THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Limb-Girdle Muscular Dystrophy

Limb girdle muscular dystrophies is a term generally used to describe progessive weakness and wasting restricted to the limb musculature (proximal greater than distal), due to a genetic defect that is distinct from X-linked dystrophinopathy (1). Muscle biopsy can show diffuse variation in fiber size, necrosis, regeneration and fibrosis (1). Onset of symptoms can range from early childhood to late adulthood, and progression and distribution of the weakness and wasting can vary considerably amongst individuals and subtypes (2).

Our Limb Girdle Muscular Dystrophy Panel includes analysis of the 31 genes listed below.

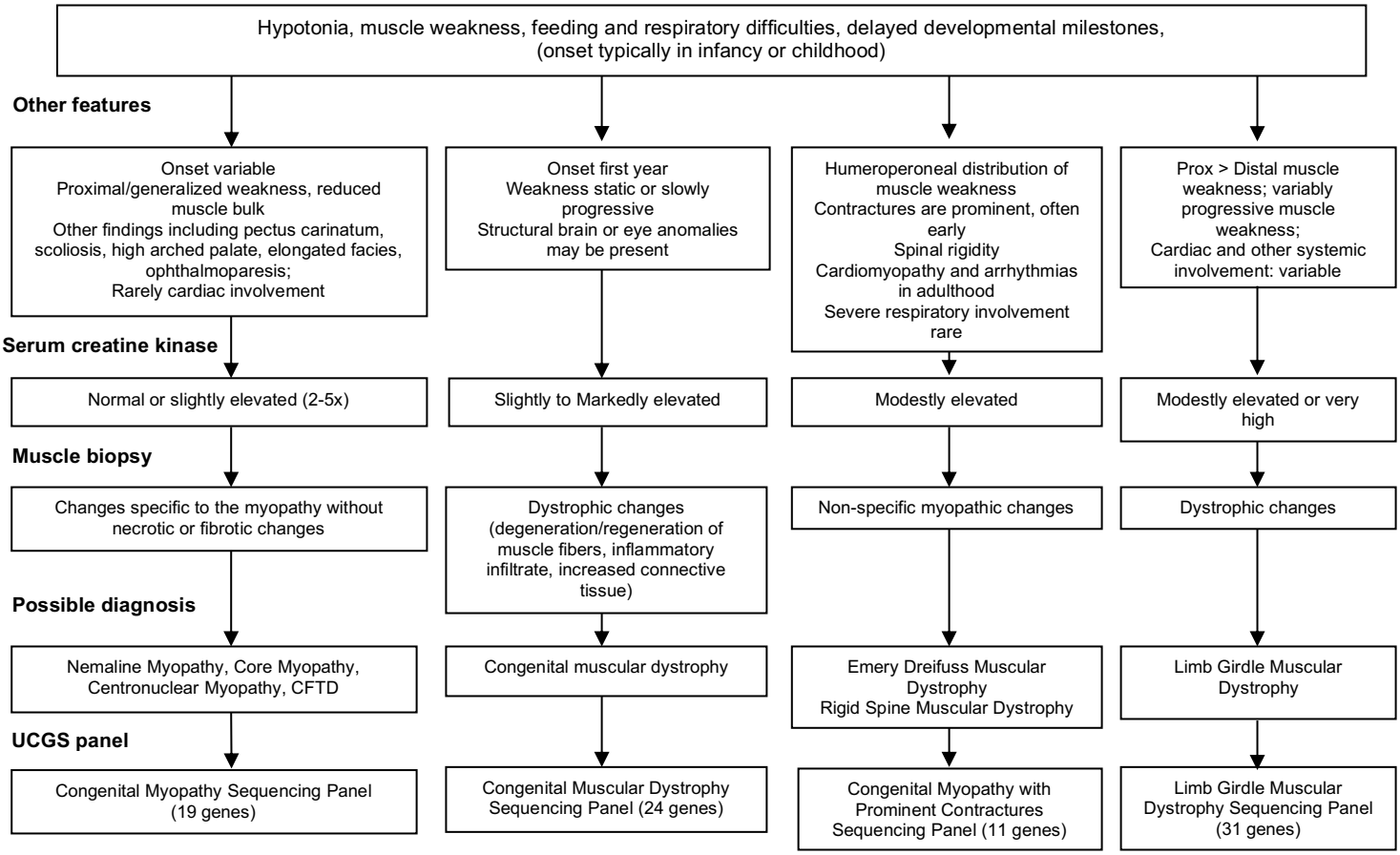
Autosomal Dominant Genes	Autosomal Recessive Genes		
CAV3	ANO5	ISPD	SGCB
DES	CAPN3	LIMS2	SGCD
DNAJB6	DAG1	PLEC	SGCG
FLNC	DYSF	POMGnT1	TCAP
HNRNPDL	FKRP	POMK	TRIM32
LMNA	FKTN	POMT1	TRAPPC11
MYOT	GAA	POMT2	TTN
TNPO3	GMPPB	SGCA	

