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 Sequencing Panel and Congenital Muscular Dystrophy Deletion/Duplication Panel include analysis of the 26 genes listed below.**

<table>
<thead>
<tr>
<th>Genes and Associated Disorder</th>
<th>Inheritance</th>
<th>Clinical Features/Molecular Pathology</th>
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</thead>
<tbody>
<tr>
<td>B3GALNT2 [OMIM#619194]</td>
<td>AR</td>
<td>Compound heterozygous and homozgyous 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).</td>
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<tr>
<td>CHKB [OMIM#612395]</td>
<td>AR</td>
<td>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).</td>
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<tr>
<td>COL6A1 [OMIM#120220]</td>
<td>AD/AR</td>
<td>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).</td>
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<tr>
<td>COL6A1 [OMIM#120240]</td>
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<td>COL6A3 [OMIM#120250]</td>
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<tr>
<td>DPM1 [OMIM#603503]</td>
<td>AR</td>
<td>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).</td>
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<td>DPM2 [OMIM#603564]</td>
<td>AR</td>
<td>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-dystroglycanopathy syndrome (8). Mutations in DPM2 are also found in patients with Congenital disorder of glycosylation type IIu [OMIM#615042].</td>
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<tr>
<td>DPM3 [OMIM#605951]</td>
<td>AR</td>
<td>Lefebre 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.</td>
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<td>DAG1 [OMIM#128239]</td>
<td>AR</td>
<td>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).</td>
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<tr>
<td>FKTN [OMIM#607440]</td>
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<td>FRK [OMIM#607596]</td>
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<td>ISPD [OMIM#614631]</td>
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<td>LARGE [OMIM#603590]</td>
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<td>POMK [OMIM#615247]</td>
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<tr>
<td>POMT1 [OMIM#607423]</td>
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<tr>
<td>POMT2 [OMIM#607439]</td>
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<tr>
<td>POMGNT1 [OMIM#606822]</td>
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<td>TMEM5 [OMIM#605862]</td>
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<td>GAA [OMIM#600830]</td>
<td>AR</td>
<td>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).</td>
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## 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. 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. 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.

### Hypotonia, muscle weakness, feeding and respiratory difficulties, delayed developmental milestones, (onset typically in infancy or childhood)

<table>
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<tr>
<th>Serum creatine kinase</th>
<th>Other features</th>
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| Normal or slightly elevated (2-5x) | Onset variable  
Proximal/generalized weakness, reduced muscle bulk  
Other findings including pectus carinatum, scoliosis, high arched palate, elongated facies, ophthalmoparesis; Rarely cardiac involvement |
| Slightly Markedly elevated | Onset first year  
Weakness static or slowly progressive  
Structural brain or eye anomalies may be present |
| Modestly elevated | Humeroperooneal distribution of muscle weakness  
Contractures are prominent, often early  
Spinal rigidity  
Cardiomyopathy and arrhythmias in adulthood  
Severe respiratory involvement rare |
| Modestly elevated or very high | Prox > Distal muscle weakness; variably progressive muscle weakness;  
Cardiac and other systemic involvement: variable |
Muscle biopsy
- Changes specific to the myopathy without necrotic or fibrotic changes

Possible diagnosis
- Nemaline Myopathy, Core Myopathy, Centronuclear Myopathy, CFTD
- Congenital muscular dystrophy
- Emery-Dreifuss Muscular Dystrophy
- Rigid Spine Muscular Dystrophy
- Limb Girdle Muscular Dystrophy

UCGS panel
- Congenital Myopathy Sequencing Panel (19 genes)
- Congenital Muscular Dystrophy Sequencing Panel (24 genes)
- Congenital Myopathy with Prominent Contractures Sequencing Panel (11 genes)
- Limb Girdle Muscular Dystrophy Sequencing Panel (31 genes)

Congenital Muscular Dystrophy Sequencing Panel (27 genes)
- Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
- Cost: $2,000
- CPT codes: 81407
- Turn-around time: 8 weeks

Note: We cannot bill insurance for the above test.

Congenital Muscular Dystrophy Deletion/Duplication Panel (27 genes)
- Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
- Cost: $1,545
- CPT codes: 81407
- Turn-around time: 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.

References:

Committed to CUSTOMIZED DIAGNOSTICS, TRANSLATIONAL RESEARCH & YOUR PATIENTS’ NEEDS