UnitedHealthcare® Commercial and Individual Exchange
Medical Policy

Genetic Testing for Neuromuscular Disorders

Policy Number: 2023T0598H
Effective Date: November 1, 2023

➲ Instructions for Use

Related Commercial/Individual Exchange Policies
- Chromosome Microarray Testing (Non-Oncology Conditions)
- Genetic Testing for Cardiac Disease
- Whole Exome and Whole Genome Sequencing

Community Plan Policy
- Genetic Testing for Neuromuscular Disorders

Medicare Advantage Coverage Summaries
- Genetic Testing
- Laboratory Tests and Services

Application

UnitedHealthcare Commercial

This Medical Policy applies to all UnitedHealthcare Commercial benefit plans.

UnitedHealthcare Individual Exchange

This Medical Policy applies to Individual Exchange benefit plans in all states except for Colorado.

Coverage Rationale

Multi-gene targeted panel testing (5 or more genes) for the diagnosis of any of the following suspected Neuromuscular Disorders is proven and medically necessary:
- Congenital myopathy, distal myopathy, metabolic myopathy (e.g., glycogen storage disease) or myofibrillar myopathy; or
- Hereditary ataxia; or
- Hereditary peripheral neuropathy; or
- Hereditary spastic paraplegia; or
- Muscular dystrophy (e.g., limb girdle muscular dystrophy, congenital muscular dystrophy including but not limited to dystroglycanopathy); or
- Mitochondrial disease (e.g., Kearns-Sayre syndrome [KSS], Leber hereditary optic neuropathy [LHON], Leigh syndrome, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes [MELAS] syndrome) in individuals with all of the following:
  - Mitochondrial testing ordered by or in consultation with a board-certified medical geneticist or neurologist; and
  - High degree of suspicion of having a mitochondrial disease based on medical history, family history, laboratory, or other clinical tests; and
  - The clinical presentation does not support use of single gene or targeted genetic analysis; and
  - The individual has clinical features consistent with a mitochondrial disease, such as one of the following conditions:
    - Proximal weakness; or

- - Muscle cramping, fatigue, or exercise intolerance; or
  - Progressive external ophthalmoplegia; or
  - Sensorineural hearing loss

Multi-gene neuromuscular disease test panels targeting multiple conditions (e.g., muscular dystrophy and mitochondrial disease) are unproven and not medically necessary due to insufficient evidence of efficacy.

Note: Whole Exome and Whole Genome Sequencing are addressed in the Medical Policy titled Whole Exome and Whole Genome Sequencing.

Documentation Requirements

Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. The documentation requirements outlined below are used to assess whether the member meets the clinical criteria for coverage but do not guarantee coverage of the service requested.

CPT Codes*	Required Clinical Information
Genetic Testing for Neuromuscular Disorders	
0216U 0217U 81440 81448 81460 81465 81479	Medical notes documenting the following, when applicable: • Name and specialty of the ordering and/ or consulting provider • Personal history of the condition, if applicable, including age at diagnosis • Family history relevant to condition being tested • Genetic testing results of family member, if applicable, and reason for testing • Ethnicity/ancestry (e.g., Ashkenazi Jewish), if reason for testing • Any prior genetic testing results • Genetic counseling (if available)

*For code descriptions, refer to the Applicable Codes section.

Definitions

Comparative Genomic Hybridization (CGH): CGH is a technology that can be used for the detection of genomic copy number variations (CNVs). Tests can use a variety of probes or single nucleotide polymorphisms (SNPS) to provide copy number and gene differentiating information. All platforms share in common that tumor (patient) and reference DNA are labelled with dyes or fluorescing probes and hybridized on the array, and a scanner measures differences in intensity between the probes, and the data is expressed as having greater or less intensity than the reference DNA (Piluso et al., 2011).

Neuromuscular Disorders (NMD): Are group of inherited diseases that represent a number of conditions that result from impairment of nerves that control the muscles, or direct impairment of the muscles (Piluso et al., 2011).

Next Generation Sequencing (NGS): High-throughput DNA sequencing of large numbers of genes in a single reaction (Efthymiou et al., 2016).

Variant of Unknown Significance (VUS): A variation in a genetic sequence that has an unknown association with disease. It may also be called an unclassified variant (Efthymiou et al., 2016).

Whole Exome Sequencing (WES): About 1% of a person's DNA makes protein. These protein-making sections are called exons. All the exons together are called the exome. WES is a DNA analysis technique that looks at all of the exons in a person's DNA at one time rather than gene by gene (MedlinePlus, 2021).

Whole Genome Sequencing (WGS): WGS determines the sequence of all the nucleotides in a person's entire DNA including the protein-making (coding) as well as non-coding DNA elements (MedlinePlus, 2021).

Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and Guidelines may apply.

CPT Code	Description
0216U	Neurology (inherited ataxias), genomic DNA sequence analysis of 12 common genes including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants
0217U	Neurology (inherited ataxias), genomic DNA sequence analysis of 51 genes including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants
81440	Nuclear encoded mitochondrial genes (e.g., neurologic or myopathic phenotypes), genomic sequence panel, must include analysis of at least 100 genes, including BCS1L, C10orf2, COQ2, COX10, DGUOK, MPV17, OPA1, PDSS2, POLG, POLG2, RRM2B, SCO1, SCO2, SLC25A4, SUCLA2, SUCLG1, TAZ, TK2, AND TYMP
81448	Hereditary peripheral neuropathies (e.g., Charcot-Marie-Tooth, spastic paraplegia), genomic sequence analysis panel, must include sequencing of at least 5 peripheral neuropathy-related genes (e.g., BSCL2, GJB1, MFN2, MPZ, REEP1, SPAST, SPG11, SPTLC1)
81460	Whole mitochondrial genome (e.g., Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS], myoclonic epilepsy with ragged-red fibers [MERFF], neuropathy, ataxia, and retinitis pigmentosa [NARP], Leber hereditary optic neuropathy [LHON]), genomic sequence, must include sequence analysis of entire mitochondrial genome with heteroplasmy detection
81465	Whole mitochondrial genome large deletion analysis panel (e.g., Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia), including heteroplasmy detection, if performed
81479	Unlisted molecular pathology procedure

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Description of Services

Technologies used for genetic testing of Neuromuscular Disorders (NMD) can vary, and can include, but are not limited to, tests that evaluate variations in the genes, such as chromosome microarray and Next Generation Sequencing (NGS), as well as others that assess the gene products, such as gene expression arrays and microRNA analysis. The number of genes evaluated can range from a single gene to the whole exome or genome of an individual. Results of genetic testing may assist individuals and healthcare providers with determining a diagnosis, prognosis, and identification of appropriate clinical interventions (Savarese et al., 2016; Piluso et al., 2011; and Ghaoui et al., 2015). This policy addresses genetic test panels with five or more genes for NMD. Neuromuscular diseases that typically present with a cardiomyopathy and are caused by a variant in a cardiomyopathy gene are addressed in the Medical Policy titled Genetic Testing for Cardiac Disease and those associated with Whole Exome Sequencing are addressed in the Medical Policy titled Whole Exome and Whole Genome Sequencing.

Clinical Evidence

Neuromuscular Disorders (NMD)

NMD are a heterogenous group of conditions that are caused by impaired muscles and impaired nerves that control the muscles. Examples of NMD include muscular dystrophies, nerve conduction disorders such as Charcot-Marie-Tooth (CMT), motor neuron disease (MND), hereditary spastic paraplegia (HSP), spinal muscular atrophies (SMA), and neuromuscular junction disease (myasthenic syndromes). Common symptoms include muscle weakness, cramps, numbness, respiratory and

cranial nerve palsies. Many of these disorders are inherited, and over 500 genes are implicated in causing NMD (Efthymiou et al., 2016).

In an observational study, Schuermans et al. (2023) evaluated the diagnostic yield of exome sequencing (ES) and multigene panel testing in individuals with adult-onset neurologic disorders, including neuromuscular disorders. A total of 1,411 individuals were tested using ES-based multigene panel testing. Panels for ataxia and spasticity, leukoencephalopathy, movement disorders, paroxysmal episodic disorders, neurodegeneration with brain iron accumulation, progressive myoclonic epilepsy, and amyotrophic lateral sclerosis were created and a total of 725 genes associated with Mendelian inheritance were included overall. Genetic diagnosis was identified in 10% of the total cases, including 71 different monogenic disorders. The highest diagnostic yield was seen in individuals demonstrating ataxia or spastic paraparesis (19%) and varied based on individual phenotype. The majority of diagnoses found included disorders with autosomal dominant inheritance (62%), and the genes that most often showed variation were NOTCH3 Z (n = 13), SPG7 (n = 11) and RFC1 (n = 8). The authors concluded that ES-based molecular testing can be successfully and efficiently used to diagnose adult-onset neurologic diseases but point out some technological limitations and recommend further studies assessing other technologies (such as genome sequencing) that could be used to assist with diagnosis of rare neurological diseases.

In a prospective, multicenter study to evaluate clinical utility and diagnostic yield of a targeted gene panel for inherited neuromuscular disorders (INMD), Barbosa-Gouveia et al. (2022) used comprehensive gene-panel analysis and next-generation sequencing (NGS) to evaluate 268 patients (both pediatric and adult) with a suspected diagnosis of INMD. Three versions of the multi-gene testing panel were designed during the three year study period, with progressive addition of genes to the panel, resulting in an exponential increase in diagnosis rate. The first version (278 genes) yielded a diagnosis rate of 31% while the third (324 genes) yielded a diagnosis rate of 40%. Mean diagnostic rate over the entire 3 year study period was 36%. Most common diagnoses included muscular dystrophies/myopathies (68.4%) and peripheral nerve diseases (22.5%). TTN, RYR1 and ANO5 were the most common causative genes found and contributed to nearly 30% of diagnosed cases. The authors assert that in the case of INMDs, reaching a definitive diagnosis requires identification of specific variants in disease-causing genes. They recommend comprehensive gene-panel testing of all neuromuscular disease-related genes, including those most commonly implicated, in individuals with suspected INMD.

In a 2021 publication, Nicolau et al. discussed approaches for genetic testing of muscle and neuromuscular junction disorders. The authors indicate that the patient's phenotype sets the guiding approach for genetic testing. Phenotypes suggesting myopathy that require targeted testing (i.e., myotonic dystrophies, FSHD, OPMD, OPDM, DMD and mitochondrial myopathies) must be identified as a first step. For remaining patients, the researchers suggest a gene panel encompassing a large number of genes related to congenital myasthenic syndromes (CMSs) and myopathies, including copy number variation analysis. Specific focus should be placed on the avoidance of missing potentially treatable neuromuscular conditions such as Pompe disease or CMSs. Unfortunately, according to this article, many patients will remain without molecular diagnosis even after testing due to such factors as disorders not amenable to detection via NGS or acquired disorders mimicking inherited myopathies. The researchers state that techniques including exome, genome and RNA sequencing will likely play a greater role in the investigation of undiagnosed patients in the near future.

Bowen et al. (2021) reported the clinical findings of a no-charge, sponsored NGS program called "SMA Identified". Eligible individuals had either a confirmed or suspected diagnosis of spinal muscular atrophy (SMA), or a family history of SMA. The study took place over a 2 year period. A total of 2,459 individuals underwent testing with an NGS based approach looking for sequence and copy number of SMN1 and SMN2. Participants were then categorized according to their test results as follows: diagnostic (two pathogenic SMN1 variants), nearly diagnostic (SMN1 exon-7 deletion with variant of uncertain significance [VUS] in SMN1 or SMN2), indeterminate VUS (one VUS in SMN1 or SMN2), carrier (heterozygous SMN1 deletion only), or negative (no pathogenic variants OR VUS in SMN1 or SMN2). Analysis was completed based on clinician reported clinical findings and genetic modifiers. Diagnostic yield for diagnostic and nearly diagnostic (combined) was 31.3% (n = 771/2459). Clinical presentation and age of onset of symptoms were variable across individuals and dependent on SMN2 copy number. The most common genetic etiology was homozygous deletions (96.2%). The authors concluded that use of a high yield panel test early in evaluation of individuals with or at higher risk for SMA may lead to earlier interventions in individuals with SMA.

Winder et al. (2020) aimed to demonstrate the clinical utility of genetic testing by creating a comprehensive data set by analyzing 25,356 unrelated individuals after testing with 266 genes using NGS. The panel was designed using published literature and genotype-phenotype associations. The patients were enrolled in the study if there was a suspicion of NMD and from the study, a definitive diagnosis was determined in 5,055 (20%) of the patients. Usual genetic studies do not routinely

include copy number variation (CNV) analysis; however, in this study, the CNVs account for 39% of the significant variants found. Multi-gene testing addressed differential diagnoses in at least 6% of individuals with positive results.

Westra et al. (2019) used WES for a NMD population with both children and adults. A cohort of 396 patients was analyzed by clinical exome sequencing and then diagnostic interpretation of variants. Significant variants were found in 75/396 patients (19%). Variants in the three COL6-genes were identified as the most common cause of the NMD followed by variants in the RYR1 gene (in total 25% of cases). Likely pathogenic variants and/or variants of uncertain significance were identified in 95 of the patients (24%).

As part of the North Carolina Clinical Genomic Evaluation by Next-Generation Exome Sequencing Study (NCGENES), Haskell et al. (2018) used WES to determine a genetic diagnosis in 93 patients with NMD. Patients were categorized into three groups based on clinical findings; primarily neuropathy, primarily myopathy, or complex. After DNA extraction and WES, variants were filtered through three different gene lists in order to compare diagnostic yield between different lists. A neuropathy list of 199 genes implicated in neuropathy phenotypes, a myopathy list of 181 genes, and a list of 482 genes implicated in NMD were used. Variants were then categorized using the American College of Medical Genetics and Genomics (ACMG) standards on pathogenicity. The overall diagnostic yield of WES for pathogenic or likely pathogenetic variants was 12.9%, and each gene list gave a different diagnostic yield. In some cases, family testing was performed to determine gene segregation and verify pathogenicity. The authors found that in patients with a clear neuropathy or myopathy, WES had the same diagnostic yield as the broader diagnostic test list. In patients with a complex phenotype, the broader list had the best diagnostic yield (9%) when compared to the neuropathy (4.9%) or myopathy (0%) diagnostic lists. Many of these patients had undergone muscle biopsy (42%), nerve conduction studies or electromyograms (86%), and genetic testing previously (68% overall and 20% had a multi-gene panel) and a definitive diagnosis had not been reached. The participants biopsy, electrodiagnostic testing, and prior genetic results were reviewed by three independent specialist reviewers who categorized the testing as informative or noninformative in the context of WES results. Sixty-three percent of the prior testing was considered informative, meaning that it correlated with the pathogenic variant identified in WES as a neuropathy, myopathy, or a complex disorder. In two cases, WES identified molecular diagnoses that directly impacted medical treatment. One patient had been clinically diagnosed with a chronic inflammatory demyelinating polyneuropathy, but WES demonstrated that the genetic diagnosis of Spastic Ataxia of Charlevoix-Saguenay, so unnecessary immunotherapy was avoided. The second patient had been thought to have a hereditary spastic paraplegia, but the genetic diagnosis was confirmed as a form of dopa-responsive dystonia, and after dopa therapy was started, she regained the ability to walk without assistance. The authors concluded that introducing genome-scale sequencing into the clinical workflow earlier may shorten the diagnostic odyssey, minimize invasive testing, and provide potential opportunities for clinical and investigational therapeutics for patients with NMD.

