THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Congenital Muscular Dystrophy

Congenital muscular dystrophies are a genetically and clinically heterogeneous group of disorders typically characterized by weakness and dystrophic pattern on muscle biopsy present at birth or during the first months of life. Affected infants typically appear 'floppy' and have more low muscle tone and poor spontaneous movements (1). The clinical course is broadly variable and can comprise the involvement of the brain and eyes (2). CMDs can be classified by the mutated gene, the respective protein's localization and the protein's predicted function (3)

Our Congenital Muscular Dystrophy Panel includes analysis of the 26 genes listed below.

Congenital Muscular Dystrophy Panel				
B3GALNT2	DPM1	POMGNT2 (GTDC2)	POMGNT1	SYNE1
CHKB	DPM2	ISPD	POMK	TMEM5
COL6A1	DPM3	ITGA7	POMT1	TRAPPC11
COL6A2	FKRP	LAMA2	POMT2	
COL6A3	FKTN	LARGE	RYR1	
DAG1	GAA	LMNA	SEPN1	

Genes and Associated Disorder	Inheritance	Clinical Features/Molecular Pathology
B3GALNT2 [OMIM#610194] Muscular dystrophy-dystroglycanopathy [OMIM#615181]	AR	Compound heterozygous and homozygous mutations in *B3GALNT2* were identified in six unrelated patients with dystroglycanopathy and structural brain abnormalities. Functional studies indicated reduced dystroglycan glycosylation in fibroblasts and muscles of affected individuals, and knockdown of *B3GALNT2* in zebrafish led to a human CMD phenotype (4).
CHKB [OMIM#612395] Muscular dystrophy, congenital, megaconial type [OMIM#602541]	AR	Mitsuhashi *et al*, 2011 identified homozygous and/or compound heterozygous mutations in *CHKB* in 15 patients with congenital muscular dystrophy, mental retardation and enlarged mitochondria (5).
COL6A1 [OMIM#120220] *COL6A2* [OMIM#120240] *COL6A3* [OMIM#120250]	AD/AR	The collagen type VI-related disorders represent a spectrum including Bethlem myopathy at the mild end, Ullrich congenital muscular dystrophy at the severe end, and autosomal dominant limb girdle muscular dystrophy and autosomal recessive myosclerosis myopathy –in between. Mutations in *COL6A1, COL6A2* and *COL6A3* account for 38, 44 and 18% of affected individuals respectively (6).
DPM1 [OMIM#603503] Congenital disorder of glycosylation, type Ie [OMIM#608799]	AR	Yang *et al.*, 2013, identified a novel missense mutation and multi-exon deletion in the *DPM1* gene in a patient with infantile-onset muscular dystrophy who later developed a CDG phenotype. Muscle biopsy findings included muscular dystrophy and reduced α-dystroglycan immunostaining with glycoepitope-specific antibodies in a pattern diagnostic of dystroglycanopathy (7)
DPM2 [OMIM#603564]	AR	Barone *et al,* 2012 identified compound heterozygous (missense and splicing) and homozygous (missense) mutations in two families with profound developmental delay, intractable epilepsy, progressive microcephaly, severe hypotonia with elevated blood CK levels, in which clinical evidence supported a muscular dystrophy-dystoglycanopathy syndrome (8). Mutations in *DPM2* are also found in patients with Congenital disorder of glycosylation type Iu [OMIM#615042].
DPM3 [OMIM#605951]	AR	Lefeber *et al,* 2009 identified a homozygous missense mutation in *DPM3* in a woman with congenital disorder or glycosylation type 1 [OMIM#612937] (9). In addition to biochemical features of CDG, this patient had mild muscle weakness and cardiomyopathy. Dolichol-phosphate-mannose is a mannosyl donor important for the biosynthesis of various glycoconjugates.
DAG1 [OMIM#128239] *FKTN* [OIMI#607440] *FRKP* [OMIM#606596] *ISPD* [OMIM#614631] *LARGE* [OMIM#603590] POMK [OMIM#615247] *POMT1* [OMIM#607423] *POMT2* [OMIM#607439] *POMGNT1* [OMIM#606822] *TMEM5* [OMIM#605862]	AR	Mutations in these genes result in a dystroglycanopathy phenotype. Dystroglycanopathies are characterized by a broad congenital muscular dystrophy phenotypic spectrum with and without ID, eye involvement and brain findings. Dystroglycanopathies are known to be caused by at least 9 different genes (10).
GAA [OMIM#606800]	AR	Biallelic mutations in GAA are associated with glycogen storage disease type II (Pompe disease). Classic infantile Pompe disease is characterized by infantile onset hypotonia, muscle weakness, cardiomegaly and hypertrophic cardiomyopathy (11). Non-classic infantile onset and late-onset forms of the disease also exist, which are also associated with slowly progressive muscle weakness (12).
POMGNT2 (GTDC2) [OMIM#614828]	AR	Manzini *et al,* 2012 identified 3 different homozygous mutations in 3 unrelated

