THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Congenital Myopathies

Congenital myopathies are typically characterized by the presence of specific structural and histochemical features on muscle biopsy and clinical presentation can include congenital hypotonia, muscle weakness, delayed motor milestones, feeding difficulties, and facial muscle involvement (1). Serum creatine kinase may be normal or elevated. Heterogeneity in presenting symptoms can occur even amongst affected members of the same family. Congenital myopathies can be divided into three main clinicopathological defined categories: nemaline myopathy, core myopathy and centronuclear myopathy (2).

Nemaline Myopathy

Nemaline Myopathy is characterized by weakness, hypotonia and depressed or absent deep tendon reflexes. Weakness is typically proximal, diffuse or selective, with or without facial weakness and the diagnostic hallmark is the presence of distinct rod-like inclusions in the sarcoplasm of skeletal muscle fibers (3).

Core Myopathy

Core Myopathy is characterized by areas lacking histochemical oxidative and glycolytic enzymatic activity on histopathological exam (2). Symptoms include proximal muscle weakness with onset either congenitally or in early childhood. Bulbar and facial weakness may also be present. Patients with core myopathy are typically subclassified as either having central core disease or multiminicore disease.

Centronuclear Myopathy

Centronuclear Myopathy (CNM) is a rare muscle disease associated with non-progressive or slowly progressive muscle weakness that can develop from infancy to adulthood (4, 5). On muscle histopathology, patients with CNM have increased frequency of central nuclei, as well as type 1 fiber predominance and hypotrophy, in the absence of other significant abnormalities. Other neuromuscular conditions can have similar findings on muscle biopsy, so these features are not always diagnostic for CNM.

Our Congenital Myopathy Sequencing Panel and Congenital Myopathy Deletion/Duplication Panel include analysis of the 28 genes listed below.

Congenital Myopathy Panel				
ACTA1	*COL12A1*	*LMOD3*	*PTPLA*	*TNNT1*
BIN1	*DNM2*	*MEGF10*	*RYR1*	*TPM2*
CFL2	*HRAS*	*MTM1*	*SCN4A*	*TPM3*
CCDC78	*KBTBD13*	*MYF6*	*SEPN1*	*TTN*
CNTN1	*KLHL40*	*MYH7*	*SPEG*	
CHKB	*KLHL41*	*NEB*	*STAC3*	

Genes and Associated Disorder	Inheritance	Clinical Features/Molecular Pathology
ACTA1 [OMIM#102610] Myopathy, Nemaline 3 [OMIM#161800]	AR/AD	Mutations in *ACTA1* account for approximately 15-25% of patients with Nemaline Myopathy (3). Mutations in *ACTA1* account for less than 6% of patients with Congenital Fiber-Type Disproportion (6). Nonsense, frameshift, missense, splicing and indels have all been reported in the *ACTA1* gene (7). The *ACTA1* gene encodes skeletal muscle alpha-actin, the principal actin isoform in adult skeletal muscle.
CFL2 [OMIM#601443] Myopathy, Nemaline 7 [OMIM#610687]	AR	Mutations in *CFL2* are a rare cause of nemaline myopathy. Agrawal *et al* 2007 screened a total of 171 patients with nemaline myopathy/congenital myopathy and identified a one homozygous missense change in 2 sibs from a consanguineous Middle Eastern family (8). The *CFL2* gene encodes a skeletal-muscle-specific isoform localized to the thin filaments where it interacts with tropomyosins.