Wu et al. (2018) evaluated a group of 169 patients referred to a Canadian neuromuscular clinic with an NGS panel of 163-183 neuromuscular disease related genes. Patients included in the study had unexplained hyperCKemia and had a CK value recorded more than 3X the usual upper limit. Patients were excluded if they were suspected of having an acquired or inflammatory cause for their symptoms like a statin induced myopathy, or had classic features of a single gene NMD, such as myotonic dystrophy or Duchenne muscular dystrophy. The ACMG guidelines were used to interpret variants, and variants identified in patients before the publication of the ACMG guidelines underwent re-interpretation in 2017. Pathogenic and likely pathogenic variants were considered in the calculation of the detection rate. Overall, pathogenic, and likely pathogenetic variants were identified in 61 (36%) of patients. In the cohort that presented with muscle weakness (n = 135), causative variants were found in 50 (37%). The detection rate in only pediatric patients (n = 47) was 38%. In individuals with recurrent rhabdomyolysis (n = 18), causative variants were found in six (33%). Sixteen patients had idiopathic hyperCKemia, and five (31%) had candidate variants identified. The authors noted that clinicians should be aware of the limitations of NGS testing, and that clinical examination and other diagnostic tools such as electromyography and muscle biopsy are still an important part of the diagnostic process. NGS may be subject to laboratory-specific limitations in detecting a variety of variant types including copy number variants, regulatory sequence variants, trinucleotide repeat expansions, and deep intronic mutations.

Nishikawa et al. (2017) studied the clinical utility of targeted NGS panels designed to identify inherited muscle diseases associated with muscular dystrophy, congenital myopathy (CM), metabolic myopathy (MM), and myopathy with protein aggregations/rimmed vacuoles (MFM). They analyzed blood samples on 188 patients who had blood and muscle biopsy submitted to their lab in 2014 and 2015. Genes for the panels were identified from the 2013 gene table of monogenic NMD, and the target gene numbers were 65 (muscular dystrophy), 41 (CM), 45 (MM), and 36 (MFM). The authors did not combine the genes into one large panel for cost and time efficiency purposes. To analyze the muscular dystrophy panel, 65 patients were recruited who had muscle biopsies and clinical findings suspicious for muscular dystrophy. Likely causative mutations were

found in 30 patients (46%), and the genotype correlated with clinical findings. Sixty-five patients were analyzed for the CM panel. Causative mutations were found in 17 patients (26%), and an additional 13 patients had variants that were consistent with their phenotype, but not enough data existed in the literature to be able to designate the mutations as pathogenic. Ten patients were analyzed for the MM panel (30%). Causative mutations were found in three patients. The MFM panel was evaluated in 48 patients who had histological profiles in biopsied muscle tissue consistent with MFM. Causative mutations were found in 12 patients (25%). Overall, the diagnostic yield was 33% for all 188 patients. The authors noted that additional genes and data that might have changed some variant classifications were found after the analysis was complete, so panels need to be updated on an ongoing basis. Their final conclusion was that a NGS panel in combination with histological, mRNA, and protein analysis is useful and efficient for determining a genetic diagnosis in patients with muscle disease.

Five hundred and four patients and eighty-four family members from the Italian Network of Congenital Myopathies and the Italian Network of Limb-Girdle Muscular Dystrophy were studied by Savarese et al. (2016) using an NGS platform designated MotorPlex. MotorPlex is made up of 93 genes that are considered causes of nonsyndromic myopathies that typically cannot be diagnosed clinically. Eighty-five percent of the patients were Italian, and 60% were male. All patients were classified according to their primary clinical presentation as LGMD (51%), congenital myopathy (CM) (32%), distal myopathy (3.8%), isolated hyperCKemia (3.4%), metabolic myopathy (MM) (1.2%), or other (8.6%). Most cases were sporadic, but 96 were familial. Bioinformatic filters took into account population frequency and current variant annotation. Variants were further scrutinized based on clinical presentation, age of onset, and segregation analysis in family members when appropriate. As a result, 218 (43.3%) cases obtained a diagnosis, and 160 patients had candidate variants identified that were interesting, but unproven. LGMD genes were responsible in 115 patients. In 30% of diagnosed cases the phenotype was atypical for that gene, expanding the understanding of the disease phenotype. The authors noted that some of the unsolved cases could be due to variants in genes not yet identified as causing NMD, and that ancillary tests such as comparative genomic hybridization (CGH) to detect copy number variants may be a necessary subsequent step. The conclusion of the study was that next generation sequencing (NGS) may become a universal first tier step in diagnosing heterogeneous conditions such as NMD.

Clinical Practice Guidelines

American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM)

AANEM developed a position statement regarding the utility of genetic testing in neuromuscular disease (NMD) (Kassardjian et al. 2016). The goal of the statement was to generally endorse genetic testing as a component of diagnosing NMD, not to endorse a specific test or testing algorithm. The authors provided a consensus opinion from an expert panel that highlighted the benefits of genetic testing that included reduced time to diagnosis, avoidance of unnecessary testing, improved surveillance and monitoring, family testing and family planning, and better access to research and clinical trials. The authors note that recommendations and guidelines exist that direct the selection of appropriate genetic tests and referenced AANEM guidelines for limb-girdle muscular dystrophies (Narayanaswami et al. 2014; reaffirmed 2022), congenital muscular dystrophy (Kang et al. 2015; reaffirmed 2021) and facioscapulohumeral muscular dystrophy (Tawil et al. 2015; reaffirmed 2021).

Metabolic Myopathies

Metabolic refers to the chemical processes in the body that utilize nutrients and energy to provide healthy functioning and growth. Metabolic myopathies are genetic disorders in which the metabolic processes for the muscles have been interrupted and can result in muscle weakness, exercise intolerance, or muscle pain. There are three primary categories of metabolic myopathies that include glycogen-storage diseases (GSD), disorders of fatty oxidation, and mitochondrial myopathies (American College of Rheumatology, 2023 and Tarnopolsky, 2016).

Glycogen Storage Diseases

Glycogen storage diseases (GSDs) that may cause metabolic myopathies and have overlapping symptoms include GSD type 2 (Pompe disease), GSD type 3 (Debrancher Deficiency), GSD type 4 (Andersen's disease), GSD type 5 (McArdle's disease), GSD type 7 (Tarui disease), and GSD type 9 (Phosphorylase Kinase Deficiency). Identifying the correct diagnosis is important because some GSDs have treatment available, such as Late Onset Pompe Disease (Lilleker et al., 2018). Symptoms often start in the second or third decade of life with muscle cramps that occur during the first few minutes of exercise. Many individuals may not see their physician at the onset of symptoms because they avoid exercise or they modify exercise by starting off slow, then ramping up activity as aerobic metabolism takes over and blood born energy is delivered to the muscle. In particular, individuals with McArdle disease report that exercise gets easier after a few minutes of activity, known as the second wind-phenomenon, and feel better and less symptomatic after a high carbohydrate meal. Patients with other forms of metabolic

myopathies do not experience a second wind phenomenon and report that they feel worse with a high carbohydrate meal and better after fasting. Some patients will experience dark urine due to the presence of muscle derived proteins. The classic diagnostic test is a forearm exercise test included pre-and post-exercise measurements of lactic acid and ammonia. This has a very high sensitivity and specificity for the presence of a glycogenic defect, with the possible exception of phosphorylase b kinase deficiency, which can be further evaluated with an aerobic cycling test. Serum CK is usually elevated in McArdle disease but is typically normal in other glycogen storage diseases. EMG is often normal, and muscle biopsy may show high glycogen, absent phosphorylase, or absent phosphofructokinase. If these tests suggest McArdle syndrome, or muscle biopsy is suggestive of a particular GSD, targeted genetic testing is suggested to confirm the diagnosis. For example, on muscle biopsy, central cores suggest RYR1 or CACNA1S mutations, abnormal dystrophin staining suggests a dystrophinopathy, ragged red fibers point to a mitochondrial disorder, and membrane bound glycogen suggests Pompe disease. Otherwise, NGS panels may be beneficial in reaching a diagnosis (Lilleker et al., 2018 and Tarnopolsky, 2016).

Sniderman King et al. (2023) reported on data from the Lantern Project, a program offering diagnostic assistance to individuals with suspected Pompe disease and LGMD as well as other lysosomal storage and neuromuscular disorders. Included in this article was information specific to an acid α-glucosidase (GAA) enzyme assay as well as GAA sequencing and lastly, the Focused Neuromuscular Panel, which includes GAA. A total of 140 individuals in the project have been confirmed to have Pompe disease. The most common symptom reported at the time of testing was proximal muscle weakness (58 individuals) and elevated creatinine kinase (29 individuals) was the most common laboratory result. Molecular results supported diagnosis in 128 individuals. The authors assert that these findings further support the use of testing with enzymatic and genetic methods to aid in the diagnosis of Pompe disease and indicate that the use of multigene NGS panels allow the critical differentiation between Pompe disease and other LGMDs.

Johnson et al. (2017) utilized WES to determine the diagnostic yield of this technology for identifying LOPD in a cohort of 606 European patients with limb-girdle weakness. Their ages ranges from 4 to 88 years old and were 46% female and 56% male. WES from blood was performed by the Genomics Platform at the Broad Institute of Harvard and MIT, and variants were filtered using a list of 169 genes associated with limb-girdle weakness. The biological relevance of the variants identified within the GAA gene was determined by considering the population frequency, deleteriousness of the variant predicted by various bioinformatics tools, ClinVar reports of pathogenicity and the published literature. The authors reported that the overall diagnostic rate for all muscle disease was still under review at the time of publication but appeared to be 49% overall. Twelve cases of LOPD were identified in this study, in eight study participants and four siblings. Four of the ten gene variants found had not been reported previously. The authors noted that GAA activity levels are typically analyzed using dried blood spot analysis, but for the subset of patients with elevated creatinine kinase and limb-girdle muscle weakness, the testing was not accurate. Nearly 8% had abnormal GAA activity levels, but only 2.4% were confirmed to have LOPD. They also highlighted one case of a woman with symptom onset in her fifties who had normal, but slightly lower GAA activity on dried blood spot, but was found to have two known pathogenic GAA mutations. The authors concluded that NGS was beneficial in the diagnosis of LOPD and has the potential for earlier diagnosis and treatment over current approaches.

Mori et al. (2017) examined the analytical and clinical validity of WES for identifying early and late onset Pompe disease. The disease is treatable by enzyme replacement therapy, but optimal outcomes are dependent on a swift and accurate diagnosis, which is challenging in the late onset form. The authors analyzed WES data in 93 patients with confirmed Pompe disease and known GAA mutations identified by Sanger sequencing. WES accurately identified both GAA variants in 77 (83%) of patients. One variant was missed in 14 (15%) and both were missed in two (2%). One patient had a complex indel that was incorrectly identified by WES due to misalignment. The authors concluded that WES may not be the most accurate approach to diagnosing Pompe disease, and clinicians should consider more targeted and specific testing in individuals with myopathy, respiratory failure, or other subtle symptoms.

The genetic lab at Centro de Diagnóstico de Enfermedades Moleculares in Madrid, Spain, reported on its experience with NGS for GSD (Vega et al., 2016). Blood samples from 47 patients suspected of having a GSD were analyzed. Two methods were employed. Sixteen patients were analyzed using a panel of 111 GSD related genes. Twelve of these patients, plus an additional 39, were analyzed by the TrueSightOne gene panel which represents all of the known disease-causing genes described in the Online Inheritance of Man (OMIM) database as of 2013. Variants were filtered by population frequency, phenotype, and inheritance pattern. Genes with potentially pathogenic mutations were assessed in the context of the patient phenotype according to OMIM criteria. Variants that met these criteria were confirmed by Sanger sequencing. In the first testing group, five of 12 patients received a genetic diagnosis (30%). In the second group, 18 of 43 patients were found to have pathogenic mutations. Fourteen were in GSD related genes and four in non-GSD genes. Eleven mutations had never been reported before

and were confirmed through segregation analysis. The authors concluded that the combination of clinical findings, biochemical test results, and NGS can provide an efficient and accurate means of making a genetic diagnosis.

Lévesque et al. (2016) studied the clinical utility of a targeted NGS panel to diagnose Late Onset Pompe Disease (LOPD). Pompe disease is an autosomal recessive disease caused by a defect in the GAA gene, resulting in a deficiency of acid alpha-glucosidase. The classic infantile form presents early in life with general muscle weakness, cardiomyopathy, and respiratory distress. The disease is treatable with enzyme replacement therapy, but without treatment, it is a fatal disease. LOPD can present at any age after infancy with limb-girdle weakness but is most commonly identified in adulthood. Patients can also have rigid spine syndrome, scoliosis and low body mass, and nocturnal hypoventilation due to diaphragmatic weakness. Because of the low incidence of LOPD and the overlap of symptoms with other neuromuscular disease, this treatable condition is often not diagnosed until 10 years after the first onset of symptoms. The authors developed a NGS panel comprised of 77 genes representing muscle disorders with a clinical overlap with LOPD. Twenty Pompe patients with known mutations were used to determine the sensitivity of the assay, and all mutations were accurately identified. Positive gene results were confirmed by measuring GAA activity. GAA activity level was measured using tandem mass spectrometry, and 15 Pompe patients were used as positive quality controls and 49 healthy controls were used to establish normal GAA activity. This pilot study included 34 patients suspected of having an inherited muscle disorder, but in whom the etiology could not be determined. Seven pediatric patients and 27 adult patients were included. Most (71%) had undergone a muscle biopsy, and 15 (44%) had at least one single gene test performed, but still did not have a diagnosis. Using the NGS panel, a genetic diagnosis was found in 32% of patients. One case of LOPD was found, confirmed by GAA activity testing. The remaining cases were various forms of LGMD, including three patients with atypical presentations. The authors concluded that targeted muscle gene panels utilized as a first-tier diagnostic test might reduce the time to diagnosis. They also note that challenges exist with the high number of VUS identified and the limited performance of bioinformatics tools for analyzing copy number variants but anticipate that these issues will be resolved as NGS technology continues to advance.