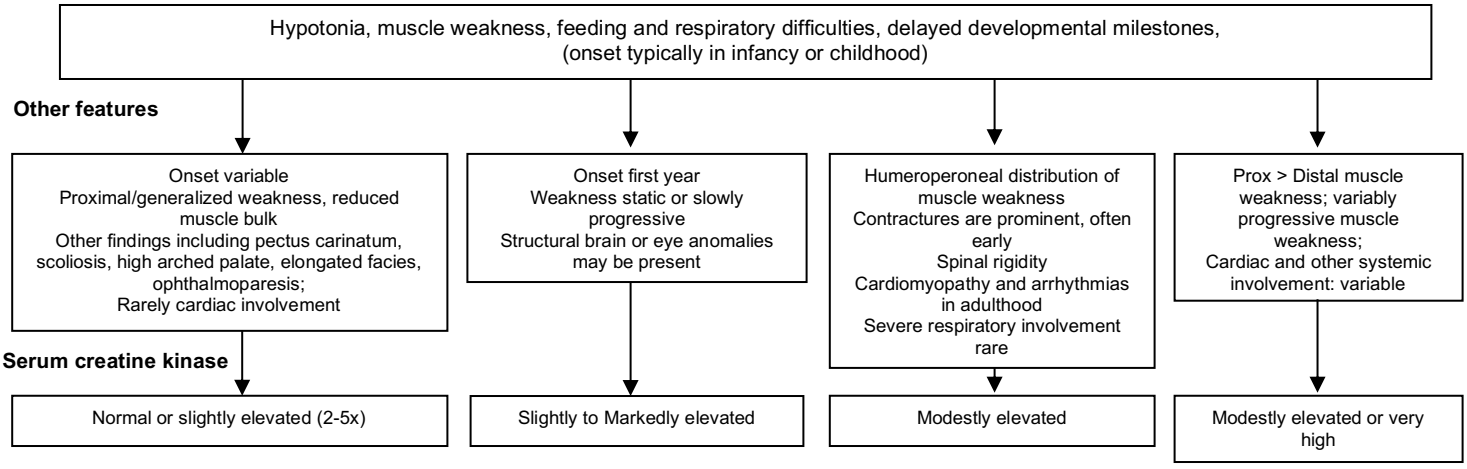
Muscular dystrophy-dystroglycanopathy, type A, 8 [OMIM#614830]		consanguineous families with MDDGA8 (13). POMGNT2 is expressed in most tissues with highest expression in pancreas, followed by adult and fetal brain.
ITGA7 [OMIM#600536] Muscular dystrophy, congenital, due to ITGA7 deficiency [OMIM#613204]	AR	Hayashi *et al,* 1998 identified compound heterozygous mutations in *ITGA7* in 3 unrelated patients with congenital muscular dystrophy due to integrin alpha-7 deficiency (14). *ITGA7* is an integrin that is a specific cellular receptor for the basement membrane protein laminin-1
LAMA2 [OMIM#156225] Muscular dystrophy, congenital merosin deficient [OMIM#607855]	AR	Mutations in *LAMA2* are identified in close to 96% of patient with LAMA2-related muscular dystrophy (15). Laminin is a heterotrimeric extracellular matrix protein and is predominantly expressed in skeletal muscle, cerebral white matter and Schwann cells.
LMNA [OMIM#150330] Muscular dystrophy, congenital [OMIM#150330]	AD	Quijano-Roy *et al,* 2008 identified 11 different de-novo heterozygous mutations in the *LMNA* gene in 15 children with *LMNA*-related CMD (16). The *LMNA* gene encodes lamin A and lamin C, structural protein components of the nuclear lamina.
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 (17). CNM-associated mutations identified in *RYR1* have included missense, frameshift, and intronic mutations (18). 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 encodes the skeletal muscle ryanodine receptor, which is the principal sarcoplasmic reticulum calcium release channel with a crucial role in excitation-contraction coupling (18).
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 (19). Homozygous or compound heterozygous mutations in *SEPN1* have also been seen in multiminicore disease, rigid spine muscular dystrophy and desmin-related myopathy with Mallory body-like inclusions.
SYNE1 [OMIM#608441]	AR/AD	In a consanguineous family with a form of congenital muscular dystrophy (described by the authors as myogenic arthrogryposis), Attali *et al,* 2009, reported a homozygous mutation in *SYNE1* (20). Dominant mutations in *SYNE1* are associated with Emery Dreifuss muscular dystrophy [OMIM#612998], the *SYNE1* gene has also been associated with autosomal recessive spinocerebellar ataxia [OMIM#610743].
TRAPPC11 [OMIM#614138] Muscular dystrophy, limb-girdle, type 2S [OMIM# 615356]	AR	Homozygous and compound heterozygous mutations in *TRAPPC11* have been reported in several different families with LGMD. This condition is characterized by childhood-onset proximal muscle weakness resulting in gait abnormalities and scapular winging. Serum creatine kinase in increased in patients with LGMD2S, and some patients are affected by movement abnormalities including chorea, dystonia, or ataxia (21).

