THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Congenital Myopathy with Prominent Contractures

Emery Dreifuss Muscular Dystrophy

Emery Dreifuss Muscular Dystrophy is characterized by joint contractures (onset in early childhood), slowly progressive muscle wasting and weakness and cardiac conduction defects (1). Muscle wasting and weakness exhibit a distinctive humero-peroneal distribution early in the course of the disease, with weakness later extending to the proximal limb girdle musculature (1). Cardiac involvement, which usually occurs after the second decade and is the most serious aspect of the disease, may manifest as palpitations, presyncope and syncope, poor exercise tolerance and congestive heart failure (2). Heterogeneity in presenting symptoms can occur even amongst affected members of the same family.

Rigid Spine Muscular Dystrophy

Rigid Spine syndrome is a condition found in a subset of patients affected by myopathy with early contractures. It is characterized by marked limitation in flexion of the whole dorsolumbar and cervical spine, owing to contracture of the spinal extensors and leading to loss of movement of the spine and thoracic cage (3). Spinal rigidity can also be seen in patient with Emery Dreifuss Muscular Dystrophy. Clinical critieria for patients with rigid spine syndrome are similar to those observed in congenital muscular dystrophies, as such, the rigid spine syndrome phenotype has been proposed as a subtype of CMD (4).

Our Congenital Myopathy with Prominent Contractures Panel includes all eleven genes listed below.

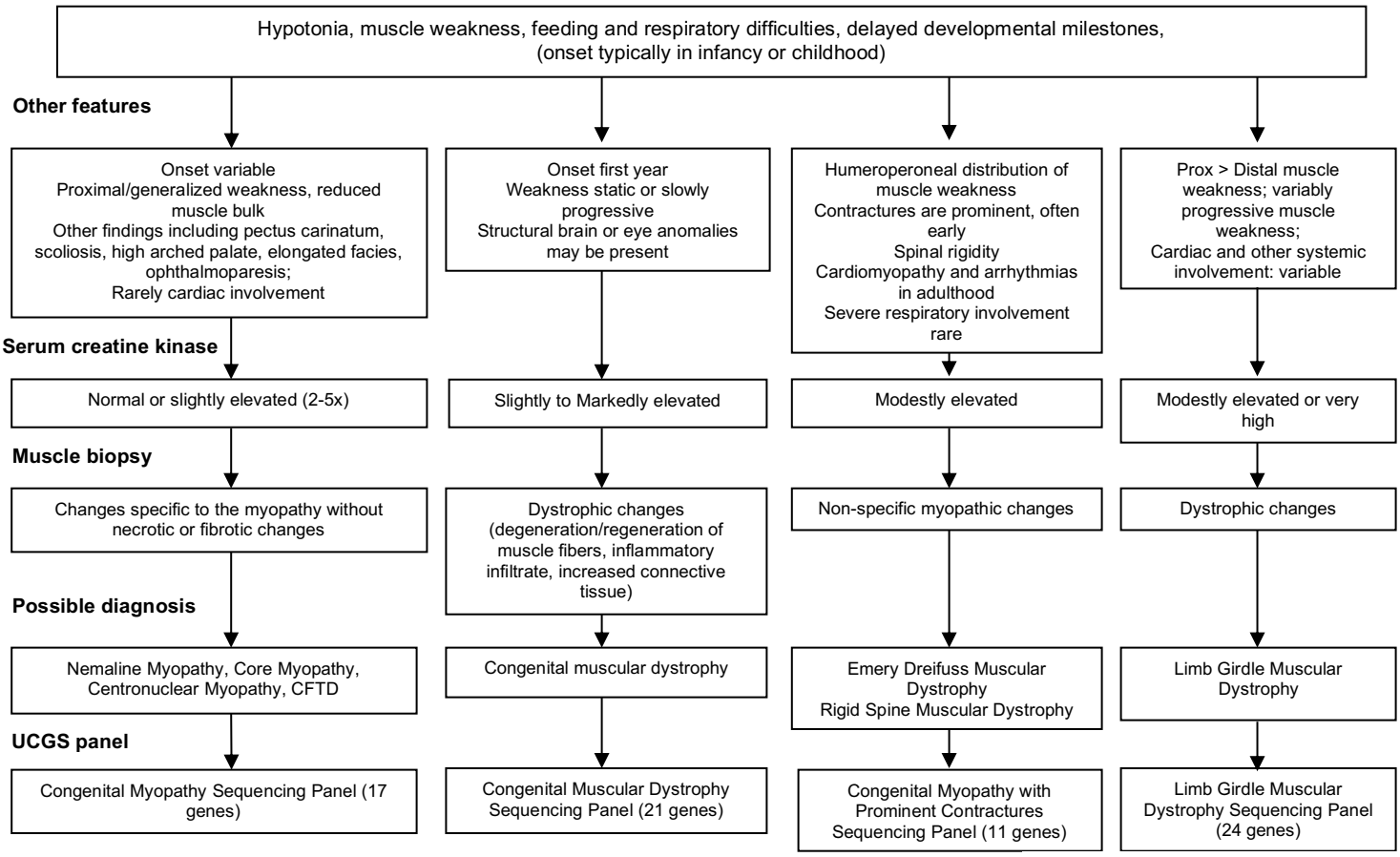
Congenital Myopathies with Prominent Contractures			
COL6A1	*EMD*	*RYR1*	*SYNE2*
COL6A2	*LMNA*	*SEPN1*	*TMEM43*
COL6A3	*MYH7*	*SYNE1*	