CHKB [OMIM#612395] Muscular dystrophy, congenital, megaconial type	AR	Homozygous and compound heterozygous mutations in *CHKB* are associated with a recessive form of congenital muscular dystrophy characterized by early-onset muscle wasting and intellectual disability. Muscle biopsy in affected patients shows enlarged mitochondria at the periphery of the fibers which are sparse towards the center (9).
COL12A1 [OMIM#120320] Bethlem myopathy 2	AD	A heterozygous missense mutation in the *COL12A1* gene was identified in a patient with Bethlem myopathy and normal collagen VI. After this mutation was identified a cohort of 24 patients with a Bethlem myopathy-like phenotype were tested, with identification of mutations in the *COL12A1* gene in 5 members of 2 families, with the mutation segregating with the disease. (10) Individuals with Bethlem myopathy are typically affected with loose joints, infantile hypotonia, and contractures of the fingers, wrists, elbows, and ankles presenting in childhood. Progressive muscle weakness typically leads to the need for walking assistance by the age of 50, and older individuals may develop respiratory complications.
HRAS [OMIM#190020] Congenital myopathy with excess of muscle spindles	AD	*HRAS*-related congenital myopathy represents an autosomal dominant variant form of Costello syndrome. Patients with this condition present with Noonan syndrome-like features, hypertrophic cardiomyopathy, and an excess of muscle spindles noted on muscle biopsy (11).
KBTBD13 [OMIM#613727] Myopathy, Nemaline 6 [OMIM609273]	AD	Sambuughin *et al,* 2010, identified 3 different missense mutations in *KBTBD13* in patients with nemaline myopathy (12). KBTBD13 is expressed in the skeletal muscle and cardiac muscle, although to date no cardiac abnormalities have been identified in individuals with *KBTBD13* mutations.
KLHL40 [OMIM#615340] Myopathy, nemaline 8 [OMIM#615348]	AR	Ravenscroft et al, 2013, identified 19 mutations in *KLHL40* in 28 unrelated kindreds with severe autosomal recessive nemaline myopathy. Clinical features included fetal akenisia and contractures, fractures, respiratory failure and swallowing difficulties at birth (13).
KLHL41 [OMIM#607701] Myopathy, nemaline 9 [OMIM#615731]	AR	Gupta VA, *et al* 2013, identified biallic deletions and missence mutations in 4 individuals with nemaline myopathy. Genotype-phenotype correlation was seen in these families, with the framshift mutations resulting in a severe phenotype with early death, and the missense mutations resulting in impaired motor function with survival into late childhood and/or early adulthood (14)
MEGF10 [OMIM#612453] Myopathy, areflexia, respiratory distress, and dysphagia, early-onset	AR	Biallelic mutations in *MEGF10* are associated with early-onset proximal and generalized muscle weakness, respiratory issues, joint contractures and scoliosis. Variable severity between affected individuals has been reported. *MEGF10* is highly expressed in satellite cells and is involved in their proliferation, differentiation and fusion into multinucleated myofibers. Skeletal muscle biopsies in affected patients show small and incompletely fused muscle fibers and decreased sparsely nucleated syncytia (15).
NEB [OMIM#161650] Myopathy, Nemaline 2 [OMIM#256030]	AR	Pie *et al,* 1999 identified 6 mutations (frameshift, nonsense and splicing) in affected members of 5 unrelated families with congenital autosomal recessive nemaline myopathy (16). It is likely that over half of nemaline myopathy cases are caused by *NEB* mutations (3). Nebulin is large protein component of the cytoskeletal matrix and accounts for up to 4% of the total myofibrillar protein.
LMOD3 [OMIM# 616165] Myopathy, Nemaline 10	AR	Yuen et al, 2014, identified homozygous or compound heterozygous mutations in the LMOD3 gene in 14 families with nemaline myopathy (17). The first two families were identified through whole exome sequencing, the others were identified through screening of 540 additional individuals with unresolved nemaline myopathy. 13 out of the 14 families identified had a severe congenital nemaline myopathy, and most patients died in the neonatal period.