Savarese et al. (2016) described the clinical validity of a targeted NGS panel (MotorPlex) for NMD in 504 patients with LGMD (51%), congenital myopathy (CM) (32%), distal myopathy (3.8%), isolated hyperCKemia (3.4%), and metabolic myopathy (MM) (1.2%) and other (8.6%). Within this subset of patients are 275 individuals with a clinical presentation of LGMD and hyperCKemia that includes LOPD within the differential diagnosis reported in a subsequent publication focusing on LOPD (Savarese et al., 2018). Ultimately, 16 patients from nine unrelated families were diagnosed with LOPD. All patients had the common c. 32 13T > G variant in the GAA gene with a second, already known mutation on the other allele. The symptoms in this cohort were primarily proximal weakness and fatigability. Exercise intolerance, myalgia, and contractures were less common. Some patients had atypical symptoms that likely confounded the clinical diagnosis, such as dysphagia, pseudohypertrophy, and calf hypertrophy. The authors concluded that with decreasing costs and technological improvements, NGS panels are likely to become important in first tier diagnostic testing in the near future.

Fatty Acid Oxidation (FAO) Disorders

Disorders of fatty acid oxidation (FAO) can result in three different presentations; hepatic, sudden infant death from hypoketotic hypoglycemia from catabolic events or cardiac disease, and a mild adult onset form. The hepatic form is severe, often lethal, and is triggered in the neonatal or infancy time period by a catabolic state, such as from frequent infections. Infants may also present with dilated or hypertrophic cardiomyopathy. These conditions may be treatable through dietary restriction of long chain triglycerides and supplementation of medium chain triglycerides, so are included in newborn screening programs. Diagnosis can be tricky, however, and may require in vivo loading tests using sunflower oil and phenylbutyrate or fasting tests. Mass spectrometry of the acyl carnitine pathway remains the gold standard for newborn screening and other diagnostic tests. Enzyme testing in lymphocytes can confirm the diagnosis, and genetic testing of the specific gene can identify the molecular problem (Houten et al., 2016). The adult onset or mild form presents with exercise-induced myalgia and may have pigmenturia within 24 hours of exercise due to rhabdomyolysis and delayed onset of muscle soreness. Symptoms may result from prolonged fasting, or prolonged exercise, especially if illness is present, too. In affected children it common to see pigmenturia during fever or fasting because of illness, or when vomiting. The exercise induced symptoms are not noted until their teen years. In these individuals, CK levels are usually normal except during rhabdomyolysis. Hyperkalemia and hypoketotic hypoglycemia can occur during rhabdomyolysis as well, and in some this might result in kidney failure. The best diagnostic test for individuals suspected of having a mild fatty oxidation disorder is a mass spectrometry analysis for acyl carnitine. A false negative can happen if the testing is performed during a non-stressed period. Targeted genetic testing can confirm a diagnosis based on the mass spectrometry results, and if acyl carnitine results are not informative, or targeted genetic testing is negative, panel genetic testing may yield additional information. For example, LPIN1 deficiency can cause rhabdomyolysis with fever or other illness, but does not cause exercise related symptoms (Tarnopolsky, 2016). In general, most FAO disorders are

diagnosed through mass spectrometry and other metabolic testing, but in some cases additional genetic testing, including exome or genome analysis, may help diagnosis unexpected phenotypes (Houten et al., 2016).

Valencia et al. (2016) examined the utility of a customized NGS panel of 26 genes in twelve pediatric patients with acute liver failure with elevated blood molar lactate/pyruvate of indeterminate etiology. The patients were selected from a retrospectively identified cohort of 74 individuals with acute liver failure because their fixed and frozen liver samples were available for additional analysis and had indeterminate etiology. The 26 genes included 15 nuclear genes involved in mitochondrial disorders, and six genes associated with FAO defects. Hepatic DNA was analyzed. Five patients were found to have significant genetic variants. Two patients had genetic variations in the RRM2B gene, not previously associated with acute liver failure. Both had patchy micro and macro-vesicular steatosis and reduced respiratory chain complex activity, and good post-liver transplant outcomes. One infant with severe lactic acidosis was a compound heterozygote for variants in ACAD9, associated with isolated complex I deficiency. Two patients had abnormal mitochondria by electron microscopy, and VUS in the POLG and DGUOK genes. Both had developed acute liver failure after drug exposure. The authors conclude that targeted NGS helped expand the understanding of genes involved in the spectrum of pediatric acute liver failure.

Sudden unexpected death in infancy (SUDI) is commonly attributed to accidents, infection, heart defects, child abuse, and metabolic disease. FAO disorders can lead to SUDI during long fasting or infection, and accounts for 5% of cases. Newborn screening allows for identification of disease before symptoms, but some may remain undetected. Yamamoto et al. (2015) examined the utility of NGS in SUDI post-mortem analysis in Japan for FAO disorders. Fifteen cases were selected where the infant did not have any identified cause of death after a typical post-mortem analysis. The ages ranged from 0 days to 11 months. All were born before 2014 and did not have screening by tandem mass spectrometry for FAO disease. DNA analysis was performed on preserved white blood cells and was a focused analysis of 13 genes associated with FAO disease. Positive results were confirmed by Sanger sequencing. One patient was found to have carnitine palmitoyltransferase (CPT) II deficiency. She was born full term and had no identified issues until she died at age 11 months after an episode of fever and vomiting. Histological examination of the liver resulted in a diagnosis of Reye's like syndrome due to the presence of diffuse and distinctive vacuoles, which were also found in the kidney and heart. Parental testing confirmed that her parents were carriers of CPT II deficiency. Four other infants were found to have at least one copy of a FAO related genetic variant that was predicted to alter protein function, consistent with being a carrier. The authors also tested post-mortem blood samples by tandem mass spectrometry and confirmed the case of CPT II deficiency. They also identified two other cases that had increases in the long-chain acylcarnitine but was considered to be a false positive by the authors because the genetic analysis was negative. The authors concluded based on this cohort that metabolic autopsy with NGS was valuable in evaluating the cause of SUDI.

Hereditary Ataxia

Ataxia is lack of muscle control or coordination of voluntary movements, and is a symptom found in a number of NMD. It is also the primary feature of a heterogenous group of disorders such as Friedreich's ataxia, and spinocerebellar ataxias 1, 2, 3, 6, 7 and 17. Over 40 genes have been implicated in ataxias. To understand the value of NGS in diagnosing genetic ataxias, Nemeth et al. (2013) identified 50 patients from unrelated families in the UK that did not have a genetic diagnosis for ataxia, where ataxia was their primary symptom. All patients had negative genetic testing for the gene expansion found in spinocerebellar ataxias 1–3, 6, 7 Friedreich's ataxia, and for mtDNA abnormalities. Multiple standard biochemical tests were run to rule out other metabolic diseases. Targeted NGS was performed for 118 genes, 42 of which were associated with the primary phenotype, and the remaining were considered good candidate genes based on their function. The overall detection rate was 18% and varied from 8.3% in those with an adult onset progressive disorder to 40% in those with a childhood or adolescent onset progressive disorder. Those that had an adolescent onset and a positive family history had a detection rate of 40%. The authors noted difficulties in variants interpretation which are being addressed with updated bioinformatics tools and the need to confirm positive variants with Sanger sequencing and functional testing. Some individuals who did not receive a genetic diagnosis may have variants in genes that were not included in the analysis, as the list of genes that are implicated in ataxia continues to grow.

In their 2021 publication, Benkirane et al. document their evaluation of the efficacy of molecular diagnoses of inherited ataxia and related diseases. In this study, the researchers analyzed 366 unrelated consecutive patients with ataxia or related disorders that had not yet been diagnosed by using clinical exome-capture sequencing. Analysis was performed via an in-house pipeline combining variant ranking and CNV searches. Variant interpretation was done according to ACMG/Association for Molecular Pathology (AMP) guidelines. A molecular diagnosis was established in 46% of the test subjects. In addition, 35 mildly affected patients with causative variants in genes classically associated with severe presentations were identified. Such cases were explained by hypomorphic variants and rarely suspected mechanisms such as C-termination truncations and translation reinitiation. Two genes, PEX10 and FASTKD2 are potential candidates for translation reinitiation, which accounted for mild

disease presentation. The authors concluded that a significant fraction of phenotypic overlap and clinical heterogeneity is explained by hypomorphic variants that are not readily predictable and difficult to identify.

Coutelier et al. (2018) assessed the analytical clinical validity of using exome targeted capture sequencing to detect mutations in genes associated with cerebellar ataxias (CA) in 319 patients from the Brain and Spine Institute, Salpetriere Hospital, Paris, France. Analysis took place between January 2014 and December 2016. Gender was equally male and female, and national origin was primarily French and European. Six clinical groups were recognized in 298 patients with full clinical information available: 1) pure ataxic phenotype (n = 62), 2) additional spastic component (spastic ataxia) (n = 100), 3) complex late-onset clinical picture with extrapyramidal signs (n = 30), 4) metabolic presentation with mitochondrial features or white matter changes (n = 70), 5) sensory ataxia (n = 19), or 6) CA with an oculomotor apraxia (AOA)–like presentation (n = 17). Consanguinity was noted in 101 of 298 patients. The 21 remaining patients were not classified. Variants were filtered to review only 209 genes associated with CA or HSP. Sanger sequencing was used to confirm variants with the read depth was below 10x, or if family members were available for segregation analysis. Pathogenic or likely pathogenic variants were achieved for 72 patients (22.6%), with an additional 19 (6.0%) harboring possibly pathogenic variants. The highest diagnostic rate was obtained for patients with an autosomal recessive CA with oculomotor apraxia–like phenotype (35.3%) or spastic ataxia (35.0%) and patients with onset before 25 years of age (31.3%). The set of 209 genes that included HSP allowed for 30% additional diagnoses.

Hadjivassiliou et al. (2017) prospectively examined 1500 patients presenting with cerebellar ataxia at the Sheffield Ataxia Centre in the United Kingdom over a period of twenty years. Each patient underwent extensive workups that were repeated at six months to yearly intervals. Baseline assessments included, but were not limited to, full blood count, erythrocyte sedimentation rate (ESR), vitamin B12, folate, vitamin E, copper, urea, electrolytes, thyroid function, anti-GAD antibodies, celiac testing, HLA typing, and immunoglobulin analysis. Genetic testing was limited to what was available at the time. Expanded NGS panels were available after June 2014. Mitochondrial testing was only performed when indicated. Overall, there were 295 patients with a familial ataxia, and 1205 patients with a sporadic form. In those with a familial ataxia, a genetic diagnosis was confirmed using genetic testing in 58% of patients. In those with sporadic ataxia, 13% were found to have a genetic diagnosis. Since June 2014, 146 patients had genetic testing using the NGS panel, of which 54 patients had a dominant family history, 17 a family history consistent with recessive inheritance, 33 had sporadic early onset ataxia and 42 had sporadic late onset ataxia. Positive results were found in 32% of patients. Of note, none of the patients with an episodic ataxia type 1 EA2 mutation had episodic ataxia, but had a progressive form. Additionally, four patients had a SPG7 mutation that helped identify a related phenotype of slurred speech, ataxia, mild spastic paraparesis, and proximal weakness. This led to the genetic testing of SPG7 in 58 additional individuals with the same phenotype. Twenty-eight of these (48%) were found to be positive. The author concluded that in their cohort the potential for a genetic diagnosis was present in 30% of cases, which included the 13% diagnosed with a genetic disease in the sporadic cases, and the familial cases. The diagnostic yield of NGS testing, when introduced, was 32% overall, but 46% in the cases with a dominant family history of disease. The authors also noted that in the sporadic ataxia group, if genetic testing was applied only to those who had other diagnoses ruled out, the diagnostic yield becomes 35%. Because genetic testing is expensive, the authors recommended a selection criterion to increase the diagnostic yield that included brain imaging, family history, routine lab tests, age of onset, physical exam, and then targeted genetic testing based on those results, if possible. If the targeted genetic testing is negative, or the other clinical tests do not point to a specific genetic diagnosis, then consider the NGS panel test.

Pyle et al. (2015). The WES approach was used to test 35 individuals from 22 families affected with ataxia, from 22 randomly selected families of white European descent. Test subjects were identified randomly through routine referrals to the regional neurogenetics service at Newcastle upon Tyne, England. Gender, age, and family history were not considered in selecting cases. Prior to inclusion, all had routine clinical investigations to exclude treatable causes of acquired ataxia, including brain MRI and CSF examination with oligoclonal band analysis. All had negative genetic testing for SCA1, 2, 3, 6, 7, 17, DRPLA, FXN, and FMR1 in adult males. The mean age was 25, and 14 were male. Family history suggested dominant inheritance in 11 individuals, and recessive inheritance in 14. No consanguinity was reported. CGH was performed in individuals who were thought to have recessive inheritance, but exome sequencing only found one causative mutation. Confirmed pathogenic variants were found in 9 of 22 probands. Possible pathogenic variants were found in five probands. The diagnostic yield was similar in patients of all age groups. Overall, the likely genetic diagnosis was found in 64% of families. Using the exome approach allows for the analysis of genes outside of the clinical context. In this cohort, this approach confirmed the diagnosis of Niemann Pick type C in two siblings with adult-onset ataxia who lacked the characteristic eye movement disorder seen in childhood. When a variant was found in an unexpected gene the authors used phenotypic and biochemical data to confirm the finding to reduce the risk of a false positive. Exome capture has limitations, noted by the authors. It does not provide complete

coverage of all coding regions of the genome, certain copy variants are not detectable, and some pathogenic variants will be found in non-coding regulatory regions.

Inherited Peripheral Neuropathies (IPN)

Inherited peripheral neuropathies (IPN) occur in about 1 in 2500 people. They are a heterogeneous group of disorders caused by over 90 genes. The main subtypes include Charcot-Marie-Tooth (CMT) disease, hereditary sensory and autonomic neuropathy (HSAN), hereditary motor neuropathy (HMN), and hereditary neuropathy with liability of pressure palsy (HNPP) (Antoniadi et al., 2015).