Genes and Associated Disorder	Inheritance	Clinical Features/Molecular Pathology
ANO5 [OMIM#608662] Muscular dystrophy, limb girdle, type 2L [OMIM#611307]	AR	Penttila *et al,* 2012 identified 11 different recessive mutations in the *ANO5* gene in 25/101 patients with undiagnosed distal calf myopathy or LGMD (3). The function of anoctamin-5 is still being elucidated.
CAPN3 [OMIM#114240] Muscular dystrophy, limb girdle, type 2A [OMIM#253600]	AR	Mutations in *CAPN3* account for approximately 10% of individuals (4) of European descent and up to 80% of individuals in the Basque country (5). *CAPN3* codes for calpain-3, a calcium-activated neutral protease, that is involved in muscle remodeling
CAV3 [OMIM#601253] Muscular dystrophy, limb girdle, type 1C [OMIM607801]	AD and AR	Caveolinopathies account for 1-2% of unclassified LGMD and other phenotypes including isolated HCK, RMD and proximal and distal myopathy (6). Caveolin-3 plays a role in muscle development and physiology.
DAG1 [OMIM#128239] Muscular dystrophy-dystroglycanopathy (limb girdle), type C [OMIM#613808]	AR	Hara *et al*, 2011 identified a homozygous mutation in *DAG1* in a Turkish woman with limb-girdle muscular dystrophy and severe cognitive impairment (7). *DAG1* codes for dystrophic-associated glycoproteins.
DES [OMIM#125660]	AD	McDonald *et al,* 2012 identified a splicing mutation in *DES* by exome sequencing in a family with LGMD (8). Most desmin mutations result in other phenotypes, such as Dilated Cardiomyopathy and Myofibrillar Myopathy.
DNAJB6 [OMIM#611332] Muscular dystrophy, limb girdle, type 1E [OMIM#603511]	AD	Heterozygous mutations in *DNAJB6* have been identified in families with autosomal dominant LGMD (9, 10). *DNAJB6* is part of a family of molecular co-chaperones involved in protecting proteins from irreversible aggregation during protein synthesis or cellular stress.
DYSF [OMIM#603009] Muscular dystrophy, limb girdle, type 2B [OMIM#253601]	AR	Mutations in *DYSF* account for approximately 5% of individuals (2). Dysferlin plays an important role in muscle fiber repair.

