZ, MT-ATP6, MTMR2, MTRFR, MT-RNR1, MT-TL1, MTTP, MYO9B, NAGA, NAGLU, NARS1, NDRG1, NEFH, NEFL, NGF, NHERF1, NMNAT2, NOTCH2NLC, NTRK1, OPA1, OPA3, PCK2, PDHA1, PDK3, PEX10, PEX7, PHYH, PLEKHG5, PMM2, PMP2, PMP22, PNKP, POLG, POLR3A, POLR3B, PPOX, PRDM12, PRNP, PRPS1, PRX, PTPN11, RAB7A, REEP1, RETREG1, RFC1, SACS, SBF1, SBF2, SCN10A, SCN11A, SCN9A, SCO2, SEPTIN9, SETX, SGPL1, SH3TC2, SIGMAR1, SLC12A6, SLC25A19, SLC25A46, SLC52A2, SLC52A3, SLC5A7, SMN1, SORD, SOX10, SPAST, SPG11, SPTBN4, SPTLC1, SPTLC2, SURF1, SYT2, TFG, TRIM2, TRPA1, TRPV4, TTPA, TTR, TUBB3, TYMP, UBA1, VCP, VPS13A, VRK1, VWA1, WARS1, WNK1, XK, XPA, YARS1, ZFHX2, ZFYVE26*
Motor Neuronopathies Panel **(33 genes)**	*ASAH1, BICD2, BSCL2, CHCHD10, DCTN1, DYNC1H1, EXOSC3, GARS1, HINT1, HSPB3, HSPB8, IGHMBP2, REEP1, SLC52A2, SLC52A3, SLC5A7, SMN1, SPG11, TRIP4, TRPV4, UBA1, VRK1, WARS1, AARS1, ASCC1, DNAJB2, FBXO38, HSPB1, PLEKHG5, SETX, SIGMAR1, SYT2, VAPB*

Appendix B: Acronyms

AChR	Acetylcholine receptor
ALS	Amyotrophic lateral sclerosis
AS	Angelman syndrome
BMD	Becker muscular dystrophy
CIDP	Chronic inflammatory demyelinating polyradiculopathy
ClinGen	Clinical Genome Resource
CM	Congenital myopathy
CMA	Chromosomal microarray analysis
CMD	Congenital muscular dystrophy
CMS	Congenital myasthenic syndrome
CMT	Charcot-Marie-Tooth disease
CNS	Central nervous system
CNV	Copy number variant
CPK	Creatinine phosphokinase
DM1	Myotonic dystrophy type 1
DM2	Myotonic dystrophy type 2
DMD	Duchene muscular dystrophy
EDMD	Emery–Dreifuss muscular dystrophy
EMG	Electromyography
ES	Exome sequencing
FRDA	Friedrich ataxia
FSHD	Facioscapulohumeral muscular dystrophy
GBS	Guillain-Barre syndrome
GS	Genome sequencing
GWS	Genome-wide sequencing
HMN	Hereditary motor neuropathy
HSAN	Hereditary sensory and autonomic neuropathy
LGMD	Limb-girdle muscular dystrophy
MG	Myasthenia gravis
MLPA	Multiplex ligation probe amplification
MRI	Magnetic resonance imaging
NBS	Newborn screening
NCS	Nerve conduction study
NMD	Neuromuscular disease
OPDM	Oculopharyngodistal myopathy
OPMD	Oculopharyngeal muscular dystrophy
PGP	Provincial Genetics Program

PGAC	Provincial Genetics Advisory Committee
P/LP	Pathogenic or likely pathogenic
PNS	Peripheral nervous system
PWS	Prader-Willi syndrome
SBMA	Spinal and bulbar muscular atrophy
SMA	Spinal muscular atrophy
VUS	Variant of uncertain significance