Limb-Girdle Muscular Dystrophy Panel
Sequence Analysis and Exon-Level Deletion/Duplication Testing of 30 Genes

Panel Gene List:
ANO5, BVES, CAPN3, CAV3, DES, DMD, DNAJB6, DOK7, DYSF, FKRP*, FKTN, GAA, GMPPB, LMNA, MYOT, POMGNT1**, POMK, POMT1, POMT2, SGCA, SGCB, SGCD, SGCG, TCAP, TNPO3, TOR1AIP1, TRAPPC11, TRIM32, TTN, VCP

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

Clinical Features:
Limb-girdle muscular dystrophies (LGMDs) are muscular dystrophies that are characterized by progressive muscle disease with proximal weakness and wasting greater than distal.[1] Disease onset is variable and ranges from childhood to adulthood. Progression and distribution of muscle weakness and wasting, as well as elevated serum CK, is variable between different subtypes. Clinical involvement is typically limited to skeletal muscle, however, dysarthric speech, cardiomyopathy, respiratory involvement, calf hypertrophy, and exercise-induced myalgia can occur in some individuals.[2] Histopathological findings include muscle degeneration and regeneration. Immunostaining of muscle can distinguish some forms of LGMD, specifically sarcoglycanopathy, calpainopathies, dysferlinopathy and glycosylation defects.[1] The prevalence of LGMD is 2.3-15/100,000 individuals.[3]

Genetics:
LGMDs are inherited in either an autosomal dominant, autosomal recessive, or X-linked manner or they may be the result of a *de novo* variant. Most of these genes encode proteins that function within the nucleus, intermediate filaments, sarcomere, sarcoplasm, or sarcolemma, and are critical to sarcolemma repair and cellular maintenance, trafficking, or signal transduction.

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: FKRP gene, no copy number testing; POMGNT1 gene, only whole gene deletions or duplications may be detected. Missense variants in regions of high homology in the TTN gene may not be reliably identified due to inherent properties of the DNA.

References:

1. Pegoraro, E., Hoffman, E.P. (2012 Aug 30). Limb-Girdle Muscular Dystrophy Overview - GeneReviews® - NCBI Bookshelf, http://www.ncbi.nlm.nih.gov/books/NBK1408/.
2. Mitsuhashi, S., Kang, P.B. (2012). Semin. Pediatr. Neurol. 19, 211-218.
3. Wicklund, M.P. (2013). Continuum (Minneap Minn) 19, 1535-1570.

Limb-Girdle Muscular Dystrophy Panel – 30 Genes

Gene	Inheritance	Disease Associations	Diagnostic Yield in Selected Population(s)
ANO5	AR	Limb-girdle muscular dystrophy (LGMD) type 2L; Miyoshi MD	Founder mutation in the Northern European populations [1, 2]
BVES	AR	Limb-Girdle Muscular Dystrophy 2X	Unknown[32]
CAPN3	AR	LGMD type 2A	~ 30% of LGMD in United States. Founder mutations in the Amish, La Reunion Island, Basque (Spain) and Turkish populations [3, 4]
CAV3	AD	LGMD type 1C	~3% of LGMD in United States [4]
DES	AD/AR	LGMD type 1D; Myofibrillar myopathy 1 (MFM1)	Rare-LGMD1D/1E caused by an intronic-splice site variant[5, 6] ~7% of MFM attributed to variants in the DES gene
DMD	X-linked	Dystrophinopathy (Duchenne / Becker muscular dystrophy)	25-30% of mutations are caused by single point variants or small rearrangements, the majority of which are detectable by this test. 65-70% of pathogenic variant in *DMD* are deletions/duplications. [28,29]
DNAJB6	AD	LGMD type 1E	Rare [7]
DOK7	AR	Congenital myasthenia syndrome, with LGMD presentation	10-21% of Congenital Myasthenia syndrome, Founder mutation in European, Canadian, and Brazilian

			populations[33,34,35,36,37]
DYSF	AR	LGMD type 2B	~19% of LGMD in United States [3, 8, 9]
FKRP *	AR	LGMD type 2; Walker–Warburg syndrome	~70% LGMD2I ~9% alpha-dystroglycanopathies [3, 10]
FKTN	AR	LGMD type 2M; Fukuyama congenital muscular dystrophy	Rare cause of LGMD. ~20% alpha-dystroglycanopathies Includes the Japanese founder mutation in the 3' UTR; does not include the intronic point variant found in the Korean population [11-14]
GAA	AR	Pompe disease (Glycogen storage disorder type II)	c.-32-13 T>G has been reported in up to 42% of alleles in the adult onset form (detectable by this test); a large deletion of exon 18 is common in some European populations [30,31]
GMPPB	AR	Muscular dystrophy-dystroglycanopathy (LGMD), type C; Walker–Warburg syndrome	Rare[15]
LMNA	AD/AR	LGMD type 1B; congenital laminopathy; Emery-Dreifuss muscular dystrophy-AD	~12% of LGMD in United States ~45% of AD-EDMD (unknown for AR-EDMD) Rare in congenital muscular dystrophies [3, 16-19,
MYOT	AD	LGMD type 1A; Myofibrillar myopathy 3 (MFM3)	~26% (69/263) individuals with LGMDhave a variant in the MYOT gene ~9% of MFM had a variants in the MYOT [5, 20]
POMGNT1 **	AR	Walker–Warburg syndrome; Muscle-Eye-Brain disease	~10-22% of the alpha-dystroglycanopathies [14, 21, 22]
POMK	AR	LGMD C12; Walker-Warburg syndrome; Muscular dystrophy-dystroglycanopathy A12	Rare[38]
POMT1	AR	LGMD type 2K; Congenital muscular dystrophy with intellectual disability; Walker–Warburg syndrome	~21-26% of the alpha-dystroglycanopathies [14, 21]
POMT2	AR	LGMD type 2N; Congenital muscular dystrophy with intellectual disability; Walker–Warburg syndrome	~11-29% of the alpha-dystroglycanopathies [14, 23]
SGCA	AR	LGMD type 2D	~9% of LGMD in United States [3, 24]
SGCB	AR	LGMD type 2E	~5% of LGMD in United States [3]
SGCD	AR	LGMD type 2F	~1% of LGMD in United States [3]
SGCG	AR	LGMD type 2C	~3% of LGMD in United States; founder mutation in the North African and Gypsy populations [3, 12]
TCAP	AR	LGMD type 2G	Rare overall; founder variant in the Italian population [12, 19]
TNPO3	AD	LGMD 1F	Rare [25, 26]
TOR1AIP1	AR	LGMD 2Y	Rare[39,40]
TRAPPC11	AR	LGMD 2S	Rare
TRIM32	AR	LGMD type 2H	Rare overall; founder variant in Manitoba Hutterites [4, 27]
TTN	AR	LGMD type 2J; Early onset myopathy with fatal cardiomyopathy	Rare overall; founder variant in the Finnish population [12]
VCP	AD	Inclusion body myopathy	Unknown [42]

* Sequence analysis only (no deletion/duplication testing) of the FKRP gene.

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