SCN4A [OMIM#603967] Fetal hypokinesia or severe congenital myopathy (18)	AD	In a study by Zaharieva *et al*, homozygous or compound heterozygous mutations in *SCN4A* were identified in 11 individuals from six unrelated families with congenital myopathy. Affected individuals presented in utero or neonatal-onset muscle weakness varying in severity. In seven cases, muscle weakness resulted in death in the third trimester or shortly after birth. Muscle biopsy showed myopathic features including variable fiber size, fibrofatty tissue, and no specific structural abnormalities (18)
SPEG [OMIM#615590] Centronucelar myopathy-5 [OMIM#615959]	AR	Biallic mutations in *SPEG* are associated with centronuclear myopathy-5, which is characterized by severe hypotonia in the neonatal period, respiratory insufficiency, and feeding difficulty. Severity varies between affected individuals, and ranges from death in infancy to survival into childhood with dilated cardiomyopathy. Muscle biopsy demonstrates the presence of hypotrophic myofibers with central nuclei (19)
STAC3 [OMIM#615521] Native American myopathy [OMIM#255995]	AR	Native American myopathy is characterized by congenital weakness and arthrogryposis, cleft palate, ptosis, myopathic facies, short stature, kyphoscoliosis, club feet, and susceptibility to malignant hyperthermia provoked by anesthesia. In five Native American families with myopathy, all five affected individuals were identified to be homozygous for a missense mutation in the *STAC3* gene. Functional studies of this missense mutation in zebrafish showed decreased excitation-contraction coupling (20)
TNTT1 [OMIM191041] Nemaline Myopathy, Amish type [OMIM#605355]	AR	Mutations in *TNTT1* have been described in a group of Old Order Amish individuals with Nemaline myopathy (21). The *TNTT1* gene encodes the slow skeletal muscle troponin.
TPM2 [OMIM190990] Myopathy, Nemaline 4 [OMIM#609285]	AD	Donner *et al,* 2002 identified missense mutations in *TPM2* in 2/66 patients with nemaline myopathy (22). Mutations in *TPM2* are also a rare cause of Congenital Fiber-Type Disproportion. Mutations in *TPM2* can also cause distal arthrogryposis. The *TPM2* gene encodes beta-troposmyosin, an isoform of tropomyosin that is mainly expressed in slow type 1 muscle fibers.
TPM3 [OMIM#191030] Myopathy, Nemaline 1 [OMIM#609284]	AD/AR	Homozygous, heterozygous and compound heterozygous mutations in *TPM3* account for approximately 2-3% of patients with Nemaline Myopathy (23). Mutations in *TPM3* have been identified in 20-40% of patients with Congenital Fiber-Type Disproportion(6). *TPM3* related CFTD has been inherited in both an autosomal dominant and autosomal recessive manner. *TPM3* produces multiple transcripts, one of which is muscle specific. The TPM3 protein is a component of the thin filament and plays a role in muscle contraction.
BIN1 [OMIM#601248] Myopathy, Centronuclear, 2 [OMIM#255200]	AR	*BIN1* mutations appear to be relatively rare, accounting for approximately 25% of cases of CNM with apparent recessive inheritance but only a small percentage of all CNM cases combined (24). To date, only a small number of mutations have been described in the *BIN1* gene, including several missense changes and nonsense mutations. The *BIN1* gene codes for Bridging Integrator 1, also known as amphiphysin II, and has a muscle specific isoform. Amphiphysin II is regulated by phosphoinositides and is believed to be involved in membrane remodeling and T tubule organization.
CCDC78 [OMIM#614666] Myopathy, centronuclear, 4 [OMIM#614807]	AD	Majczenko *et al,* 2012, identified a heterozygous mutation in *CCDC78* in a family with AD CNM (25). The *CCDC78* gene encodes a protein that is important in skeletal muscle function.
CNTN1 [OMIM#600016] Myopathy, congenital, Compton-North [OMIM#612540]	AR	Compton *et al*, 2008 identified a homozygous frameshift mutation in the *CNTN1* gene in affected individuals of a large consanguineous Egyptian family (26). The *CNTN1* gene encodes for contactin-1, a neural adhesion molecular of the immunoglobulin superfamily.