CMT is a common neuromuscular disorder affected 40 per 100,000 individuals. Classical symptoms include slow, progressive distal muscle weakness, muscle atrophy, and sensory loss over time in the distal limbs. Electrodiagnostic testing has been used to classify CMT as demyelinating or axonal. One gene can result in multiple phenotypes, and all forms of inheritance have been reported. Over 50 genes have been associated with CMT. Traditional Sanger sequencing is cost-prohibitive in investigating all the genes associated with CMT, making targeted NGS an attractive option. In a pilot study of 22 unrelated Chinese CMT patients, the common PMP22 duplication was analyzed first, and found in eight. The remaining individuals underwent target NGS analysis of 44 genes. Genetic variants were classified using ACMG standards. Eleven patients were found to have a total of ten possible pathogenic variants, including seven previously reported variants. The three novel variants identified underwent functional testing, and two were found to be likely pathogenic and one likely benign. The authors concluded that NGS has the potential to make a more rapid and precise diagnosis for CMT patients and that functional analysis of novel variants is critical (Li et al., 2016).

Examining the impact of targeted NGS panels on the molecular diagnosis of CMT disease in standard clinical practice and demonstrating the importance and limitations of the use of NGS related to the diagnosis of CMT was the focus of a retrospective study by Ceylan et al. (2023). Molecular techniques including multiplex ligation probe amplification (MLPA), NGS and WES were used to identify variations related to CMT disease. After molecular evaluation with MLPA, 25 of 64 individuals with suspected CMT disease (39%) were positively diagnosed. Duplication in PMP22 was seen in 14 participants and PMP22 deletion was seen in 11 participants. Fifty individuals had NGS with targeted gene panels specific to CMT and 36% had pathogenic or likely pathogenic variants. Lastly, five individuals with normal NGS results underwent WES; diagnostic yield for those who had WES was reported to be 80%. The authors determined that in this study, targeted NGS panel use was diagnostic in approximately one-third of participants after exclusion of PMP22 deletion/duplication assessment. They advocate for an algorithmic molecular approach for genetic evaluation along with genetic counseling and pedigree analysis and further study to uncover additional information related to the etiology of CMT.

IPNs have been associated with a variety of genomic variants which include large duplication and/or deletion and repeat expansion; this has made molecular diagnosis difficult. In a large case series, Ando et al. (2022) sought to pinpoint genetic features in a group of Japanese individuals with IPNs. Clinical information was obtained for 2,695 individuals with IPN in Japan; no individuals with a finding of PMP22 CNV were included in this case series. Several technologies were used for genetic evaluation, including DNA microarrays, NGS-based gene panels, WES, CNV analysis and RFC1 repeat expansion analysis. Overall, 909 individuals with suspected IPNs and pathogenic or likely-pathogenic variations were detected. For individuals with early-onset disease, MFN2 was found to be the most frequent finding. GJB1 and MPZ were most frequently identified as the cause of middle- and late-onset disease and GJB1 and MFN2 were most common in demyelinating and axonal subtypes. Overall, the most commonly identified genes linked to IPNs were MFN2, GJB1, MPZ and MME. CNVs in MPZ and FJB1 genes and RFC1 repeat expansions were also detected. The authors concluded that completing a comprehensive genetic evaluation for individuals with suspected IPNs revealed genetic origins in this case series. They recommend further study focused on clinical features and their relationship to genetic variations to continue to aid in development of best practices for assessment of affected individuals in order to obtain early diagnosis.

Volodarsky et al. (2021) performed genetic testing in a cohort of patients with suspected CMT with 34 related genes. The researchers used genetic sequencing and copy number analysis and identified a large number of pathogenic variants that were novel and of unknown significance. The 2517 patients resulted in a diagnostic yield of 15% in males and 21% in females. Vogt et al. (2020) evaluated genetic testing in patients who presented with neuropathy without a definite etiology in a neuromuscular clinic. The testing consisted of an inherited neuropathy panel (72-81 genes) by NGS. Of the 200 patients that were screened, 30 had pathogenic mutations and 83.3% of the positive mutations were in PMP22, TTR and GJB1. In four patients, the determination of pathogenic mutation altered the treatment management. Two of these patients were diagnosed with CMT1A and CMTX.

Between 2010 and 2015, patients with inherited neuropathies in the genetic neuropathy clinic at Newcastle Hospitals NHS Foundation in Northern England were evaluated by NGS or WES (Bansagi et al. 2017). Genetic testing to rule out common mutations for PMP22 deletion/duplication, MFN2, and GJB1 was performed for all patients, and neurological and electrophysiological tests were used to identify candidates. One hundred and five patients from 73 families were ultimately included in the study, including distal motor neuropathy (n = 64), axonal neuropathy (n = 16), or complex disease impacting the motor nerves (n = 25). Variant classification was based on the 2013 Association for Clinical Genetic Science Practice Guidelines. Twelve index patients were diagnosed from the NGS panel (26%) and 18 index patients from WES (45%). Candidate gene sequencing based on clinical presentation alone would have found a genetic diagnosis in only five of the 105 patients (4.7%). Overall, causative mutations were found in 26 of 73 families, resulting in a 35.6% detection rate. The diagnostic rate in the distal motor neuropathy group was 32.5%, which was higher than what was reported previously. Many of the positive genes were the same between the distal motor neuropathy and axonal neuropathy group, suggesting that disease classifications may need to change.

Nam et al. (2016) researched the clinical utility of a 73 IPN gene targeted NGS panel in 78 Korean families affected with IPN (89 affected and 46 unaffected individuals). Fifteen individuals were already known to have the common PMP22 duplication variant in CMT1. In addition, 300 health Korean controls were included in the analysis. Clinical information collected included age of onset, motor and sensory impairments, deep tendon reflexes, muscle atrophy, and nerve conduction studies. Variants were filtered using population frequency and pathogenicity scores from various bioinformatics tools. Putative causative variants were confirmed using Sanger sequencing. In the 15 individuals with a known PMP22 duplication, the gene panel results for read depth for PMP22 and TEKT3 in the duplicated region, when compared to 5 non-duplicated health controls, were significantly higher at 1.49 and 1.47, respectively. This demonstrates that this panel can detect the common CMT1 variant. In remaining study participants 15 pathogenic or likely pathogenic variants with identified in 25 patients from 17 families. Eight mutations had not been previously identified as pathogenic but segregated with disease in affected family members. In this remaining group, the diagnostic yield was 27%. If the common PMP22 duplication is taken into account, 32 of 78 families could be diagnosed using this panel.

Antoniadi et al. (2015) explored the clinical validity of a targeted NGS panel of 56 genes associated with IPN. From July 2013 to December 2014, 448 samples referred from neurologists (67%) or geneticists (33%) for evaluation were included that met clinical criteria. The criteria included idiopathic peripheral neuropathy with progressive weakness in hands/wrists, feet/ankles, or associated pes cavus or finger contractures, and/or peripheral sensory loss. Supportive nerve conduction studies were required, with an absence of other non-genetic causes or central nervous system involvement. Most patients (70%) were over the age of 18. Variants were classified using the Association of Clinical Genetics Science Practice Guidelines. Genetic diagnosis was made in 137 (31%) patients, involving 195 pathogenic variants in 31 genes. Nearly half of the diagnosed patients had a pathogenic variant in a gene not previously available for testing, or in a gene whose primary clinical association was not IPN. The authors conclude that this approach overcomes the limitations of a sequential single gene approach and is an efficient tool for obtaining a genetic diagnosis in IPN.

Clinical Practice Guidelines

American Academy of Neurology (AAN), American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM), and American Academy of Physical Medicine and Rehabilitation (AAMPR)

Distal symmetric polyneuropathy (DSP) is the most common variety of neuropathy. Since the evaluation of this disorder is not standardized, the available literature was reviewed to provide evidence-based guidelines regarding the role of laboratory and genetic tests for the assessment of DSP in a report of the AAN, AANMEM and AAMPR (England et al. 2009, reaffirmed 2022).

- Screening laboratory tests may be considered for all patients with polyneuropathy (Level C). Those tests that provide the highest yield of abnormality are blood glucose, serum B12 with metabolites (methylmalonic acid with or without homocysteine), and serum protein immunofixation electrophoresis (Level C). If there is no definite evidence of diabetes mellitus by routine testing of blood glucose, testing for impaired glucose tolerance may be considered in distal symmetric sensory polyneuropathy (Level C).
- Genetic testing should be conducted for the accurate diagnosis and classification of hereditary neuropathies (Level A). Genetic testing may be considered in patients with cryptogenic polyneuropathy who exhibit a hereditary neuropathy phenotype (Level C). Initial genetic testing should be guided by the clinical phenotype, inheritance pattern, and electrodiagnostic features and should focus on the most common abnormalities which are CMT1A duplication/HNPP deletion, Cx32 (GJB1), and MFN2 mutation screening.

- There is insufficient evidence to support routine genetic testing in cryptogenic polyneuropathy patients without a classical hereditary phenotype.

The majority of hereditary DSPs are variants of Charcot Marie Tooth (CMT) disease, which has a wide range of phenotypic expression, and can be caused by de novo mutations, so a family history may not always be informative. When a hereditary DSP is suspected, the authors recommend a stepwise approach based on family history and electrodiagnostic test results as follows:
- Electrodiagnostic testing
  - Demyelinating
    - Positive family history, autosomal dominant
      - PMP22 duplication, first tier
      - PMP22 and MPZ testing, second tier
      - EGR2 and LITAF testing, third tier
    - Positive family history, autosomal recessive
      - PRX and GDAP1 testing
    - Positive family history, X-linked
      - GJB1 testing
    - Negative or uninformative family history
      - PMP22 duplication and GJB1 testing, first tier
      - MPZ and PMP22 sequencing, second tier
      - EGR2, LITAF, PRX, and GDAP1 testing, third tier
  - Axonal
    - Positive family history, autosomal dominant
      - MFN2 testing, first tier
      - MPZ testing, second tier
      - RAB7,GARS, NEFL, and HSPB1 testing, third tier
    - Positive family history, autosomal recessive
      - GDAP1 testing
    - Positive family history, X-linked
      - GJB1 testing
    - Negative or uninformative family history
      - MFN2 and GJB1 testing, first tier
      - MPZ sequencing, second tier
      - RAB7,GARS, NEFL, HSPB1, and GDAP1 testing, third tier

The authors concluded that there was insufficient evidence to support routine genetic testing in cryptogenic polyneuropathy patients without a classical hereditary phenotype.

Hereditary Spastic Paraplegias (HSP)

Hereditary Spastic Paraplegias (HSP) is a group of genetic diseases characterized by spastic paralysis of the legs, typically caused by selective distal axonal degeneration. They are rare, chronic disorders that occur in about 1 to 9 in 100,000 people and present in childhood and young adulthood. The typical clinical picture is of a slowly progressive, symmetrical, spastic paraplegia. Minor sensory abnormalities (such as absent vibration sensation) and neurological bladder involvement, are common. Arm involvement is not usually seen, and if present, it is minimal and does not extend beyond hyperreflexia and minor weakness (e.g., difficulty unscrewing a tight bottle top). HSP is categorized into the subtypes of "pure" and "complex." The pure form is most common in European populations and can be autosomal dominant or recessive. The complex form is typically autosomal recessive and is more commonly found in populations with a high rate of consanguinity. Over 70 genes have been identified for HSP (Hensiek et al., 2015).

Iqbal et al. (2017) researched the use of NGS in the diagnosis of 105 hereditary ataxia (HA) and HSP probands identified through the HA and HSP registry in the Department of Neurology, Oslo University Hospital. HA and HSP have phenotypic overlap. HA is characterized by progressive limb and gait ataxia, loss of coordination and disturbances of speech and oculomotor control, and HSP is characterized by progressive spasticity and weakness of the lower limbs, the weakness often being mild relative to the spasticity. The HA/HSP registry has 446 probands, and 77 HSP and 41 HA individuals had a molecular diagnosis at the time of the study. Of those without a genetic diagnosis, 48 HSP and 58 HA patients were selected for

NGS, and eight individuals with a known diagnosis were included as a positive control. Variants were classified per the joint consensus recommendations of the ACMG and AMP and were confirmed by Sanger sequencing. The NGS panel identified all eight positive controls. In the test group, 12 HSP patients had pathogenic or likely pathogenic variants, and two had VUS. Eight HA patients had had pathogenic or likely pathogenic variants, and eight had VUS. Overall, 19% had a definitive molecular diagnosis.

Chrestian et al. (2016) conducted a multi-center observational study of patients who met the clinical criteria for the diagnosis of HSP in Alberta, Ontario and Quebec from 2012-2015 and reported on the genetic test results. Five hundred and twenty-six patients were identified with HSP during this time period. DNA testing was conducted on peripheral blood samples. Fifty-one families, representing 108 individuals, had WES, and the variants were filtered against all known HSP genes. Thirty-seven patients, a cohort from Ontario, had NGS of 51 HSP related genes. Patients with cerebellar signs were screened for mutations in FXN, SACS, and the common spinocerebellar ataxias (SCAs 1–8) prior to being included in the study. Overall, 150 (28.5%) of patients from 58 families had a confirmed genetic diagnosis. Mutations from 15 different genes were identified, and the most common were in SPAST (78%), ALT1 (16%), and SPG11 (8%). Genotype/phenotype correlations were noted with SPAST mutations (SPG4) and were more likely to have a later age of onset but also have bladder dysfunction. SPG11 mutations were more strongly associated with the presence of learning dysfunction and cognitive deficits.

Kara et al. (2016) investigated the genetic cause of disease in a series of 97 index cases with complex spastic paraplegia referred to a tertiary referral neurology center in London for diagnosis or management. Patients were enrolled who had clinical details and DNA available prior to 2015. Only the proband was included in the analysis where a family had more than one affected member. Inclusion criteria included slowly progressive HSP as the primary clinical finding, along with at least one other neurological feature; peripheral neuropathy, cognitive decline, epilepsy, skeletal/bony abnormalities, visual problems, parkinsonism, dystonia, or ataxia. Acquired and metabolic causes of HSP were ruled out. Patients were classified by symptoms as severe, moderate, or mild. Sanger sequencing of the SPG11 gene was conducted. In patients who were negative for SPG11 or had only one mutation identified, NGS was employed using the TrueSightOne platform of 4813 genes. Filtering and variant analysis focused on a subset of genes related to spastic paraplegia, neurodegeneration, ataxia, peripheral neuropathy, Parkinson's disease, and pallidopyramidal syndromes. Except for one case without available DNA, Sanger sequencing was used to confirm identified variants. A likely pathogenic variant was found in 48 of 97 patients (49%). Mutations in SPG11 were the most common, found in 30 patients. No copy number variants of SPG11 were identified with NGS. Numerous VUS were detected, which is a frequent problem with high throughput NGS studies.

