

Practical guidelines for molecular testing in Limb-girdle muscular dystrophies

Disease definition : Limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of muscular dystrophies that are restricted to the limb musculature.

Frequency : LGMD affects approximately 1 in 50.000 persons.

Main clinical symptoms : Patients with limb-girdle muscular dystrophies typically show weakness and wasting restricted to the limb musculature, with the proximal muscles (shoulder, pelvic girdle, upper thighs, and upper arms) being more severely affected than the distal muscles, with relative sparing of the heart and bulbar muscles. The symptoms start in childhood or adulthood, and are slowly progressive. The diseases is caused by progressive dystrophic changes (degeneration/regeneration) of the muscles, which is usually associated with elevated serum creatine kinase (CK) values.

Clinical diagnosis : If LGMD is suspected, serum creatine kinase (CK) concentration should be determined, and a muscle CT scan or MRI, electromyogram (EMG) and muscle biopsy should be performed.

Clinical-biochemical classification :

In the differential diagnosis of LGMD are dystrophinopathies type Duchenne or Becker (DMD gene), Facio-scapulo-humeral muscular dystrophy (FSHD gene), Emery-Dreifuss muscular dystrophy (EMD and LMNA genes), Bethlem-Ullrich disorders (COL6A1, COL6A2, and COL6A3 genes) and the congenital muscular dystrophies (FCMD, POMGNT1, POMT1, POMT1 and

LARGE genes). Protein testing by immunostaining (immunoblot) performed on a muscle biopsy can establish the diagnosis of LGMD subtypes dysferlinopathy, calpainopathy, and sarcoglycanopathy (alpha-sarcoglycanopathy, beta-sarcoglycanopathy, delta-sarcoglycanopathy, gamma-sarcoglycanopathy). However, immunoblot analysis needs to be interpreted with caution, as the analysis is neither completely sensitive (i.e., it can yield false negative results) nor completely specific (i.e., it can yield false positive results).

Inheritance : Whereas the most frequent muscular dystrophy type Duchenne / Becker is X-linked, LGMD can be inherited in an autosomal dominant (40 %) or autosomal recessive (60 %).

Molecular testing : Up to 20 loci with 14 nuclear genes have been shown to be implicated in LGMD. All loci have been classified as LGMD, followed by a number indicating the chronological order.

- **Autosomal dominant LGMD:** 3 genes (TTID, LMNA and CAV3) have been shown to cause autosomal dominant LGMD. As altogether these genes only comprise 38 exons autosomal dominant LGMD is amenable to cost-effective molecular testing.
- **Autosomal recessive LGMD:** 11 genes (CAPN3, DYSF, SGCA, SGCA, SGCD, SGCG, TCAP, TRIM32, FKRP, TTN, POMT1) have been shown to cause autosomal recessive LGMD. Up to 70 % of patients with childhood onset and about 10% of patients with adult onset LGMD have a sarcoglycanopathy with recessive mutations in SGCA, SGCB, SGCD, or SGCG. As each of these genes is small (< 10 exons), sarcoglycanopathies are amenable to molecular diagnosis. The FKRP gene (6 % of mutations, 4 exons), the TCAP gene (3 % of mutations, 2 exons), and the TRIM32 gene (unknown % of mutations, 2 exons) are all small and can therefore be analysed cheaply. The CAPN3, DYSF, and POMT1 genes contribute to more than 20 % of recessive LGMD, but are more expensive to analyse due to their larger

genes (24, 55 and 20 exons, respectively). The TTN gene encoding titin is the largest human gene with 363 exons encoding a protein of 38.138 amino acids, but all affected patients (mostly of Finnish ancestry) have a homozygous 11-bp deletion/insertion in the last exon of TTN.

References

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Table 1. Different types of LGMD

| Type | Type | Gene | Protein | Gene contribution (%) | Number of exons (AA) | Price (Euro) |
|-----------------------|--------|--------|-----------------------------|-----------------------|---|--------------|
| Recessive LGMD | LGMD2A | CAPN3 | Calpain-3 | 10% | 24 exons (822 AA) | 900 |
| | LGMD2B | DYSF | Dysferlin | 10% | 55 exons (2081 AA) | 2400 |
| | LGMD2C | SGCG | Gamma-sarcoglycan | 10% | 8 exons (291 AA) | |
| | LGMD2D | SGCA | Alpha-sarcoglycan | 10% | 10 exons (387 AA) | 670 |
| | LGMD2E | SGCB | Beta-sarcoglycan | Unknown | 6 exons (318 AA) | 500 |
| | LGMD2F | SGCD | Delta-sarcoglycan | Unknown | 9 exons (289 AA) | 600 |
| | LGMD2G | TCAP | Telethonin | 3% | 2 exons (167 AA) | |
| | LGMD2H | TRIM32 | Tripartite motif protein 32 | Unknown | 2 exons (653 AA) | |
| | LGMD2I | FKRP | Fukutin-related protein | 6% | 4 exons (495 AA) | 600 |
| | LGMD2J | TTN | Titin | Unknown | 363 exons (38138 AA) Common 11 bp deletion/insertion | 250 |
| | LGMD2K | POMT1 | O-mannosyl-transferase | Unknown | 20 exons (747 AA) | |
| Dominant LGMD | LGMD1A | TTID | Myotilin | Rare | 24 exons (822 AA) | 1500 |
| | LGMD1B | LMNA | Lamin A/C | Rare | 12 exons (664 AA) | 700 |
| | LGMD1C | CAV3 | Caveolin-3 | Rare | 2 exons (151 AA) | 300 |