DNM2 [OMIM#602378] Myopathy, Centronuclear, 1 [OMIM#160150]	AD	The majority of patients with autosomal dominant or later onset CNM, including *DNM2*-associated CNM, are ambulatory into adulthood (4). Intelligence is usually normal but at least one family with a *DNM2* mutation has been reported to have mild cognitive impairment, as well as mild axonal peripheral nerve involvement (27). *DNM2* mutations account for most, but not all, cases of CNM with autosomal dominant inheritance or later onset (28). NADH staining of patients with *DNM2* mutations often reveals radial arrangement of sarcoplasmic strands, which is highly characteristic, but not diagnostic, of *DNM2*-associated CNM.
MTM1 [OMIM#300415] Myopathy, Centronuclear, X-linked [OMIM#310400]	X-linked	Patients with X-linked myotubular myopathy (XLMTM) generally present with hypotonia, feeding difficulties, respiratory distress, and delayed motor milestones. Death in infancy is common in males with the classic form of this condition. Milder forms of XLMTM have been identified and are characterized by fewer respiratory complications and longer life expectancy than observed in the severe cases (29). Truncating and splice site *MTM1* mutations are more likely to be associated with the severe neonatal form, whereas the milder phenotypes are often caused by missense mutations outside of the functional domains (29). Missense mutations may result in a mild or severe phenotype based on their position in the *MTM1* gene (30). Approximately 80% of males with a diagnosis of myotubular myopathy by muscle biopsy will have a mutation in *MTM1* identifiable by sequence analysis. About 7% of mutations in *MTM1* are deletions (31).
MYF6 [OMIM#159991] Myopathy, centronuclear, 3 [OMIM#614408]	AD	Kerst *et al,* 2000 identified a heterozygous missense mutation in the *MYF6* gene in a boy with myopathy and an increase of muscle fibers with central nuclei (32). The *MYF6* gene is a novel member of the human gene family of muscle determination factors
MYH7 [OMIM#160760] Myopathy, myosin storage [OMIM#608358]	AD	Heterozygous mutations in *MYH7* have been associated with isolated hypertrophic/dilated cardiomyopathy, Laing distal myopathy and myosin storage myopathy (33). Inheritance is generally dominant, but recessive inheritance has been reported in at least one family with a more severe presentation that included cardiomyopathy.
PTPLA [OMIM# 610467]	AR	Muhammad *et al,* 2013, identified a homozygous truncating mutation in *PTPLA* (also known as *HACD1*) in a consanguineous family with congenital myopathy (34). Functional studies showed this mutation completely abrogated the activity of the enzyme encoded for by PTPLA, leading to a reduction in very long chain fatty acid (VLCFA) synthesis (34). The authors hypothesized that this reduction of VLCFAs, which are essential components of membrane lipids, leads to the congenital myopathy phenotype (34).
RYR1 [OMIM#180901]	AR	*RYR1* is typically associated with autosomal recessive CNM, although a *de novo* autosomal dominant mutation in this gene has also been reported (35). CNM-associated mutations identified in *RYR1* have included missense, frameshift, and intronic (36). Mutations in *RYR1* have also been associated with malignant hyperthermia [OMIM#145600], central core disease [OMIM#117000] and multi-minicore disease [OMIM#255320]. The *RYR1* gene, located at 19q13.2, encodes the skeletal muscle ryanodine receptor, which is the principal sarcoplasmic reticulum calcium release channel with a crucial role in excitation-contraction coupling (36).
SEPN1 [OMIM#606210] Myopathy, congenital with fiber-type disproportion [OMIM#255310]	AR	Clarke *et al,* 2006 identified a homozygous mutation in the *SEPN1* gene in two sisters with congenital fiber type disproportion (37). Homozygous or compound heterozygous mutations in *SEPN1* have also been seen in multi-minicore disease, rigid spine muscular dystrophy and desmin-related myopathy with Mallory body-like inclusions.
TTN [OMIM#188840]	AR/AD	Mutations in *TTN* have been described in patients with hereditary myopathy with early respiratory failure, tibial muscular dystrophy, and dilated cardiomyopathy type 1G. Titin is a muscle protein expressed in the cardiac and skeletal muscles and plays a key role in muscle assembly.

