Congenital Myopathy and Muscular Dystrophy Panel
Sequence Analysis and Exon-Level Deletion/Duplication Testing of 34 Genes

Panel Gene List: ACTA1, BICD2, CCDC78, CFL2, CHKB, COL6A1, COL6A2, COL6A3, COL12A1, DYNC1H1, FKRP*, FKTN, GBE1, IGHMBP2, ITGA7, KBTBD13, KLHL40, KLHL41, LAMA2, LMNA, LMOD3, MEGF10, MICU1, MTM1, NEB, RYR1, SEPN1, TNNT1**, TPM2, TPM3, TRIP4, TRPV4, UBA1, VRK1

*Sequence analysis only of FKRP gene.

**Only whole gene deletions or duplications may be detected for TNNT1.

Clinical Features:

Congenital myopathies and congenital muscular dystrophies are a clinically and genetically heterogeneous group of disorders, characterized by hypotonia and poor reflexes at birth or in the first years of life. They were traditionally classified by clinical phenotypes, histopathology, and creatine kinase levels, but currently molecular diagnosis is used to distinguish subtypes. Variable age of onset and disease severity is observed between the congenital myopathies and congenital muscular dystrophies.[1,2] The congenital myopathies are clinically defined by stable or slowly progressive muscle weakness and hypotonia that typically occurs within the first year after birth, that may be accompanied by delayed motor milestones and breathing difficulties. Variable underlying histologic features have been noted for the congenital myopathies, but histopathology is likely to include structural changes without the dystrophic features seen in muscular dystrophies.[1] The primary histological findings include central nuclei, cores (areas devoid of mitochondria), and nemaline bodies (rod-like inclusions in the sarcoplasm).[3,4] Congenital myopathies have an estimated prevalence of 1 per 25,000.[4]

The congenital muscular dystrophies are clinically defined by low muscle tone and poor reflexes. Disease progression is variable, but progressive, with some individuals showing short-term improvement or stabilization. Common features include progressive weakness and joint contractures, spinal deformities, and respiratory involvement.[2] Histopathology may include dystrophic features and rarely includes structural changes.[2] The congenital muscular dystrophies are subdivided into categories by protein function or gene and have an estimated prevalence ranging from 0.68-2.5/100,000.[2, 5, 6] In addition to congenital myopathies and congential muscular dystrophies, spinal muscular atrophies can also present with similar clinical features. Spinal muscular atrophies are characterized by the degeneration of the antenatal anterior horn cells, leading to progressive muscle weakness and wasting.[7] Individuals typically present with symmetric proximal extremity weakness that may progress to

distal, axial, intercostal, and bulbar muscles; however age-of-onset, disease severity, and additional clinical findings can be variable.[7,8]

Genetics:
Congenital myopathies, congenital muscular dystrophies, and spinal muscular atrophies are inherited in either an autosomal dominant, autosomal recessive, or X-linked manner or they may be the result of a de novo variant. Many of the genes associated with these disorders encode proteins that are critical components of the extracellular matrix, nuclear envelope, or endoplasmic reticulum. Several others are involved in the processes of glycosylation or ubiquitin-mediated degradation.

Test Methods:
Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the genes on this panel are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). For FKTN, nucleotides surrounding the insertion site of an ALU-based founder mutation in the 3' UTR are also captured to determine if the insertion is present or absent. Presence of an insertion is confirmed using multiplex PCR, followed by gel electrophoresis. The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be > 99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. Gene specific exclusions for exon-level deletion/duplication testing for this panel are: FKRP gene, no copy number testing; TNNT1 gene, only whole gene deletions or duplications may be detected.

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Congenital Myopathies and Muscular Dystrophies Panel – 34 Genes

Gene	Inheritance	Disease Associations	Diagnostic Yield in Selected Population(s)
ACTA1	AD	Congenital fiber-type disproportion myopathy; Nemaline myopathy (NM)	~15-25% of NM [1]
BICD2	AD	Autosomal dominant congenital spinal muscular atrophy (DCSMA)	Rare[32,33]
CCDC78	AD	Centronuclear Myopathy	Rare[36]
CFL2	AR	Nemaline myopathy	Rare[1, 2]
CHKB	AR	Congenital megaconial type muscular dystrophy	Rare- Identified in Japanese, Turkish, British, French and African American populations[3]
COL6A1	AD/AR	Ullrich CMD/Bethlem myopathy	~62% of patients with Ullrich or Bethlem myopathy have been found to have a mutation in one of the three collagen VI genes (COL6A1, COL6A2, COL6A3)[4]
COL6A2	AD/AR	Ullrich CMD/Bethlem myopathy	~62% of patients with Ullrich or Bethlem myopathy have been found to have a mutation in one of the three collagen VI genes (COL6A1, COL6A2, COL6A3)[4]

