Syndromic Congenital Muscular Dystrophy Panel
Sequence Analysis and Exon-Level Deletion/Duplication* Testing of 19 Genes

Panel Gene List: B4GAT1*, B3GALNT2, DAG1, DPM1, DPM2, DPM3, FKTN, FKRP*, GMPPB, ISPD, ITGA7, LARGE, LMNA, POMGNT1**, POMK, POMT1, POMT2, TMEM5, VRK1

* Sequence analysis only of B4GAT1 and FKRP gene.
** Only whole gene deletions or duplications may be detected for POMGNT1 gene.

Clinical Features:

Syndromic congenital muscular dystrophies (SCMDs) are a clinically and genetically heterogeneous group of disorders that typically present at birth with low muscle tone and poor reflexes and are also associated with intellectual disability and brain or eye abnormalities. SCMDs with brain involvement are more commonly due to glycosylation defects of the alpha dystroglycan protein and are referred to as the alpha-dystroglycanopathies.[1,2] The alpha-dystroglycanopathies include Fukuyama congenital muscular dystrophy, Muscle-eye-brain disease, and Walker-Warburg syndrome. Disease progression is variable for each of these disorders and individuals may show progressive muscle weakness, joint contractures, spinal deformities, and respiratory complications.[2] Variable disease severity is observed for these disorders.[2] Congenital muscular dystrophies have an estimated prevalence ranging from 0.68-2.5/100,000.

Genetics:

The syndromic congenital muscular dystrophies described here are inherited in an autosomal recessive manner, with the exclusion of *LMNA* pathogenic variants that can be inherited in an autosomal recessive or dominant manner. Most of these genes encode proteins that are involved in the process of glycosylation, but others are critical components of the extracellular matrix or endoplasmic reticulum.

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. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data. 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: B4GAT1 and FKRP genes, no copy number testing; POMGNT1 gene, only whole gene deletions or duplications may be detected.

References:

1. Kirschner, J. (2013). Congenital muscular dystrophies. Handb. Clin. Neurol. 113, 1377-1385.
2. Sparks, et al. Congenital Muscular Dystrophy Overview. 2001 Jan 22 [Updated 2012 Aug 23]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2014. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1291/
3. Darin and Tulinius (2000) Neuromuscular disorders in childhood: A descriptive epidemiological study from western Sweden. Neuromuscul Disord 10:1-9.

Syndromic Congenital Muscular Dystrophy Panel – 19 Genes

Gene	Inheritance	Disorder	Diagnostic Yield in Selected Population(s)
B4GAT1 (B3GNT1)*	AR	Walker-Warburg syndrome	Rare[25]
B3GALNT2	AR	Walker–Warburg syndrome; Muscle-eye-brain disease (MEB)	Rare[1]
DAG1	AR	Primary dystroglycanopathy, Limb-girdle muscular dystrophy (LGMD) with early onset and intellectual disability	Rare[2]
DPM1	AR	Congenital disorder of glycosylation type Ie	Rare[3]
DPM2	AR	Congenital muscular dystrophy (CMD) with intellectual disability and severe epilepsy	Rare[4]
DPM3	AR	CMD with intellectual disability and severe epilepsy	Rare[3]
FKRP*	AR	Walker–Warburg syndrome; LGMD type 2	~70% LGMD2I

			~9% of the alpha-dystroglycanopathies[5, 6]
FKTN	AR	Fukuyama congenital muscular dystrophy; LGMD type 2M	~7% of the alpha-dystroglycanopathies Includes the Japanese founder mutation in the 3' UTR and an intronic point mutation found in the Korean population[7-10] Rare cause of LGMD
GMPPB	AR	Muscular dystrophy-dystroglycanopathy (LGMD), type C; Walker–Warburg syndrome	Rare[11]
ISPD	AR	Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies type A7	~30% of Walker-Warburg syndrome and ~11% of the alpha-dystroglycanopathies [8, 12, 13] Rare-LGMD
ITGA7	AR	Integrin alpha-7-related CMD	Rare [14]
LARGE	AR	Walker–Warburg syndrome; MEB disease	~3% of the alpha-dystroglycanopathies[10,16]
LMNA	AD/AR	Emery-Dreifuss muscular dystrophy - Autosomal dominant; 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]
POMGNT1**	AR	Walker–Warburg syndrome; MEB disease	~11-18% of the alpha-dystroglycanopathies[10,20, 21]
POMK	AR	Walker-Warburg syndrome; Muscular dystrophy-dystroglycanopathy A12; LGMD C12	Rare[64]
POMT1	AR	Walker–Warburg syndrome; CMD with intellectual disability and microcephaly; LGMD type 2K	~9-21% of the alpha-dystroglycanopathies[10,20]
POMT2	AR	Walker–Warburg syndrome; CMD with intellectual disability and microcephaly; LGMD type 2N	~9-11% of the alpha-dystroglycanopathies[10,22]
TMEM5	AR	Walker–Warburg syndrome; MEB disease	Rare[23]
VRK1	AR	Spinal muscular atrophy with pontocerebellar hypoplasia/ Pontocerebellar hypoplasia type 1 (Norman disease)	Rare overall; founder mutation in Ashkenazi Jewish [24]