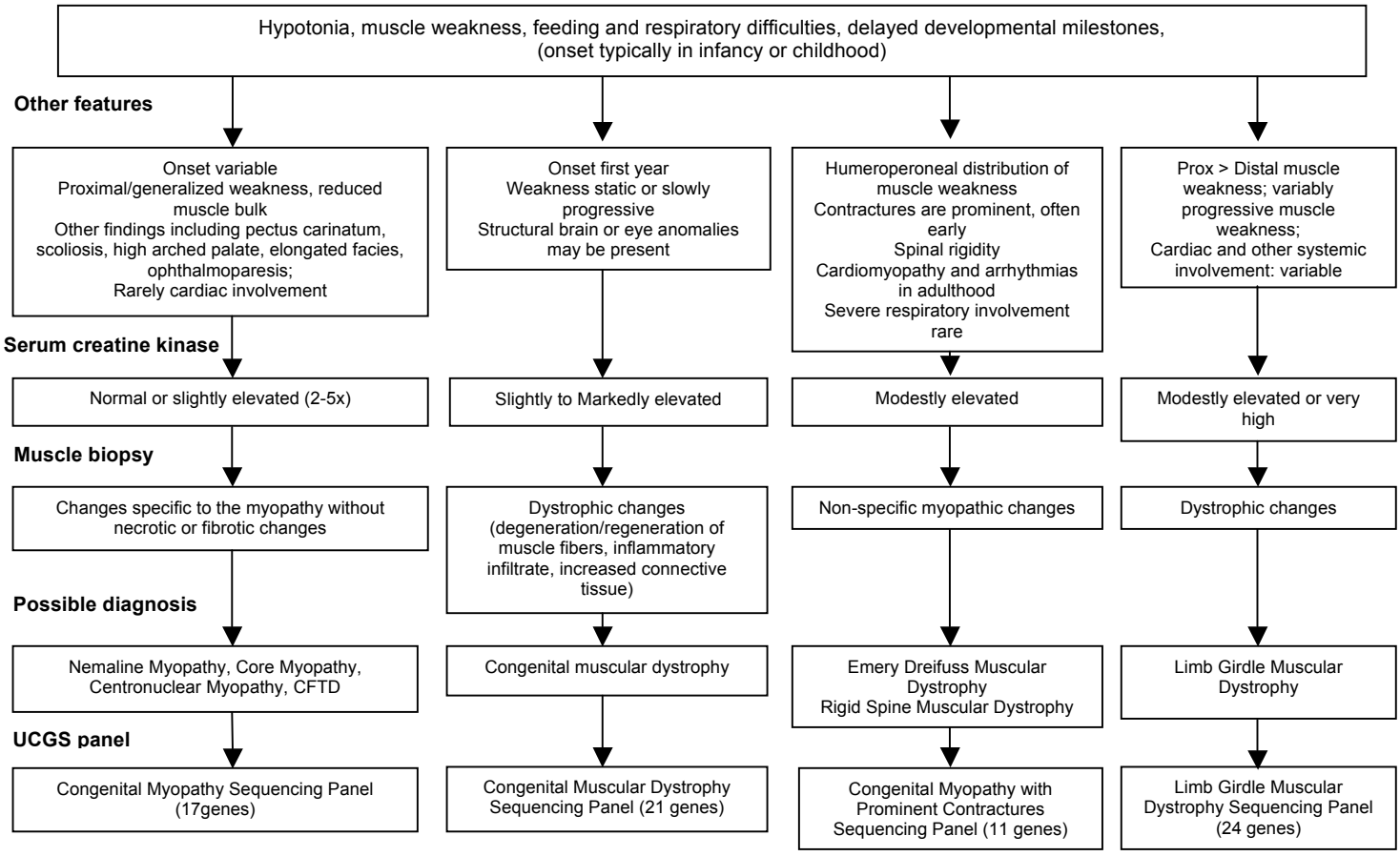
Test methods:

Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interest are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All novel and/or potentially pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp.

Deletion/duplication analysis is performed by oligonucleotide array-CGH. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by array-CGH. Array-CGH will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory.

Testing algorithm:

There is wide variation in onset, presentation and severity of congenital myopathies/muscular dystrophies. The flowchart below is only intended to be a general guide in considering which UCGS test may be most appropriate for your patient. Physicians should utilize their discretion and medical expertise in determining which testing panel to order.



Cardamone et al., Semin Neurol. 28:250-9, 2008

Congenital Myopathy Sequencing Panel (28 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2,000
CPT codes:	81407
Turn-around time:	8 – 10 weeks

Note: We cannot bill insurance for the above test.

Congenital Myopathy Deletion/Duplication Panel (28 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$1,545
CPT codes:	81407
Turn-around time:	4 – 6 weeks

Results:

Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

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