Gene	Inheritance	Disease Associations	Diagnostic Yield in Selected Population(s)
COL6A3	AD/AR	Ullrich CMD/Bethlem myopathy	~62% of patients with Ullrich or Bethlem myopathy identified to have a mutation in one of the three collagen VI genes (COL6A1, COL6A2, COL6A3)[4]
COL12A1	AD/AR	Ullrich CMD/Bethlem myopathy	Rare[37]
DYNC1H1	AD	Spinal muscular atrophy lower extremity	Rare [38-40]
FKRP*	AR	Walker–Warburg syndrome; CMD with/without intellectual disability and microcephaly; Limb-girdle muscular dystrophy (LGMD) type 2I	~70% LGMD2I ~9% alpha-dystroglycanopathies [5, 6]
FKTN	AR	Fukuyama congenital muscular dystrophy; LGMD type 2M	~7% alpha-dystroglycanopathies; a founder mutation in the 3' UTR in the Japanese population, and an intronic point mutation found in the Korean population[7-10] Rare cause of LGMD
GBE1	AR	Glycogen Storage Disease IV	3% of glycogen storage disease[41]
IGHMBP2	AR	Spinal muscular atrophy with respiratory distress 1 (SMARD)	~33% of patients with SMARD[42]
ITGA7	AR	Integrin alpha-7-related CMD	Rare [11]
KBTBD13	AD	Nemaline myopathy	Unknown [13, 14]
KLHL40	AR	Nemaline myopathy	~20% of severe NM[44]
KLHL41	AR	Nemaline myopathy	Rare [43]
LAMA2	AR	Merosin-deficient congenital muscular dystrophy, (MDC1A)	~30-50% of congenital muscular dystrophy are MDC1A in the European population[15-17]
LMNA	AD/AR	Emery-Dreifuss muscular dystrophy (EDMD) - AD; LGMD type 1B; congenital laminopathy	~45% of AD-EDMD (unknown for AR-EDMD) ~12% of LGMD in United States. Rare in congenital muscular dystrophies[5, 6, 17-19]
LMOD3	AR	Nemaline myopathy	Rare[45]

Gene	Inheritance	Disease Associations	Diagnostic Yield in Selected Population(s)
MEGF10	AR	Early onset myopathy areflexia, respiratory distress, dysphagia	Rare[20-22]
MICU1	AR	Myopathy with extrapyramidal signs	Rare[46]
MTM1	XL	Centronuclear/Myotubular myopathy	Rare[34]
NEB	AR	Nemaline myopathy	~ 50% of Nemaline myopathy; Ashkenazi Jewish founder mutation (2.5kb deletion in exon 55)[23]
RYR1	AD/AR	Central core disease; minicore myopathy with external ophthalmoplegia	~89 % of central core disease[24-26]
SEPN1	AR	CMD with spinal rigidity (RSMD1); Minicore/multicore disease	~30-50% of minicore/multicore disease[24,27]
TNNT1**	AR	Nemaline myopathy	Rare overall; founder mutation in the Old Order Amish population[29, 30]
TPM2	AD	Nemaline myopathy	<1% of NM [13]
TPM3	AD/AR	Nemaline myopathy	~2-3% of NM 1[3, 31]
TRIP4	AR	Spinal Muscular Atrophy	Unknown [47]
TRPV4	AD	Scapuloperoneal spinal muscular atrophy	Rare[48-51]
UBA1	X-linked	X-linked SMA with arthrogryposis and congenital contractures; Spinal muscular atrophy- X-linked 2	Rare[52]
VRK1	AR	Spinal muscular atrophy with pontocerebellar hypoplasia; pontocerebellar hypoplasia type 1 (Norman disease)	Rare overall; founder mutation in the Ashkenazi Jewish [52]

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