Genes and Associated Disorder	Inheritance	Clinical Features/Molecular Pathology
COL6A1 [OMIM#120220] *COL6A2* [OMIM#120240] *COL6A3* [OMIM#120250]	AD and 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 (5).
EMD [OMIM#300384] Emery-Dreifuss muscular dystrophy 1, X-linked [OMIM#310300]	X-linked	Mutations in *EMD* account for approximately 61% of XL-EDMD (2). Emerin is a ubiquitous protein that is found along the nuclear rim of many cell types and is a member of the nuclear lamina-associated protein family.
LMNA [OMIM#150330] Emery-Dreifuss muscular dystrophy 2, AD [OMIM#181350] Emery-Dreifuss muscular dystrophy 3, AR [OMIM#181350]	AD/AR	Mutations in *LMNA* account for approximately 45% of AD-EDMD (2). AR-EDMD is rare, Raffaele Di Barletta *et al,* 2000 identified a homozygous mutation in *LMNA* leading to AR-EDMD (6). The *LMNA* gene encodes lamin A and lamin C, structural protein components of the nuclear lamina
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 (7). Inheritance is generally dominant, but recessive inheritance has been reported in at least one family with a more severe presentation that included cardiomyopathy.
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 (8). CNM-associated mutations identified in RYR1 have included missense, frameshift, and intronic mutations (9). 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 (9).
SEPN1 [OMIM#606210] Muscular dystrophy, rigid spine, 1	AR	Mutations in the *SEPN1* gene have been identified in patients with rigid spine muscular dystrophy (10). Homozygous or compound heterozygous mutations in *SEPN1* have also been seen in multiminicore disease,

[OMIM#602771]		congenital myopathy with fiber-type disproportion and desmin-related myopathy with Mallory body-like inclusions.
SYNE1 [OMIM#608441] *SYNE2* [OMIM#608442] Emery-Dreifuss muscular dystrophy 4 [OMIM#612998] Emery-Dreifuss muscular dystrophy 5, autosomal dominant	AD	Zhang *et al,* 2007 identified a heterozygous mutation in *SYNE2* in a father and his 2 children with EDMD and identified 2 different heterozygous mutations in *SYNE1* in two unrelated patients (11).
TMEM43 [OMIM#612048] Emery-Dreifuss muscular dystrophy 7 [OMIM#614302]	AD	Liang *et al,* 2011 identified heterozygous missense mutations in 2/41 individuals with Emery-Dreifuss muscular dystrophy (12).

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

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 pathogenic and likely 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. In addition, targeted sequence analysis for the recurrent c.930+189C> variant in the *COL6A1* gene is performed by Sanger sequencing.
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.

Congenital Myopathy with Prominent Contractures Panel (11 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2500
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.

References:

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8. Jungbluth H, Zhou H, Sewry CA et al. Centronuclear myopathy due to a de novo dominant mutation in the skeletal muscle ryanodine receptor (RYR1) gene. Neuromuscul Disord 2007: 17: 338-345.
9. Wilmshurst JM, Lillis S, Zhou H et al. RYR1 mutations are a common cause of congenital myopathies with central nuclei. Ann Neurol 2010: 68: 717-726.
10. Okamoto Y, Takashima H, Higuchi I et al. Molecular mechanism of rigid spine with muscular dystrophy type 1 caused by novel mutations of selenoprotein N gene. Neurogenetics 2006: 7: 175-183.
11. Zhang Q, Bethmann C, Worth NF et al. Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss muscular dystrophy and are critical for nuclear envelope integrity. Hum Mol Genet 2007: 16: 2816-2833.
12. Liang WC, Mitsuhashi H, Keduka E et al. TMEM43 mutations in Emery-Dreifuss muscular dystrophy-related myopathy. Ann Neurol 2011: 69: 1005-1013.

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