Muscular Dystrophies

Congenital Muscular Dystrophies (CMD)

CMD are disorders of muscle weakness and hypotonia that have an age of onset in the first two years of life. The prevalence is variable and not all geographies have epidemiological data. In the European populations about 1 in 100,000 people are affected. Serum creatine kinase (CK) levels are often, but not always, elevated. Muscle biopsy usually shows abnormalities such as necrosis, regenerating fibers, variable fiber size, and increased permysial and enomysial connective tissue. The three major categories of CMD are collagenopathies, such as Ullrich CMD and Bethlem myopathy, merosinopathies (merosin-deficient CMD), and dystroglycanopathies. Collagenopathies involving muscular dystrophy are typically associated with the COL6A1, COL6A2, COL6A3 genes and have recessive as well as dominant forms of inheritance. The age of onset and severity can vary widely, but involves a combination of progressive muscle weakness, joint hypermobility, and contractures. Merosinopathies are also known as laminin α2 related CMDs are caused by defects in the LAMA2 gene and feature congenital weakness, elevated CK levels, and brain magnetic resonance imaging (MRI) evidence of white matter signal abnormalities. Dystroglycanopathies include Walker Warburg syndrome, muscle-eye-brain disease, Fukuyama congenital muscular dystrophy, and congenital muscular dystrophy 1C and 1D. Multiple genes are responsible for these disorders. Originally it was thought that each individual dystroglycanopathy was a distinct disorder that could be defined solely by clinical findings and mutations in a specific gene. However, it is now known that there can be significant phenotypic overlap in clinical findings and variable disease severity. Common symptoms remain, however, within dystroglycanopathies and typically have muscular dystrophy, elevated CK, abnormal muscle biopsy. Most have some degree of ocular and brain abnormality as well. (Kang et al., 2015; reaffirmed 2021; Bönnemann et al., 2014, Jobling et al., 2014 and Martin et al., 2005).

O'Grady et al. (2016) researched the use of a targeted NGS panel vs. candidate gene sequencing on CMD patients who were identified retrospectively and prospectively through clinical records and the Institute for Neuroscience and Muscle Research Biospecimen Bank. Patients were identified from a 35-year period, and were included for study if there was evidence of muscle

weakness and hypotonia in the first two years of life, and clinical features were consistent with CMD. Only the proband from a family was included when a sibling was identified in the cohort. Exclusion criteria included identification of structural changes in skeletal muscle diagnostic of a congenital myopathy, or if the case was identified from many years prior and re-contact could be considered insensitive. A total of 123 CMD patients were included. Patients underwent histological studies for laminin-a2, glycosylated a-dystroglycan, and collagen VI. Microarray analysis was performed, and candidate gene sequencing was driven by the histological classification and clinical phenotype and included analysis of FKRP, LARGE, POMT1, POMT2, FKTN, and POMGNT1, the 3 collagen VI genes, LAMA2, SEPN1, LMNA, DNM2, and ACTA1. This approach yielded a firm genetic diagnosis in 39 (32%) of patients, and two patients had a probable diagnosis. The remaining undiagnosed patients were offered additional genetic analysis. Targeted NGS was performed with a research based-45 gene panel, a commercial 336 gene panel, or WES. Twenty-eight patients, who were identified clinically from 1993 or later, consented to additional studies. Two patients had the 45 gene panel, four had the 336 gene panel, and one patient had both. The remaining 21 patients had WES. Eleven of this cohort had causative variants identified. Overall, 59 of the 123 (48%) probands had a genetic diagnosis established by this study. The authors felt this data supported NGS as a first-line tool for genetic evaluation of patients with CMD, with muscle biopsy reserved as a second-tier investigation.

Ceyhan-Birsoy et al. (2015) examined the role of WES in identifying the cause of CMD in five Turkish CMD patients from three families who presented with early-onset rapidly progressive weakness without brain or eye abnormalities. Variants were filtered and analyzed by population frequency and the use of various bioinformatics tools to predict pathogenicity. All patients were found to have causative variants. In Family A, a mutation in the DYSF gene was identified, and pathogenicity was confirmed through re-examination of muscle biopsy for the absence of dysferlin, and immunohistochemistry for the dysferlin antibody confirmed the diagnosis. Families B and C had compound heterozygous mutations in the FKTN and ISPD genes. These genes are typically associated with brain and eye involvement, but these patients did not have cognitive ocular abnormalities. The authors highlight that the diverse clinical spectrum of CMD may prevent the accurate selection of targeted genes for analysis, and that an unbiased approach such as WES may be the best diagnostic tool.

Clinical Practice Guidelines

American Academy of Neurology (AAN) and the American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM)

In many situations, CMD can be diagnosed clinically based on a characteristic phenotype, histological results, and other clinical tests (Kang et al., 2015; reaffirmed 2021). However, genetic diagnoses are beneficial to the patient, as they often enable physicians to provide more accurate prognoses and facilitate genetic counseling and family-planning discussions and may enable patients to become more aware of future clinical trials for which they may be eligible. In 2015 (reaffirmed 2021), Kang et al. published an evidence-based guideline for the AAN and AANEM that included the use of genetic testing in the evaluation and diagnosis of CMD, and make the following recommendations:
- When available and feasible, physicians might order targeted genetic testing for specific CMD subtypes that have well-characterized molecular causes (Level C).
- In individuals with CMD who either do not have a mutation identified in one of the commonly associated genes or have a phenotype whose genetic origins have not been well characterized, physicians might order whole-exome or whole genome sequencing when those technologies become more accessible and affordable for routine clinical use (Level C).

Limb Girdle Muscular Dystrophies (LGMD) and Myofibrillar Myopathies (MFM)

LGMD are a relatively rare group of diseases impacting up to .43 per 100,000 individuals. Incidence can vary by ethnicity (Narayanaswami et al., 2014; reaffirmed 2022). LGMD are characterized by proximal muscle weakness (shoulders, upper arms, pelvic area, and thighs), muscle wasting, and myopathic or dystrophic myopathological features (Kuhn et al., 2016). There are many subtypes of LGMD which can vary with age of onset, severity, and additional co-morbidities such as weakness of the heart muscles (MedlinePlus, 2019). There are at least thirty genes associated with LGMD; seven are autosomal dominant, and twenty-three are autosomal recessive (Kuhn et al., 2016). LGMD are classified according to inheritance pattern. LGMD1 are autosomal dominant, and LGMD2 are recessive. Further subtyping is delineated using a letter. In their most recent guidelines, the American Academy of Neurology (AAN) identified LGMD1A-LGMD1F, and LGMD2A-LGMD2S (Narayanaswami et al., 2014; reaffirmed 2022).

In a retrospective evaluation, Çavdarlı et al. (2023) assessed the diagnostic rate of a 47-gene, NGS-based panel (created by the research team) to identify genetic variations in a population of 146 individuals in Turkey (ages 6 months to 67 years) suspected to have a neuromuscular disorder based on clinical examination, laboratory findings and imaging. Individuals who had been

diagnosed with dystrophinopathy based on genetic evaluation of dystrophin by MLPA were excluded. The genes included in the panel targeted variations related to muscular dystrophy and myopathies that have been suggested for first-tier testing. Based on the study results, 67 individuals were found to have a genetic basis for their disorder, correlating to a diagnostic yield of 46%. Twenty-three genes showed variations associated with neuromuscular disorders; these included CAPN3(11), DYSF(9), DMD(8), SGCA(5), TTN(4), LAMA2(3), LMNA(3), SGCB(3), COL6A1(3), DES (2), CAV3(2), FKRP(2), FKTN(2), ANO5, COL6A2, CLCN1, GNE, POMGNT1, POMGNT2, POMT2, SYNE1, TCAP, and FLNC. Novel variants were identified in 16 genes. Indeterminate results were found in 27 participants, including those with VUS, only one heterozygous variant for an autosomal recessive disease and individuals with two variants in different genes. Based on the results of the study, the authors assert that targeted NGS testing is a viable option for molecular diagnosis of neuromuscular conditions such as muscular dystrophy and could reduce the need for WES.

Winckler et al. (2022) examined the diagnostic yield of an NGS panel made up of 39 genes to be used as a first-tier test for diagnosing individuals with genetic myopathies. This cross-sectional study took place in Brazil and included 51 cases where genetic myopathies were suspected based on clinical findings. In this study, the diagnostic yield of the NGS panel was found to be 52.9%; when candidate variants were included in the evaluation, the diagnostic yield increased to 60.8%. *LGMD* was identified in 12/25 individuals (48%), 7/14 individuals (50%) with congenital muscular diseases were identified and 7/10 (70%) with muscular dystrophy including prominent joint contractures. The researchers indicate that these results show that the customized NGS panel studied produced high diagnostic yields when used early in the exploration of gene-related myopathies, which could result in earlier diagnosis and potential treatments.

Töpf et al. (2020) established an international consortium, MYO-SEQ, aiming to aid in the workups and improve the diagnostic pathway for patients with limb-girdle muscle diseases and better understand the etiology of these rare diseases. The authors note that gene-by-gene and small panel testing strategies are typically dictated by phenotype, which leaves little room for the expansion of disease associations and new disease characterizations, and that NGS technologies have been integral in the diagnosis of some of the 955 distinct NMD. As of publishing date, 535 genes are known to be associated with these disorders. The researchers applied a sequential targeted exome sequencing to a large cohort of 1001 undiagnosed patients with proximal muscle weakness and/or elevated serum creatine kinase. Exomes were analyzed for variants in 429 genes that are associated with muscle conditions. Suspected pathogen variants were found in 52% of patients across 87 genes. Four-hundred one novel variants were detected and 116 of these were recurrent. Variants in CAPN3, DYSF, ANO5, DMD, RYR1, TTN, COL6A2, and SGCA together made up over half of the solved cases. Variants in newer disease genes, such as BVES and POGLUT1, were also found. The authors concluded that their data suggest exome sequencing as an appropriate method for pathogenic variant detection in patients where genetic muscle disease is suspected, focusing first on common disease genes and subsequently in rarer or newly characterized genes.

In the United Kingdom, individuals suspected to have LGMD are evaluated at a central clinical known as the UK LGMD clinic. Harris et al. (2017) reported that in this population a genetic diagnosis is achieved in 63% of patients using standard clinical approaches, and her team explored the use of WES to increase the diagnostic yield in the remaining third of patients. They examined 104 affected individuals from 75 families. Patients had already undergone targeted genetic testing with an average of eight genes screened, as well as other extensive clinical investigations such as muscle biopsy and electrodiagnostic testing. In some cases, the ongoing clinical analysis had taken place over a decade or longer without reaching a definitive diagnosis. The WES genetic variants were filtered and analyzed against a list of known muscle disease genes, and if no variants were found, the scope of variant analysis was widened to include variants in novel genes. The genetic findings, clinical features, muscle MRI and muscle biopsy results were then integrated at a multidisciplinary meeting to reach a consensus as to whether variants were likely to be disease causing. Overall, the WES group achieved a diagnostic yield of 37%. By comparison, 91 individuals from 84 families were tested using the standard genetic testing procedures in place in the clinic during the 24-month investigation period. This standard genetic testing group had a diagnostic yield of 33%. The authors concluded that earlier application of WES in the diagnostic pathway would reduce the time to diagnosis and may also reduce the costs incurred by ongoing investigations, as well as affording opportunities for detection of low-level mosaicism and novel disease gene identification.

Kuhn et al. (2016) examined the clinical utility of a NGS panel for LGMD in a group of fifty-eight German patients who were suspected to have a LGMD. The panel focused on 23 genes known to cause LGMD and 15 genes known to cause a similar phenotype. The age of onset ranged from 3 to 63 years of age. Four patients had autosomal dominant forms of disease, and sixteen patients had affected siblings, suggesting autosomal recessive. X-linked inheritance was most likely in two patients. The remaining patients were considered to have sporadic cases. All patients had a muscle biopsy that confirmed myopathic or dystrophic changes, but LGMD immunohistochemistry or immunoblotting was not possible on the remaining sample. NGS was

performed on the 38 targeted genes with an average 20X coverage. All pathogenic variants and VUS were confirmed by Sanger sequencing. Disease causing mutations that explained the phenotype were found in 19 of 58 patients (33%). In 28% of patients with autosomal recessive disease, only a single pathogenic mutation was found. Additional sequencing and copy number variant analysis on the relevant gene to identify another pathogenic mutation, consistent with recessive inheritance, was negative. VUS were found in 10% of patients, and the remainder had no mutations identified.

Monies et al. (2016) studied a NGS panel of 759 genes associated with neurological disorders in patients from 50 families presenting with muscle weakness affecting the pelvic girdle and shoulder, of which 36 had an autosomal recessive form of inheritance. These families were identified through the Neurosciences Clinic of King Faisal Specialist Hospital and Research Centre, Saudi Arabia. Variants were analyzed and classified using the ACMG and AMP guidelines. Thirty-eight families (76%) received a genetic diagnosis from this study. Thirty-four had LGMD related mutations, and four had novel genetic variants not usually associated with LGMD. Families with negative results had follow up WES, but no additional variants were found. The authors concluded that their panel was sensitive, cost-effective, and rapid; significantly assisting the clinical practice.

Ankala et al. (2015) reported on the design and validation of several NGS panels at Emory Genetics Lab (EGL), developed to help expedite the diagnosis of NMD, including LGMD. The authors report that in their experience, clinicians must go through an extensive diagnostic workup in order to determine a small gene list to pursue for NMD, and patients may opt out of the process before a diagnosis is finalized. A combination of a targeted NGS panel and a targeted CGH test to identify copy number variants may reduce the burden of invasive tests. From October 2009 to May 2014, the authors analyzed the data for LGMD single gene and NGS/CGH panels to determine the difference in clinical utility. Exome analysis was also compared for 20 random patients to determine how well the exome covered the NMD genes in the study. In this timeframe, 343 LGMD single gene tests were ordered which included 250 sequencing tests and 93 CGH tests. The diagnostic yield was 19% overall. It was very low for CGH, with only eight positives of 93 tested. Ninety-six patients had a LGMD eleven gene NGS panel with a diagnostic yield of 26%. Eighty-one patients had a broad NGS panel that covered 41 genes for NMD, including genes that can cause overlapping phenotypes with LGMD, Emery-Dreifuss muscular dystrophy (EDMD), metabolic myopathies, congenital myopathies, dystrophinopathies, and congenital disorders of glycosylation (CDG). The diagnostic yield of the NMD panel was 46%. The authors also compared WES to the LGMD and NMD panel. Based on the low coverage of WES for some key NMD genes, they concluded that WES would miss variants in five key LGMD genes, whereas the NMD panel would miss one.

