SUB-TYPING LIMB GIRDLE MUSCULAR DYSTROPHIES

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ABSTRACT

Limb girdle muscular dystrophy (LGMD) encompasses a number of different inherited muscular dystrophies that are grouped under the label "limb girdle" because they generally affect the pelvic and shoulder girdles. To date, over 20 different types of LGMD have been identified, each one caused by mutations in a different gene. Despite the predominant involvement of the limb girdle musculature in all types of LGMD, each subtype has some specific features, such as age of onset of symptoms, rate of progression, particular muscles involved, and whether there is heart, respiratory or CNS involvement. These differences should be used to define the most appropriate standard of care and disease management among the different subtypes of LGMDs. It is therefore vital to go beyond the general diagnosis of LGMD and diagnose the patient's particular LGMD subtype in order to determine how most effectively to manage the patient.

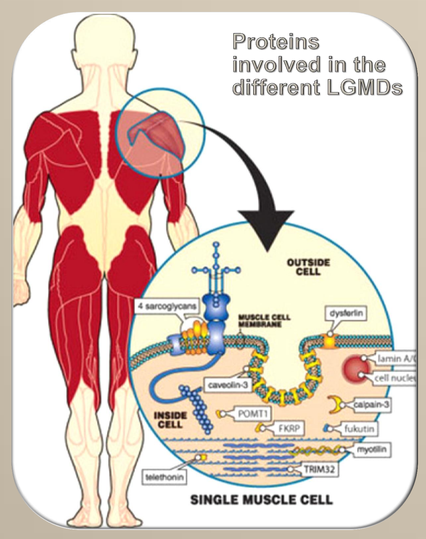
Sub-typing LGMDs is done by gathering information such as family history, clinical presentation including CK levels, immunostaining of muscle biopsy and genetic testing. Here, we present a framework based on specific clinical indicators that helps narrow down the diagnosis to particular subtypes. We also review the antibodies available for immunostaining of muscle biopsies, and genetic testing options that should be performed to obtain a definite diagnosis. In summary, our goal is to provide guidelines to use in the sub-typing of limb girdle muscular dystrophies.

THE DIFFERENT LGMD SUB-TYPES

The LGMDs are a group of heterogeneous diseases that share the characteristic feature of muscle weakness mainly affecting shoulder and pelvic girdles.

Their classification has been revised in recent years due to the elucidation of the underlying genetic mutations in the various subtypes.

LGMD can begin in early childhood (congenital forms), adolescence, young adulthood or even later. Both genders are affected equally.



LGMD forms	Gene symbol	Protein
LGMD1A	MYOT	Myotilin
LGMD1B	LMNA	Lamin A/C
LGMD1C	CAV3	Caveolin-3
LGMD1D	DNAJB6	DNAJB6
LGMD1E	DES	Desmin
LGMD1F	?	?
LGMD1G	?	?
LGMD1H	?	?
LGMD2A	CAPN3	Calpain3
LGMD2B	DYSF	Dysferlin
LGMD2C	SGCG	g-Sarcoglycan
LGMD2D	SGCA	a-Sarcoglycan
LGMD2E	SGCB	b-Sarcoglycan
LGMD2F	SGCD	d-Sarcoglycan
LGMD2G	TCAP	Telethonin
LGMD2H	TRIM32	Trim32
LGMD2I	FKRP	Fukutin-Related Protein
LGMD2J	TTN	Titin
LGMD2K	POMT1	POMT1
LGMD2L	ANO5	Anoctamin 5
LGMD2M	FCMD	Fukutin
LGMD2N	POMT2	POMT2
LGMD2O	POMGnT1	POMGnT1

IMPORTANCE OF SUB-TYPING LGMDS:

Differences in disease management

Among the LGMDs, there are different types of disease presentation, with important implications for management. Prognosis for LGMD is not uniform and therefore timely intervention through early identification of potential complications may improve survival. For example, the risks of cardiomyopathy and pulmonary problem varies greatly among LGMDs, with these issues being frequent in LGMD2I patients, while very uncommon in LGMD2A or 2B patients. Also, anecdotal reports of prednisone use in LGMDs show striking differences among subtypes: while prednisone seems to be beneficial for LGMD2I patients, negative effects have been reported when this treatment was administered to some LGMD2B patients.

Participation in clinical trials

At least half of ongoing clinical trials for these diseases are sub-type specific. Moreover, clinical trials often need mutation information as a pre-requisite for enrollment.

Some potential treatments are mutation-specific

There are some new treatments currently under clinical trials that would benefit patients only with some specific types of mutations (i.e. stop-codon read-through approaches that would only benefit patients with a non-sense mutation). It is thus important to know the patient's specific type of mutation in order to assess whether or not these treatments could help the patient.

Collection of natural history data

In order to obtain a greater understanding of how each disease progresses, it is important to gather natural history data on each specific sub-type.

HOW TO SUB-TYPE LGMD:

NEW

Online LGMD Sub-typing Tool

Making the diagnosis of a particular sub-type of LGMD is challenging, but there are several clues in the patient's clinical presentation that can help differentiate the various sub-types.

The Jain Foundation has developed an algorithm, available as an online question/answer based-tool that uses clinical features to narrow down and help direct final diagnostic testing.