***Sequence analysis only of B4GAT1 and FKRP genes**

**** Only whole gene deletions or duplications may be detected for POMGNT1 gene**

References: 1. Agrawal, et al. (2007). Am. J. Hum. Genet. 80, 162-167. 2. Godfrey et al. (2011). Dystroglycanopathies: coming into focus. Current Opinion in Genetics & Development 21, 278-285 (PMID: 21397493) 3. Mitsuhashi, S., Nishino, I. (2013). Curr. Opin. Neurol. 26, 536-543. 4. Butterfield, R.J., et al. (2013). Hum. Mutat. 34, 1558-1567. 5. Wicklund, M.P. (2013). Continuum (Minneap Minn) 19, 1535-1570. 6. Clement, et al. (2012). Neuromuscular Disord. 22, 522-527. 7. Yis, et al. (2011). Neuromuscular Disord. 21, 20-30. 8. Anonymous Limb-Girdle Muscular Dystrophy Overview - GeneReviews® - NCBI Bookshelf, http://www.ncbi.nlm.nih.gov/books/NBK1408/. 9. Saito, Fukuyama Congenital Muscular Dystrophy-GeneReviews® - NCBI Bookshelf, http://www.ncbi.nlm.nih.gov/books/NBK1206/. 10. Godfrey, et al. (2011). Curr. Opin. Genet. Dev. 21, 278-285. 11. Peat, et al. (2008). Neurology 71, 312-321. 12. Valencia, et al. (2013). PLoS 1:8. 13. Romero, N.B., Sandaradura, S.A., Clarke, N.F. (2013). Curr. Opin. Neurol. 26, 519-526. 14. Mayer et al. (1997) Absence of integrin alpha 7 causes a novel form of muscular dystrophy. Nature Genetics 17 (3):318-23 (PMID: 9354797) 16. Di Blasi, C., et al. (2005). Arch. Neurol. 62, 1582-1586. 17. Sarkozy, A., Bushby, K., Mercuri, E. (2013). Muscular Dystrophies. Emery and Rimoin's Principles and Practice of Medical Genetics, 1-58. 18. Anonymous Emery-Dreifuss Muscular Dystrophy - GeneReviews® - NCBI Bookshelf, http://www.ncbi.nlm.nih.gov/books/NBK1436/. 19. Quijano-Roy, et al. (2008). Ann. Neurol. 64, 177-186. 20. Mitsuhashi, S., et al. (2013). FEBS Lett. 587, 2952-2957. 21. Boyden, S.E., et al. (2012). Neurogenetics 13, 115-124. 22. Mitsuhashi, S., Kang, P.B. (2012). Semin. Pediatr. Neurol. 19, 211-218. 23. North, K.N., et al. (2014). Neuromuscular Disord. 24, 97-116. 24. Brislin, R.P., Theroux, M.C. (2013). Paediatr. Anaesth. 23, 834-841. 25. Wu, S., et al. (2006). Brain 129, 1470-1480. 26. Malicdan, M.C.V., Nishino, I. (2010 May 11). Central Core Disease - GeneReviews® - NCBI Bookshelf, http://www.ncbi.nlm.nih.gov/books/NBK1391/. 27. Treves, S., et al. (2008). Curr. Opin. Pharmacol. 8, 319-326. 28. Attali, R., et al. (2009). Hum. Mol. Genet. 18, 3462-3469. 29. Johnston, J.J., et al. (2000). Am. J. Hum. Genet. 67, 814-821. 30. Wallgren-Pettersson, C., et al.

(2011). Semin Pediatr Neurol. 18, 230-238. 31. Durling, H.J., et al. (2002). Neuromuscular Disord. 12, 947-951. 32. Synofzik et al. (2014) J. Neurol. Neurosurg. Psychiatr. 85 (5):590-2 (PMID: 24336790). 33. Peeters et al. (2013) American Journal Of Human Genetics 92 (6):955-64 (PMID: 23664119). 34. Biancalana, V., et al.(2012). Eur. J. Hum. Genet. 20, 1101. 63 . Jungbluth, H., Wallgren-Pettersson, C. (2013). Emery and Rimoin's Principles and Practice of Medical Genetics, 1-51. 64. Di et al. (2014) Human Molecular Genetics 23 (21):5781-92 (PMID: 24925318)