Clinical Practice Guidelines

American Academy of Neurology (AAN) and the American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM)

When presented with a patient with a possible LGMD or other distal myopathies like MFM, the AAN and AANEM (Narayanaswami et al., 2014: reaffirmed 2022) recommend referring patients to a specialized neuromuscular center for evaluation, management, and diagnosis because of the complex nature of NMDs and the need for a multi-disciplinary team. They recommend utilizing an approach focused on a clinical evaluation to narrow down the possible forms of LGMD or other muscular dystrophies. Their evidence based review found that utilizing information such as pattern of muscle weakness, hypertrophy, or atrophy of certain muscle groups, cardiac or respiratory involvement, muscle biopsy findings, electromyogram (EMG) results, and creatinine kinase (CK) serum levels, can narrow down the differential to just a few disorders. Verification of the specific disorder through genetic testing is recommended, as this will direct the most efficient care path and identify necessary prophylactic interventions, such as the correct timing for placing a pacemaker, or the monitoring interval for cardiorespiratory function.

The following phenotype and genotyping recommendations (evidence level B, expert consensus based on moderate evidence) were provided for those with limb girdle weakness and probable autosomal dominant inheritance:
● If cardiomyopathy, respiratory involvement, EMG with myotonic or "pseudomyotonic" discharges, foot drop, and myofibrillar myopathy on muscle biopsy are present; test for mutations in the genes desmin (LGMD1E), myotilin (LGMD1A), DNAJB6 (LGMD1D), ZASP, filamin C, αB-crystallin, and titin.
● If rippling muscles and percussion-induced rapid contractions are present; test for mutations in the caveolin-3 gene (LGMD1C).
● If early humeroperoneal weakness, contractures (neck, elbows, knee, ankle), and cardiomyopathy are present; test for mutations in the lamin A/C gene (LGMD1B or AD-EDMD).
● If distal weakness, myotonic discharges on EMG, past or family history of Paget disease, frontotemporal dementia, or motor neuron disease are present; test for mutations in VCP (hIBMPFD).

- If no clinical features suggest a specific form of dystrophy, or if initial genetic testing is not informative, perform a muscle biopsy to direct further genetic testing (such as immunohistochemistry/immunoblotting for various sarcolemmal proteins, calpain-3, or features of myofibrillar myopathy) or to exclude an alternative diagnosis (e.g., a metabolic myopathy, mitochondrial myopathy, congenital myopathy, or inflammatory myopathy).

The following phenotype and genotyping recommendations (evidence level B, expert consensus based on moderate evidence) were provided for those with limb girdle weakness and probable autosomal recessive inheritance:
- If scapular winging but no calf hypertrophy, and normal cardiorespiratory function are present; test for mutations in calpain-3 (LGMD2A). Patients of English, French, Spanish, Italian, Portuguese, or Brazilian descent may have a higher pretest probability of this disorder.
- If calf atrophy and weakness (i.e., inability to stand on toes) are present; test for mutations in anoctamin-5 (LGMD2L) or dysferlin (LGMD2B).
- If the onset of symptoms is in the teens or early twenties or the patient is from Asia, clinicians should assess for dysferlin mutations first and, if negative, test for anoctamin-5 mutations. If the onset of symptoms is in the 30s or later or the patient is of English or northern European ancestry, clinicians should assess for anoctamin-5 mutations first and, if negative, test for dysferlin mutations.
- If muscle biopsy immunohistochemistry showing reduction in 90 α-, β-, γ-, or δ-sarcoglycans is present; test for mutations in the sarcoglycan genes, SGCA, SGCB, SGCG, and SGCD (LGMD2C–2F).
- If the patient is of Hutterite descent; test for mutations in TRIM32.
- If scapular winging, calf hypertrophy, and early cardiorespiratory involvement are present; test for mutations in FKRP.
- If mental retardation is present; test for mutations in genes that cause primary or secondary deficiency of α-dystroglycan, POMT1, POMT2, FKTN, FKRP, LARGE1, POMGNT1, and ISPDI genes (LGMD2K, LGMD2M, LGMD2N, LGMD2O, and LGMD2P).
- If epidermolysis bullosa or pyloric atresia; test for mutations in plectin, PLEC.
- If no other specific clinical features are identified, or the muscle biopsy does not inform genetic testing, clinicians should perform a dried blood spot test for α-glucosidase (acid maltase) deficiency or Pompe disease.

The following phenotype and genotyping recommendations (evidence level B, expert consensus based on moderate evidence) were provided for those with limb girdle weakness and probable X-linked inheritance:
- If male, perform testing for mutations in the dystrophin (DMD) gene.
- If female, test for DMD gene mutations or perform a muscle biopsy and immunostaining for dystrophin to assess for a mosaic pattern of staining. If positive, confirm diagnosis with DMD gene testing.

The following phenotype and genotyping recommendations (evidence level B, expert consensus based on moderate evidence) were provided for those with humeroperoneal weakness and probable autosomal dominant inheritance:
- If early cardiac involvement and no joint laxity are present; perform genetic testing for mutations in the lamin A/C gene (AD-EDMD, LGMD1B).
- If joint laxity, protuberant calcaneus, and no cardiac involvement are present; test for mutations in the collagen VI gene (Bethlem myopathy).

The following phenotype and genotyping recommendations (evidence level B, expert consensus based on moderate evidence) were provided for those with humeroperoneal weakness and probable autosomal recessive inheritance:
- If congenital onset, joint laxity, protuberant calcaneus, and no cardiac involvement are present; test for mutations in the collagen VI gene (Ullrich myopathy).

The following phenotype and genotyping recommendations (evidence level B, expert consensus based on moderate evidence) were provided for those with humeroperoneal weakness and probable X-linked inheritance:
- If joint laxity, protuberant calcaneus, and no cardiac involvement are present; test for mutations in the emerin (EMD) gene.

If humeroperoneal weakness and suspected muscular dystrophy with early cardiac involvement and no joint laxity are present, and there are no mutations found in the lamin A/C or emerin gene, clinicians should perform muscle biopsy to delineate characteristic abnormalities that direct further genetic testing (evidence level B, expert consensus based on moderate evidence).

If late adult onset of index finger and wrist extensor weakness, followed by atrophy and weakness of hand muscles, and muscle biopsy showing rimmed vacuoles are present; a diagnosis of Welander distal myopathy is most likely and should be confirmed through genetic testing for Welander myopathy (evidence level B, expert consensus based on moderate evidence).

The following phenotype and genotyping recommendations (evidence level B, expert consensus based on moderate evidence) were made for patients with suspected distal muscular dystrophy and probable autosomal recessive inheritance:
● If early onset of calf weakness is present; test for mutations in the anoctamin-5 and dysferlin genes.
● If early onset (< 30 years of age) of progressive foot drop is present in individuals who are of Japanese or Middle Eastern Jewish descent; test for GNE mutations (AR-hIBM).
● If none of the clinical features above are noted, clinicians should perform a muscle biopsy to direct further genetic testing.

In patients with muscular dystrophy who have proximal as well as distal weakness, clinicians should use specific clinical features (e.g., rippling muscles, cardiomyopathy, atrophy of specific muscle groups, irritability on EMG) and biopsy features (myofibrillar myopathy [MFM]), reduction of emerin immunostaining, presence of rimmed vacuoles) to guide genetic testing, which may include mutations in the genes causing the various forms of MFM; LGMD2B (dysferlin), LGMD2L (anoctamin-5), LGMD2J (titin), LGMD1C (caveolin-3), and EDMD (emerin and lamin A/C).

In patients with suspected muscular dystrophy in whom initial genetic testing, muscle biopsy, and dried blood spot test for Pompe disease do not provide a diagnosis, clinicians may obtain genetic consultation or perform parallel sequencing of targeted exomes, Whole-Exome Sequencing, Whole-Genome sequencing, or Next-Generation Sequencing to identify the genetic abnormality (Level C, expert consensus based on modest evidence).

Mitochondrial Disease

Mitochondria are organelles, and hundreds to thousands of mitochondria are found in each cell. Mitochondrial Myopathies (MM) are a group of genetic disorders with a primary defect in electron transport chain function, resulting in abnormal energy production from fat and carbohydrate oxidation pathways. Symptoms such as muscle weakness, muscle cramping, or pain often appear during periods where these pathways are relied upon most, such as endurance sports activities, illness, or periods of fasting. The mitochondria have their own DNA (mtDNA) that exists in a double stranded circle, and multiple copies of mtDNA can occur in each mitochondrion. mtDNA codes for about 37 genes, but the transcription, translation, and function of the mitochondrial DNA is dependent on a number of nuclear genes. Therefore, MM are a constellation of diseases that can have their root cause in either the mitochondrial or the nuclear DNA (Tarnopolsky 2016).

Because each cell can have multiple copies of normal and abnormal mitochondria, called heteroplasmy, mitochondrial based diseases are known to have a wide range of phenotypic expression. DNA testing can be challenging as a result, but the advent of NGS allows for better detection of heteroplasmy in blood, ranging from 1-10% depending on the methodology and tissue type (Parikh et al. 2015).

The prevalence of most types of MMs is unknown; however, mitochondrial disease is one of the most common groups of genetic diseases with a minimum prevalence of greater than 1 in 5000 in adults. Examples of MM with neuromuscular manifestations include, but are not limited to, the following:
● Kearns-Sayre syndrome (KSS) is a mitochondrial disorder characterized by the onset of progressive external ophthalmoplegia (PEO) younger than age 20, pigmentary retinopathy, heart block, and cerebellar ataxia. There is wide phenotypic expression, and some may experience myopathy, deafness, dysphagia, hypoparathyroidism, diabetes, and dementia (Chinnery, 2021).
● Chronic progressive external ophthalmoplegia (CPEO) is characterized by external ophthalmoplegia, bilateral ptosis, and mild proximal myopathy (Chinnery, 2021). This is often the canonical symptom representing mitochondrial disease. It can be caused by a mutation or large rearrangement of mitochondrial DNA that accumulate throughout life in the skeletal muscle and cause disease. Nuclear genes that interact with the mitochondria such as SPG7 have also been implicated in the disease (Pfeffer et al., 2014).
● Progressive external ophthalmoplegia (PEO) is part of a spectrum of disorders, including CPEO, and has an unknown prevalence. Similar disorders include ataxia neuropathy spectrum and KSS. They are typically clinical diagnoses that are made through history and examination. Imaging studies, blood and cerebral spinal fluid tests, electromyography of the limbs, and muscle biopsy can help refine the differential diagnosis if there is doubt. About 50% of PEO is inherited and caused by mutations in mtDNA and nuclear genes such as POLG1, POLG2, ANT1, Twinkle, RRM2B, DNA2, and OPA1.

The remaining 50% is sporadic, and mtDNA testing often shows an accumulation of a single large mtDNA deletion. The size of the deletion may be associated with disease severity (McClelland et al., 2016).

- MERRF (myoclonic epilepsy with ragged red fibers [RRF]) is a multisystem disorder with a childhood onset that presents with myoclonus followed by generalized epilepsy, ataxia, weakness, and dementia. Additional findings can include hearing loss, short stature, optic atrophy, cardiomyopathy, pigmentary retinopathy, and lipomatosis. Diagnosis is usually clinical and based on the presence of four primary features; myoclonus, generalized epilepsy, ataxia, and RRF identified in a muscle biopsy. Genetic testing can be used to confirm a diagnosis, and often the genetic variants in MERRF are found in white blood cells. However, because of mitochondrial heteroplasmy, it is possible that the mutation is not detectable in blood and another tissue type should be tested if blood is negative (DiMauro and Hirano, 2015).
- MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) is a multisystem disorder with onset typically in childhood although it can begin at any age. The first symptoms can be exercise intolerance or proximal limb weakness, followed by generalized tonic-clonic seizures, recurrent headaches, anorexia, and recurrent vomiting. Seizures manifest as stroke like episodes which may involve transient blindness or hemiparesis. Over time, the recurrent seizures may result in deafness, impaired motor abilities, and vision and intellectual capabilities. Diagnosis is usually clinical and based on presentation of stroke-like episodes, typically before age 40 years, encephalopathy with seizures and/or dementia and mitochondrial myopathy, evidenced by lactic acidosis and/or RRF on muscle biopsy. In addition, two of the three following symptoms are also required for diagnosis; normal early psychomotor development, recurrent headache, or recurrent vomiting. Genetic testing can be used to confirm a diagnosis (DiMauro and Hirano, 2013).
- Leigh syndrome has onset of symptoms in the first year of life, often after a viral infection. Clinical manifestations include hypotonia, spasticity, movement disorders, cerebellar ataxia, and peripheral neuropathy. Cardiomyopathy may occur as well. About 50% of children die by age 3 as a result of respiratory or cardiac failure. A diagnosis is usually accomplished clinically through characteristics features on brain imaging, typical neuropathologic changes, and similar symptoms in an affected sibling. The detection of a pathogenic variant in one of the 14 mitochondrial genes that are known to be involved in mtDNA-associated Leigh syndrome can confirm the diagnosis. (Thorburn et al., 2023).
- NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa) first appears in childhood or early adulthood and is characterized by proximal neurogenic muscle weakness with sensory neuropathy, ataxia, and pigmentary retinopathy. Learning disabilities may also be present. Diagnosis can be suspected through clinical means but may require DNA testing to confirm a diagnosis. The suggested approach is to look for two common variants that cause NARP in the MT-ATP-6 gene, and if negative consider mtDNA genome sequencing (Thorburn et al., 2023).

Select articles below review the experience of researchers and clinical labs with targeted NGS and WES and the clinical validity for diagnosing mitochondrial diseases.

In a retrospective, multi-center study, Wu et al. (2023) analyzed the diagnostic yield of dual genomic sequencing along with mitochondrial disease criteria (MDC) in a pediatric population. Enrollees included 503 children aged less than 18 years with an unknown neuromuscular disorder or a multisystem progressive disease that was suspected to be related to a mitochondrial abnormality. The children underwent dual genomic analysis and the results were evaluated in terms of potential relationship of the variants to clinical features seen in the children and also to previously reported clinical features, leveraging the ACMG classification of variants and segregation patterns. If the genes were associated with mitochondrial diseases in the literature, they were classified as "mitochondria-related." If not, they were classified as "non-mitochondria-related." Overall, causative variants were detected in 177/503 (35.2%) of participants. Mitochondrial-related variants were found in 46 individuals (9.1%). Of these. 25 individuals had nuclear DNA variants, 15 had mitochondrial DNA variants and six had dual genomic variants. When MDC was applied, 15.2% of individuals with mitochondrial-related variations were found "unlikely to have mitochondrial disorder." Additionally. 4.5% of individuals with non-mitochondrial related variants and 1.43% with no genetic abnormality were classified with MDC as "probably has a mitochondrial disorder." The authors propose using MDC to guide dual genomic sequencing, particularly in cases where an individual is assessed as "possibly having a mitochondrial disorder" and "probably having a mitochondrial disorder."