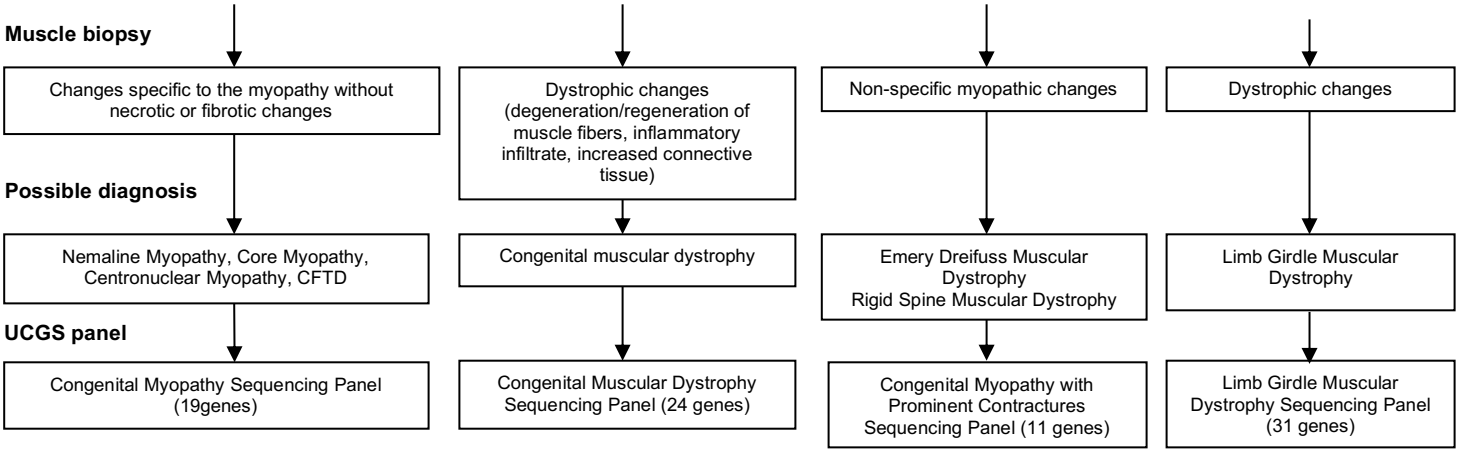
Test methods:

Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests 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. Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided. **This assay also includes analysis for the recurrent c.930+189C>T deep intronic variant in the *COL6A1* gene.**

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 Muscular Dystrophy Panel (27 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2,000
CPT codes:	81406
	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for the above test.

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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