The sub-typing tool can be directly accessed online through the Jain Foundation and the LGMD2I Research Fund websites:
www.jain-foundation.org www.lgmd2ifund.org

In order to sub-type patients with suspected LGMDs , we recommend the following steps:

Assess Clinical Presentation

- Pattern of muscles involved
- Additional clinical features
- Creatine Kinase levels

Run LGMD Sub-typing software

- Use the online tool to narrow down diagnosis

Choose the adequate test to confirm diagnosis

- Immunostaining
- Genetic testing

With appropriate testing, it should be possible to reach a precise diagnosis in around 75% of the LGMD patients.

Below are examples of the application of the tool to different LGMD cases:

Patient 1

Clinical presentation:
- High CK levels, age of onset 14y, waddling gait, calf hypertrophy, difficulty breathing when lying flat, no family history.

Predicted LGMDs:
2I (25.12%)
or
2A (24.53%)

Final diagnosis through genetic testing:
LGMD2I

Patient 2

Clinical presentation:
- High CK, age of onset 22y, distal muscle weakness, no family history, no signs of cardiomyopathy or respiratory issues.

Predicted LGMDs:
2B (57.76%)
or
2A (13.45%)

Final diagnosis through immunostaining and genetic testing:
LGMD2B

Patient 3

Clinical presentation:
- High CK, scapular winging, age of onset 15y, no family history, no signs of cardiomyopathy or respiratory issues.

Predicted LGMDs:
2A (38.93%)
or
2B (21.55%)

Final diagnosis through immunostaining and genetic testing:
LGMD2A

Patient 4

Clinical presentation:
- Very high CK, age of onset 34y, highly athletic prior to onset, difficulty getting up from a chair, no family history.

Predicted LGMDs:
2B (25.46%)
or
2A (18.40%)

Final diagnosis through immunostaining and genetic testing:
LGMD2B

POTENTIAL CHALLENGES AND SOLUTIONS:

It is not always possible to get the appropriate testing done for the reasons cited below, but here are alternatives that help deal with these challenges:

Challenges	Possible Solutions
High Cost of genetic testing	Do comprehensive immunostaining testing to narrow down diagnosis
Insurance does not cover genetic testing	Look for sponsorship by Foundations
Insurance only partially covers cost	Use clinical features algorithm to narrow down diagnosis
Muscle biopsy not available	Use clinical features algorithm to narrow down diagnosis and use genetic testing for confirmation

DIAGNOSTIC TESTS AVAILABLE:

There are several places where immunostaining and genetic testing are available for LGMD proteins and genes.

For immunostaining information, contact University of Iowa Diagnostic Laboratory in Iowa City, IA, or Neuromuscular Clinical Laboratory at Washington University in St. Louis, MO.

			LGMD Gene Sequencing Centers (USA)				
LGMD TYPE	Genes	Antibody Available	Emory Genetics Lab	Athena	Prevention Genetics	Nationwide Children's Hospital	Utah Genome Depot
1A	MYOT/ TTID	✓†	✓	✓	✓	✓	
1B	LMNA	✓‡	✓	✓	✓	✓	
1C	CAV3	✓	✓	✓	✓	✓	✓
1D	DNAJB6	✓†					
1E	DES	✓†					
1F	unknown						
1G	unknown						
1H	unknown						
2A	CAPN3	✓§	✓	✓	✓	✓	
2B	DYSF	✓¶	✓	✓	✓	✓	✓
2C	SGCG	✓**	✓	✓	✓	✓	
2D	SGCA		✓	✓	✓	✓	
2E	SGCB		✓	✓	✓	✓	
2F	SGCD		✓	✓	✓	✓	
2G	TCAP	✓	✓		✓		
2H	TRIM32		✓		✓		
2I	FKRP	††	✓	✓	✓	✓	
2J	TITIN				✓‡‡		
2K	POMT1	††	✓	✓	✓		
2L	ANO5		✓		✓	✓	
2M	FKTN	††		✓	✓		
2N	POMT2	††	✓	✓	✓		
2O	POMGnT1	††	✓	✓	✓		
SG panel	SGCG, SGCA, SGCB, SGCD		$3,700	Call for Pricing	NA	Call for Pricing	NA
LGMD panel	Indicated by color		$5,800	Call for Pricing	~ $7,920	Call for Pricing	$1,500

† Staining pattern can be slightly altered in affected individuals.
‡ Not informative, staining is rarely altered in affected individuals.
§ Used for western blotting, not immunostaining.
¶ Reduced levels by immunostaining can be a secondary effect.
** Can have secondary deficiencies in non-mutated SGs.
†† Alpha dystroglycan can give indirect evidence.
‡‡ Sequencing only available for exons 307-312.

Sponsorship for certain genetic testing can be obtained through some LGMD Foundations:

The Jain Foundation offers **sponsorship of dysferlin protein and mutational analysis** where this diagnostic step is warranted and isn't covered by insurance. For more information, see the Jain Foundation poster "Clinical Studies for Dysferlinopathies (LGMD2B/Miyoshi)".

The LGMD2I Research Fund **supports FKRP genetic testing** for patients presenting clinical features compatible to the dystroglycanopathies (LGMD2I, 2K, 2M, 2N, 2O).

LGMD FOUNDATIONS:

Below is the list of foundations dedicated to finding treatments for the different forms of LGMD.

They are great sources of information about these diseases and of support to the patients.

Foundation	Disease focus	Contact
Coalition to Cure Calpain 3	LGMD2A	www.curecalpain3.org
Jain Foundation	LGMD2B	www.jain-foundation.org
LGMD2I Research Fund	LGMD2I	www.lgmd2ifund.org
Cure CMD	LGMD2I, 2K, 2M, 2N, 2O	www.curecmd.org
Kurt + Peter Foundation	LGMD2C	www.kurtpeterfoundation.org

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