Mavraki et al. (2023) published United Kingdom consensus guidelines developed by a working group of clinical scientists from the NHS Highly Specialized Service followed by national laboratory consultation. The guidelines highlight the current technologies and methods recommended for evaluation of mitochondrial DNA and nuclear-encoded genes in individuals with suspected mitochondrial diseases. The guidelines state that nuclear gene analysis can be comprehensively performed through the use of multi-gene panels with NGS. Genes included in the panels are based on associated clinical phenotype data and may include a comprehensive mitochondrial disorder nuclear gene panels or targeted nuclear gene panels. Consultation with a genetic counselor is recommended to assist with determining testing recommended for family members.

In a 2018 article, Witters et al. detailed their retrospective study which aimed to validate the diagnostic value of mitochondrial disease criteria (MDC). The study included a multicenter cohort of patients with genetically confirmed primary mitochondrial disease (MD). A total of 136 patients were studied of which 91 had nuclear DNA (nDNA) mutations. Of these, 51 % had definite MD according to MDC, and 33% had probable MD. Muscle biopsy was performed in 63 (47%) of the patients as well. The researchers found that patients with (nDNA) mutations versus mitochondrial DNA mutations were younger (6.4 ±9.7 versus 19.5 ±17.3 years) and had higher MDC scores. Using a cutoff of 6.5/8 on the MDC scale, sensitivity to diagnose individuals with nDNA mutations was 72.5% and positive predictive value was 69.5%. In the group of subjects with nDNA mutations, the researchers found that whole exome sequencing was better able to diagnose patients with lower scores compared to Sanger sequencing; 7/8 individuals diagnosed with possible MD by MDC were diagnosed by whole exome sequencing. The authors concluded that MDC continue to be very useful in clinical diagnosis of MD, assisting with decision-making regarding muscle biopsy and aiding in interpretation of whole exome sequencing results.

Plutino et al. (2018) utilized NGS in a cohort of 80 patients who were clinically diagnosed with mitochondrial disease tests to determine the clinical validity of targeted panel approach to a genetic diagnosis. The patients were diagnosed through clinical, biochemical, and histological analysis. They included 24 children and 56 adults, 38 males and 32 females. Patients first underwent mtDNA testing, and if negative, a custom nuclear gene panel was run. Single deletions and point mutations in the mtDNA were identified using XL-PCR and NGS and confirmed by Southern blot. The custom panel was comprised of 281 genes known to be involved in mitochondrial disease and were analyzed by NGS and confirmed with Sanger sequencing. Variant filtering and interpretation focused on rare mutations that were predicted to be missense, frame-shift, stop-gain, stop-loss, or splice site variants. Pathogenic variants were found in mtDNA in the first step in one child and 14 adults. The remaining 65 patients had the targeted NGS panel and an additional five children and three adults achieved a genetic diagnosis for an overall diagnostic rate of 29%. The authors reviewed their panel to other studies involving WES and larger panels with reported diagnostic rates of 8-24% and concluded that larger gene panels are not necessary in mitochondrial diseases because of their high heterogeneity, the ongoing discovery of novel genes, and genes that may not appear to be related to mitochondrial function could lead to secondary respiratory chain deficiency.

Lilleker et al. (2018) suggested an approach to diagnosing identifying and diagnosing metabolic myopathies that focuses on the following priorities; identify those that might have a 'genuine' metabolic myopathy, determine clinically the most likely biochemical process, identify which patients need a further work up such as a muscle biopsy or genetic test, identify those patients with conditions for that have treatment available, and offer genetic counseling to the patient and family members, as appropriate. To meet these goals, the authors recommend obtaining a thorough history, which might include obtaining historical medical records to determine how symptoms and lab results change over time, developmental and exercise history. Some symptoms could be attributed by the patient or family to something normal, such as "growing pains," when in fact this is a subtle yet important clue to a diagnosis. It is important to ask specific and targeted questions to prevent missing possible symptoms. A physical exam, including neurological elements, is key. If a metabolic myopathy is suspected, the early involvement of an experienced multidisciplinary team will be important to help tailor further investigations and reduce the time to diagnosis and treatment. This team may help further rule out "pseudomyopathies" and recommend further CK testing, muscle biopsy, skin biopsy, exercise testing, or EMG. Enzymatic testing based on history, symptoms, and specialist analysis may lead to the diagnosis, or identify the most likely pathway that is affected in the patient. Genetic testing is proving to be a valuable tool in diagnosing metabolic myopathies, and targeted genetic testing can help confirm a diagnosis at this point. There is a shift towards NGS panel genetic testing as a first-tier diagnostic investigation of choice by some; however genetic testing sometimes produces results that are difficult to interpret. The authors conclude that the diagnostic workup and management of patients with metabolic myopathies is complex and early referral to a specialist neuromuscular multidisciplinary clinic is strongly recommended.

The Neurological Institute C. Besta in Milan, Italy reported on its experience using a combination of targeted NGS and WES in a cohort of 125 patients strongly suspected to have mitochondrial disease (Legati et al. 2016). The patients were divided into respiratory chain complex groups based on their histological findings, mtDNA testing, and biochemical testing results; complex I (n = 5), complex II (n = 18), complex III (n = 15), complex IV (n = 21), complex V (n = 5), multiple defects (n = 26), CoQ10 deficiency (n = 3), mtDNA deletions or depletion determined by Sanger sequencing (n = 8) and pyruvate dehydrogenase (PDH) complex defects (n = 14). DNA was extracted from blood and underwent targeted NGS sequencing for 132 genes associated with mitochondrial disease for all patients. Overall, targeted NGS found causative mutations in 19 patients (15%). Two were patients with defective complex I, two with defective complex II, two with defective complex III, two with defective complex IV, two with defective PDH complex, six with multiple defects, one with mtDNA depletion whereas two were biochemically undefined. Additionally, 27 patients had candidate genetic variants that were suspicious but not conclusively pathogenic. Ten

patients who had negative panel results were selected for WES based on the accuracy of family history, clinical description, parental consanguinity, and availability of other family members for testing. Variants found on WES were confirmed by Sanger sequencing. Six patients who had WES had pathogenic mutations (60%) that were confirmed by Sanger sequencing and family segregation studies. The authors concluded that the approach of a targeted NGS panel followed by WES in select patients was a powerful approach and predicted that if used as a first line test, the detection rate would be about 25%, but noted that the choice of which approach may be best for these disorders depends on several institutional factors, such as availability of funding, space, personnel, and bioinformatics expertise.

Pronicka et al. (2016) published their experience using WES to identify a genetic variant in 113 patients who appeared to have a mitochondrial disease when routine genetic testing was negative. Eighty-eight of the patients were tested retrospectively. The patients all scored high on the Mitochondrial Disease Criteria (MDC) scale and met at least one of the following criteria: neonatal onset, basal ganglia involvement, increased 3-methylglutaconic aciduria in urine, and additionally had genetic counseling. WES using TruSeqExome enrichment was performed. The preferred sample was muscle biopsy, but included fibroblast cultures, frozen tissue samples obtained from muscle or liver biopsy, or autopsy. Sixty-seven (59%) patients were positive for likely deleterious genetic variants in 49 different genes with a known disease link, including 47 patients who had changes in 31 mitochondrial related genes. The rate of WES positivity was directly correlated to the MDC score. The higher the score, the more likely it was a disease-causing variant was found, and it was also more likely to be a mitochondrial related gene. Overall, 40 patients had an MDC score above 4, and 90% had a pathogenic variant found. They found six rare mtDNA variants not typically included on targeted mtDNA mutation panels and reported disease causing variants in 13 genes that were not well understood at the time of publication and were not included on targeted mitochondrial gene panels. The authors reviewed their experience at their center in Poland from 1996-2013 using single gene Sanger sequencing on similar patients and reported that only eight disease causing genes were identified in that time. The authors also compared their results to similar studies in the literature using targeted NGS panels. They concluded that WES was a superior first line genetic test because not all mitochondrial disease genes are known, and targeted NGS may miss important mutations or genes.

The role of WES in the diagnosis of mitochondrial disease was explored by Wortmann et al. (2015). The Nijmegen Centre for Mitochondrial Disorders (NCMD) referred 109 patients between December 2011 and June 2013 for WES. Patients were included that met three criteria; high degree of suspicion of mitochondrial disease by the referring physician, absence of mtDNA deletions or point mutations, and absence of copy number variations by microarray. The most common symptoms reported were intellectual disability, developmental delay, myopathy/exercise intolerance, and mitochondrial dysfunction in muscle. Retrospectively the cohort was divided into high, medium, and low groups based on the clinical degree of suspicion of mitochondrial disease. Sequencing was performed and variants were filtered first to look at 238 genes known to be associated with mitochondrial disease. If no variants of interest were identified in this panel, the whole exome was examined. A pathogenic mutation explaining the clinical findings was identified in 42 (39%) patients. The highest diagnostic yield was in the high suspicion group, in which a genetic diagnosis was reached in 24 of 42 patients (57%). Sixteen had a mutation in a known mitochondrial disease gene on the targeted gene panel, and eight had a mutation in a gene outside of the targeted panel. For the medium risk group, four patients had a mutation on the targeted panel, and six had a mutation outside of the panel. In the low risk group, one patient had a mutation on the targeted mitochondrial panel, and seven had a genetic diagnosis outside of the panel. The authors noted that the genetic variants identified by WES were evaluated in relation to the available clinical, metabolic, neuroradiological and biochemical data, by a multidisciplinary team of metabolic pediatricians, clinical geneticists, and laboratory specialists. In several cases the diagnosis was only made after a specific sign or symptom of the patient was considered. The authors concluded that WES has an advantage over a targeted NGS panel because it allows for an analysis beyond mitochondrial genes if needed, but noted that at the time of publication, WES has poorer coverage than a targeted NGS panel.

Clinical Practice Guidelines
Mitochondrial Medicine Society (MMS)
There are no published consensus-based practice parameters that clinicians can utilize for initiating diagnosis or management of patients with mitochondrial diseases. In 2015, Parikh et al. reviewed the literature on mitochondrial disease and made the following consensus-based recommendations for the diagnosis and management of these patients:
● Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.

- Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling.
- Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m.3243A > G mutation.
- mtDNA deletion and duplication testing should be performed in cases of suspected mitochondrial disease via NGS of the mtDNA genome, especially in all patients undergoing a diagnostic tissue biopsy.
  - If a single small deletion is identified using polymerase chain reaction–based analysis, then one should be cautious in associating these findings with a primary mitochondrial disorder.
  - When multiple mtDNA deletions are noted, sequencing of nuclear genes involved in mtDNA biosynthesis is recommended.
- When a tissue specimen is obtained for mitochondrial studies, mtDNA content (copy number) testing via real-time quantitative polymerase chain reaction should strongly be considered for mtDNA depletion analysis because mtDNA depletion may not be detected in blood.
  - mtDNA proliferation is a nonspecific compensatory finding that can be seen in primary mitochondrial disease, secondary mitochondrial dysfunction, myopathy, hypotonia, and as a by-product of regular, intense exercise.
- When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease genes is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no known mutation is identified via known NGS gene panels, then WES should be considered.

U.S. Food and Drug Administration (FDA)

This section is to be used for informational purposes only. FDA approval alone is not a basis for coverage.

Laboratories that perform genetic tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988. More information is available at:
https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm124105.htm.
(Accessed June 20, 2023)

References

American College of Rheumatology. Metabolic Myopathies. Available at: https://rheumatology.org/patients/metabolic-myopathies. Updated February 2023. Accessed June 20, 2023.

Ando M, Higuchi Y, Yuan J, et al. Comprehensive genetic analyses of inherited peripheral neuropathies in Japan: making early diagnosis possible. Biomedicines. 2022 Jun 29;10(7):1546.

Ankala A, da Silva C, Gualandi F, et al. A comprehensive genomic approach for neuromuscular diseases gives a high diagnostic yield. Ann Neurol. 2015 Feb;77(2):206-14.

Antoniadi T, Buxton C, Dennis G, et al. Application of targeted multi-gene panel testing for the diagnosis of inherited peripheral neuropathy provides a high diagnostic yield with unexpected phenotype-genotype variability. BMC Med Genet. 2015 Sep 21;16:84.

Bansagi B, Griffin H, Whittaker RG, et al. Genetic heterogeneity of motor neuropathies. Neurology. 2017 Mar 28;88(13):1226-1234.

Barbosa-Gouveia S, Vázquez-Mosquera ME, González-Vioque E, et al. Rapid molecular diagnosis of genetically inherited neuromuscular disorders using next-generation sequencing technologies. J Clin Med. 2022 May 12;11(10):2750.

Benkirane M, Marelli C, Guissart C, et al. High rate of hypomorphic variants as the cause of inherited ataxia and related diseases: Study of a cohort of 366 families. Genet Med. 2021 Jul 7. Epub ahead of print.

Bönnemann CG, Wang CH, Quijano-Roy S, et al.; Members of International Standard of Care Committee for Congenital Muscular Dystrophies. Diagnostic approach to the congenital muscular dystrophies. Neuromuscul Disord. 2014 Apr;24(4):289-311.

Bowen BM, Truty R, Aradhya S, et al. SMA identified: Clinical and molecular findings from a sponsored testing program for spinal muscular atrophy in more than 2,000 individuals. Front Neurol. 2021 May 6;12:663911.

Çavdarlı B, Köken ÖY, Satılmış SBA, et al. High diagnostic yield of targeted next-generation sequencing panel as a first-tier molecular test for the patients with myopathy or muscular dystrophy. Ann Hum Genet. 2023 May;87(3):104-114.Ceyhan-Birsoy O, Talim B, Swanson LC, et al. Whole exome sequencing reveals DYSF, FKTN, and ISPD mutations in congenital muscular dystrophy without brain or eye involvement. J Neuromuscul Dis. 2015;2(1):87-92.

Ceylan GG, Habiloğlu E, Çavdarlı B, et al. High diagnostic yield with algorithmic molecular approach on hereditary neuropathies. Rev Assoc Med Bras (1992). 2023 Feb 10;69(2):233-239.

Chinnery PF. Primary Mitochondrial Disorders Overview. 2000 Jun 8 (Updated 2021 July 29). In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2023. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1224/. Accessed June 20, 2023.

Chrestian N, Dupré N, Gan-Or Z, et al. Clinical and genetic study of hereditary spastic paraplegia in Canada. Neurol Genet. 2016 Dec 5;3(1):e122.

Coutelier M, Hammer MB, Stevanin G, et al. Spastic Paraplegia and Ataxia Network. Efficacy of exome-targeted capture sequencing to detect mutations in known cerebellar ataxia genes. JAMA Neurol. 2018 May 1;75(5):591-599.