FLNC [OMIM#102565]	AD	McDonald *et al,* 2012 identified a nonsense mutation in *FLNC* by exome sequencing in a family with LGMD (8). Most *FLNC* mutations result in other phenotypes, such as Distal and Myofibrillar Myopathy.
FKRP [OMIM#606596] *GMPPB* [OMIM 615320] *ISPD* [OMIM# 614631] *POMT1* [OMIM#607423] *FKTN* [OMIM#607440] *POMK* [OMIM# 616094] *POMGNT1* [OMIM#606822] *POMT2* [OMIM#607439]	AR	Mutations in these genes are associated with a wide spectrum of muscular dystrophies ranging from congenital muscular dystrophies with various eye and brain involvement, to milder forms with later onset limb girdle muscular dystrophies. Relatively few individuals with an LGMD phenotype and mutations in these genes have been reported (2).
GAA [OMIM#606800] Glycogen storage disease II [OMIM#232300]	AR	Late-onset Pompe disease may present from the second to as late as the seventh decade of life with progressive proximal muscle weakness primarily affecting the lower limbs, as in a limb-girdle muscular dystrophy or polymyositis (11).
HNRNPDL [OMIM# 607137]	AD	Heterozygous mutations in *HNRNPDL* have been reported in two families with LGMD type 1G (12).
LIMS2 [OMIM# 607908]	AR	Exome sequencing of two siblings with severe LGMD revealed compound heterozygous mutations in *LIMS2* (13). In addition to features of LGMD these siblings also had distinctive triangular shaped tongues.
LMNA [OMIM#150330] Muscular dystrophy, limb girdle, type 1B [OMIM#159001]	AD	Quijano-Roy et al, 2008 identified 11 different de-novo heterozygous mutations in the *LMNA* gene in 15 children with *LMNA*-related CMD (14). The *LMNA* gene encodes lamin A and lamin C, structural protein components of the nuclear lamina
MYOT (also known as *TTID*) [OMIM#604103] Muscular dystrophy, limb girdle, type 1A [OMIM#159000]	AD	Hauser *et al,* 2000 identified a mutation in the myotilin gene in a large North American Family of German ancestry (15). Mutations in *MYOT* have also been identified in patients with myofibrillar myopathy and spheroid body myopathy. Myotilin is a sarcomeric protein that binds to alpha-actinin and is associated with the Z-line.
PLEC [OMIM#601282] Muscular dystrophy, limb girdle, type 2Q [OMIM#613723]	AR	Gundesli *et al,* 2010 identified a homozygous 9bp deletion in the *PLEC1* gene in a Turkish family with AR LGMD (16). PLEC1 is believed to provide mechanical strength to cells and tissues by acting as a crosslinking element of the cytoskeleton.
SGCA [OMIM#600119] *SGCB* [OMIM#600900] *SGCG* [OMIM#608896] *SGCD* [OMIM#601411]	AR	Mutations in *SGCA, SGCB, SGCG* and *SGCD* account for up to 68% of individuals with childhood onset and 10% of individuals with adult onset (17). The sarcoglycanopathy genes encode proteins that form a tetrameric complex at the muscle cell plasma membrane.
TCAP [OMIM#604488] Muscular dystrophy, limb girdle, type 2G [OMIM#601954]	AR	Moreira *et al,* 2000 identified homozygous T*CAP* mutations in four Brazilian families (18). The TCAP protein is found exclusively in striated and cardiac muscle and serves as both a structural anchor and a signaling center.
TNPO3 [OMIM# 610032]	AD	A heterozygous mutation in *TNPO3* has been described in a large Spanish family with LGMD type 1F (19).
TRAPPC11 [OMIM# 614138]	AR	Homozygous mutations in TRAPPC11 have been reported in a Syrian family with LGMD type 2S, and also in 2 Hutterite families with overlapping phenotypes including neuromuscular dysfunction (20).
TRIM32 [OMIM#602290] Muscular dystrophy, limb girdle, type 2H	AR	Frosk *et al,* 2002 identified a homozygous missense mutation in the *TRIM32* in Manitoba Hutterites with mild autosomal recessive myopathy (21). Saccone *et al,* 2008 identified homozygous mutations in *TRIM32* gene in non-Hutterite patients with LGMD2H (22). TRIM32 is a widely expressed ubiquitin ligase that is localized to the Z-line in skeletal muscle.
TTN [OMIM#188840] Muscular dystrophy, limb girdle, type 2J [OMIM#608807	AR	Hackman *et al,* 2002 identified a homozygous 11bp indel of the TTN gene in a large Finnish family with LGMD (23). This mutation is a common founder mutation in the Finnish population. Mutations in *TTN* have also been described in patients with hereditary myopathy with early respiratory failture, tardive tibial muscular dystrophy, and dilated cardiomyopathy type 1G. Titin is a muscle protein expressed in the cardiac and skeletal muscles and plays a key role in muscle assembly.

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.

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.

Limb Girdle Muscular Dystrophy Panel (31 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2000
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

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