Efthymiou S, Manole A, Houlden H. Next-generation sequencing in neuromuscular diseases. Curr Opin Neurol. 2016 Oct;29(5):527-36. Erratum in: Curr Opin Neurol. 2018 Apr;31(2):232.

England JD, Gronseth GS, Franklin G, et al.; American Academy of Neurology; American Association of Neuromuscular and Electrodiagnostic Medicine; American Academy of Physical Medicine and Rehabilitation. Practice parameter: the evaluation of distal symmetric polyneuropathy: the role of laboratory and genetic testing (an evidence-based review). Report of the American Academy of Neurology, the American Association of Neuromuscular and Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. PM R. 2009 Jan; 1(1):5-13. Reaffirmed January 22, 2022.

Ghaoui R, Cooper ST, Lek M, et al. Use of whole-exome sequencing for diagnosis of limb-girdle muscular dystrophy: outcomes and lessons learned. JAMA Neurol 2015;72:1424–32.

Hadjivassiliou M, Martindale J, Shanmugarajah P, et al. Causes of progressive cerebellar ataxia: prospective evaluation of 1500 patients. J Neurol Neurosurg Psychiatry. 2017 Apr;88(4):301-309.

Harris E, Topf A, Barresi R, et al. Exome sequences versus sequential gene testing in the UK highly specialised Service for Limb Girdle Muscular Dystrophy. Orphanet J Rare Dis. 2017 Sep 6;12(1):151.

Haskell GT, Adams MC, Fan Z, et al. Diagnostic utility of exome sequencing in the evaluation of neuromuscular disorders. Neurol Genet. 2018 Feb 1;4(1):e212.

Hensiek A, Kirker S, Reid E. Diagnosis, investigation and management of hereditary spastic paraplegias in the era of next-generation sequencing. J Neurol. 2015 Jul;262(7):1601-12.

Houten SM, Violante S, Ventura FV, et al. The biochemistry and physiology of mitochondrial fatty acid β-oxidation and its genetic disorders. Annu Rev Physiol. 2016;78:23-44.

Iqbal Z, Rydning SL, Wedding IM, et al. Targeted high throughput sequencing in hereditary ataxia and spastic paraplegia. PLoS One. 2017 Mar 31;12(3):e0174667. Erratum in: PLoS One. 2017 Oct 12;12 (10):e0186571.

Jobling R, D'Souza R, Baker N, et al. The collagenopathies: review of clinical phenotypes and molecular correlations. Curr Rheumatol Rep. 2014 Jan;16(1):394.

Johnson K, Töpf A, Bertoli M, et al. Identification of GAA variants through whole exome sequencing targeted to a cohort of 606 patients with unexplained limb-girdle muscle weakness. Orphanet J Rare Dis. 2017 Nov 17;12(1):173.

Kang PB, Morrison L, Iannaccone ST, et al. Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Evidence-based guideline summary: evaluation, diagnosis, and management of congenital muscular dystrophy: Report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Neurology. 2015 Mar 31;84(13):1369-78. Reaffirmed September 2021.

Kara E, Tucci A, Manzoni C, et al. Genetic and phenotypic characterization of complex hereditary spastic paraplegia. Brain. 2016;139(7):1904-1918.

Kassardjian CD, Amato AA, Boon AJ, et al.; AANEM Professional Practice Committee. The utility of genetic testing in neuromuscular disease: A consensus statement from the AANEM on the clinical utility of genetic testing in diagnosis of neuromuscular disease. Muscle Nerve. 2016 Dec;54(6):1007-1009.

Kuhn M, Glaser D, Joshi PR, et al. Utility of a next-generation sequencing-based gene panel investigation in German patients with genetically unclassified limb-girdle muscular dystrophy. J Neurol 2016;263:743–50.

Legati A, Reyes A, Nasca A, et al. New genes and pathomechanisms in mitochondrial disorders unraveled by NGS technologies. Biochim Biophys Acta. 2016 Aug;1857(8):1326-1335.

Lévesque S, Auray-Blais C, Gravel E, et al. Diagnosis of late-onset Pompe disease and other muscle disorders by next-generation sequencing. Orphanet J Rare Dis. 2016 Jan 25;11:8.

Li LX, Zhao SY, Liu ZJ, et al. Improving molecular diagnosis of Chinese patients with Charcot-Marie-Tooth by targeted next-generation sequencing and functional analysis. Oncotarget. 2016 May 10;7(19):27655-64.

Lilleker JB, Keh YS, Roncaroli F, et al. Metabolic myopathies: a practical approach. Pract Neurol. 2018 Feb;18(1):14-26.

Martin PT. The dystroglycanopathies: the new disorders of O-linked glycosylation. Semin Pediatr Neurol. 2005;12(3):152-8.

Mavraki E, Labrum R, Sergeant K, et al. Genetic testing for mitochondrial disease: the United Kingdom best practice guidelines. Eur J Hum Genet. 2023 Feb;31(2):148-163.

McClelland C, Manousakis G, Lee MS. Progressive external ophthalmoplegia. Curr Neurol Neurosci Rep. 2016 Jun;16(6):53.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); [updated 2020 Jun 24]. Limb-girdle muscular dystrophy; [updated 2019 Sept 1]. Available at: https://medlineplus.gov/genetics/condition/limb-girdle-muscular-dystrophy/. Accessed June 20, 2023.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); [updated 2020 Jun 24]. What are whole exome sequencing and whole genome sequencing?; [updated 2021 Jul 28]. Available at: https://medlineplus.gov/genetics/understanding/testing/sequencing/. Accessed June 20, 2023.

Monies D, Alhindi HN, Almuhaizea MA, et al. A first-line diagnostic assay for limb-girdle muscular dystrophy and other myopathies. Hum Genomics. 2016 Sep 27;10(1):32.

Mori M, Haskell G, Kazi Z, Zhu X, et al. Sensitivity of whole exome sequencing in detecting infantile- and late-onset Pompe disease. Mol Genet Metab. 2017 Dec;122(4):189-197.

Nam SH, Hong YB, Hyun YS, et al. Identification of genetic causes of inherited peripheral neuropathies by targeted gene panel sequencing. Mol Cells. 2016 May 31;39(5):382-8.

Narayanaswami P, Weiss M, Selcen D. et al.; Guideline Development Subcommittee of the American Academy of Neurology; Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Neurology. 2014 Oct 14;83(16):1453-63. Reaffirmed April 30, 2022.

Németh AH, Kwasniewska AC, Lise S, et al. Next generation sequencing for molecular diagnosis of neurological disorders using ataxias as a model. Brain. 2013 Oct;136(Pt 10):3106-18.

Nicolau S, Milone M, Liewluck T. Guidelines for genetic testing of muscle and neuromuscular junction disorders. Muscle Nerve. 2021 Sep;64(3):255-269.

Nishikawa A, Mitsuhashi S, Miyata N, et al. Targeted massively parallel sequencing and histological assessment of skeletal muscles for the molecular diagnosis of inherited muscle disorders. J Med Genet. 2017 Feb;54(2):104-110.

O'Grady GL, Lek M, Lamande SR, et al. Diagnosis and etiology of congenital muscular dystrophy: We are halfway there. Ann Neurol. 2016 Jul;80(1):101-11.

Parikh S, Goldstein A, Koenig et al. Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. Genet Med. 2015 Sep;17(9):689-701.

Pfeffer G, Gorman GS, Griffin H, et al. Mutations in the SPG7 gene cause chronic progressive external ophthalmoplegia through disordered mitochondrial DNA maintenance. Brain. 2014 May;137(Pt 5):1323-36.

Piluso G, Dionisi M, Del Vecchio Blanco F, et al. Motor chip: a comparative genomic hybridization microarray for copy-number mutations in 245 neuromuscular disorders. Clin Chem. 2011 Nov;57(11):1584-96.

Plutino M, Chaussenot A, Rouzier C, et al. Targeted next generation sequencing with an extended gene panel does not impact variant detection in mitochondrial diseases. BMC Med Genet. 2018 Apr 7;19(1):57.

Pronicka E, Piekutowska-Abramczuk D, Ciara E, et al. New perspective in diagnostics of mitochondrial disorders: two years' experience with whole-exome sequencing at a national paediatric centre. J Transl Med. 2016 Jun 12;14(1):174.

Pyle A, Smertenko T, Bargiela D, et al. Exome sequencing in undiagnosed inherited and sporadic ataxias. Brain. 2015 Feb;138(Pt 2):276-83.

Savarese M, Di Fruscio G, Torella A, et al. The genetic basis of undiagnosed muscular dystrophies and myopathies: Results from 504 patients. Neurology. 2016 Jul 5;87(1):71-6. Erratum in: Neurology. 2018 Jun 5;90(23):1084. Erratum in: Neurology. 2019 Aug 20;93(8):371.

Savarese M, Torella A, Musumeci O, et al. Targeted gene panel screening is an effective tool to identify undiagnosed late onset Pompe disease. Neuromuscul Disord. 2018 Jul;28(7):586-591.

Schuermans N, Verdin H, Ghijsels J, et al.; for Program for Undiagnosed Rare Diseases (UD-PrOZA). Exome sequencing and multigene panel testing in 1,411 patients With adult-onset neurologic disorders. Neurol Genet. 2023 Apr 26;9(3):e200071.

Sniderman King L, Pan Y, Nallamilli BRR, et al. Pompe disease ascertained through The Lantern Project, 2018-2021: Next-generation sequencing and enzymatic testing to overcome obstacles to diagnosis. Mol Genet Metab. 2023 May;139(1):107565.

Tarnopolsky MA. Metabolic Myopathies. Continuum (Minneap Minn). 2016 Dec;22(6, Muscle and Neuromuscular Junction Disorders):1829-1851.

Tawil R, Kissel JT, Heatwole C, et al. Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology; Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Evidence-based guideline summary: Evaluation, diagnosis, and management of facioscapulohumeral muscular dystrophy: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Neurology. 2015 Jul 28;85(4):357-64. Reaffirmed September 18, 2021.

Thorburn DR, Rahman J, Rahman S. Mitochondrial DNA-associated Leigh Syndrome and NARP. 2003 Oct 30 [Updated 2023 May 4]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2023. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1173/. Accessed June 20, 2023.

Töpf A, Johnson K, Bates A, et al. Sequential targeted exome sequencing of 1001 patients affected by unexplained limb-girdle weakness. Genet Med. 2020 Sep;22(9):1478-1488.

Valencia CA, Wang X, Wang J, et al. Deep sequencing reveals novel genetic variants in children with acute liver failure and tissue evidence of impaired energy metabolism. PLoS One. 2016 Aug 2;11(8):e0156738.

Vega AI, Medrano C, Navarrete R, et al. Molecular diagnosis of glycogen storage disease and disorders with overlapping clinical symptoms by massive parallel sequencing. Genet Med. 2016 Oct;18(10):1037-43.

Vogt B, Chahin N, Wiszniewski W, et al. Screening for genetic mutations in patients with neuropathy without definite etiology is useful. J Neurol. 2020 May 12. Epub ahead of print.

Volodarsky M, Kerkhof J, Stuart A, et al. Comprehensive genetic sequence and copy number analysis for Charcot-Marie-Tooth disease in a Canadian cohort of 2517 patients. J Med Genet. 2021 Apr;58(4):284-288.

Westra D, Schouten MI, Stunnenberg BC, et al. Panel-based exome sequencing for neuromuscular disorders as a diagnostic service. J Neuromuscul Dis. 2019;6(2):241-258.

Winckler PB, Chwal BC, Dos Santos MAR, et al. Diagnostic yield of multi-gene panel for muscular dystrophies and other hereditary myopathies. Neurol Sci. 2022 Jul;43(7):4473-4481.

Winder TL, Tan CA, Klemm S, et al. Clinical utility of multigene analysis in over 25,000 patients with neuromuscular disorders. Neurol Genet. 2020 Mar 9;6(2):e412.

Witters P, Saada A, Honzik T, et al. Revisiting mitochondrial diagnostic criteria in the new era of genomics. Genet Med. 2018 Apr;20(4):444-451.

Wortmann SB, Koolen DA, Smeitink JA, et al. Whole exome sequencing of suspected mitochondrial patients in clinical practice. J Inherit Metab Dis. 2015 May;38(3):437-43.

Wu L, Brady L, Shoffner J, et al. Next-generation sequencing to diagnose muscular dystrophy, rhabdomyolysis, and hyperckemia. Can J Neurol Sci. 2018 May;45(3):262-268.

Wu TH, Peng J, Yang L, et al. Use of dual genomic sequencing to screen mitochondrial diseases in pediatrics: a retrospective analysis. Sci Rep. 2023 Mar 14;13(1):4193.

Yamamoto T, Mishima H, Mizukami H, et al. Metabolic autopsy with next generation sequencing in sudden unexpected death in infancy: Postmortem diagnosis of fatty acid oxidation disorders. Mol Genet Metab Rep. 2015 Oct 2;5:26-32.

Policy History/Revision Information

Date	Summary of Changes
11/01/2023	**Coverage Rationale** ● Added list of examples for mitochondrial disease: 　○ Kearns-Sayre syndrome (KSS) 　○ Leber hereditary optic neuropathy (LHON) 　○ Leigh syndrome 　○ Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome **Documentation Requirements** ● Updated list of CPT codes with associated documentation requirements; added 81448 **Applicable Codes** ● Updated list of applicable CPT codes: 　○ Added 0417U* and 81448 (*quarterly edit) 　○ Revised description for 81479 **Supporting Information** ● Updated *Clinical Evidence* and *References* sections to reflect the most current information ● Archived previous policy version 2023T0598G

Instructions for Use

This Medical Policy provides assistance in interpreting UnitedHealthcare standard benefit plans. When deciding coverage, the member specific benefit plan document must be referenced as the terms of the member specific benefit plan may differ from the standard plan. In the event of a conflict, the member specific benefit plan document governs. Before using this policy, please check the member specific benefit plan document and any applicable federal or state mandates. UnitedHealthcare reserves the right to modify its Policies and Guidelines as necessary. This Medical Policy is provided for informational purposes. It does not constitute medical advice.

This Medical Policy may also be applied to Medicare Advantage plans in certain instances. In the absence of a Medicare National Coverage Determination (NCD), Local Coverage Determination (LCD), or other Medicare coverage guidance, CMS allows a Medicare Advantage Organization (MAO) to create its own coverage determinations, using objective evidence-based rationale relying on authoritative evidence (Medicare IOM Pub. No. 100-16, Ch. 4, §90.5).

UnitedHealthcare may also use tools developed by third parties, such as the InterQual® criteria, to assist us in administering health benefits. UnitedHealthcare Medical Policies are intended to be used in connection with the independent professional medical judgment of a qualified health care provider and do not constitute the practice of medicine or medical advice.