Limb-girdle muscular dystrophies (LGMD) are a highly heterogeneous group of muscle disorders, which first affect the voluntary muscles of the hip and shoulder areas. The definition is highly descriptive and less ambiguous by exclusion: non-X-linked, non-FSH, non-myotonic, non-distal, nonsyndromic, and non-congenital. At present, the genetic classification is becoming too complex, since the acronym LGMD has also been used for a number of other myopathic disorders with overlapping phenotypes. Today, the list of genes to be screened is too large for the gene-by-gene approach and it is well suited for targeted next generation sequencing (NGS) panels that should include any gene that has been so far associated with a clinical picture of LGMD.

The present review has the aim of recapitulating the genetic basis of LGMD ordering and of proposing a nomenclature for the orphan forms. This is useful given the pace of new discoveries. Thirty-one loci have been identified so far, eight autosomal dominant and 23 autosomal recessive. The dominant forms (LGMD1) are: LGMD1A (myotilin), LGMD1B (lamin A/C), LGMD1C (caveolin 3), LGMD1D (DNAJB6), LGMD1E (desmin), LGMD1F (transportin 3), LGMD1G (HNRPDL), LGMD1H (chr. 3). The autosomal recessive forms (LGMD2) are: LGMD2A (calpain 3), LGMD2B (dysferlin), LGMD2C (γ sarcoglycan), LGMD2D (α sarcoglycan), LGMD2E (β sarcoglycan), LGMD2F (δ sarcoglycan), LGMD2G (telethonin), LGMD2H (TRIM32), LGMD2I (FKRP), LGMD2J (titin), LGMD2K (POMT1), LGMD2L (anoctamin 5), LGMD2M (fukutin), LGMD2N (POMT2), LGMD2O (POMTnG1), LGMD2P (dystroglycan), LGMD2Q (pectin), LGMD2R (desmin), LGMD2S (TRAPPC11), LGMD2T (GMPPB), LGMD2U (ISPD), LGMD2V (Glucosidase, alpha ), LGMD2W (PINCH2).

Key words: Limb-girdle muscular dystrophies, LGMD, NGS

Introduction

The term limb-girdle muscular dystrophy refers to a long list of Mendelian disorders characterized by a progressive deterioration of proximal limb muscles. Very often, other muscles are affected, together with the heart and the respiratory muscles. The clinical course and the expressivity may be variable, ranging from severe forms with rapid onset and progression to very mild forms allowing affected people to have fairly normal life spans and activity levels (1). The term LGMD is becoming descriptive and also comprises clinical pictures of different diseases. The original definition was given as muscular dystrophies milder that DMD and inherited as autosomal traits (2). However, the most severe forms with childhood onset also result in dramatic physical weakness and a shortened life-span. The advent of next generation sequencing approaches has accelerated the pace of discovery of new LGMD genes. Ten years ago the list included 16 loci (3), while today the LGMD loci so far identified are thirty-one, eight autosomal dominant and 23 autosomal recessive.

Autosomal dominant LGMD

The LGMD1, i.e. the autosomal dominant forms, have usually an adult-onset and are milder, because affected parents are usually in quite good health at reproductive age. They are relatively rare representing less than 10% of all LGMD. Sometimes, they correspond to particular cases of mutations in genes involved in other disorders, such as myotilin, lamin A/C or caveolin 3 (Table 1).

LGMD1A - LGMD1A may be caused by mutations in the myotilin (MYOT) gene at chr. 5q31.2. The cDNA is of 2.2 kb and contains 10 exons. Myotilin is a Z-disk-associated protein. LGMD1A may be considered as an occasional form of LGMD (4). The first clinical report was in 1994 (5). The gene was identified in 2000 (6), but myotilin mutations have been rather associated with myofibrillar myopathy. LGMD1A is characterized by late
| Gene    | Disease | Locus | Name | Exons | Protein (protein function)                                           | Typical onset | Progression | Cardiomiopathy | sCK | Allelic disorders (OMIM, #)                                                                 |
|---------|---------|-------|------|-------|----------------------------------------------------------------------|---------------|-------------|----------------|-----|-------------------------------------------------------------------------------------------|
| LGMD1A  | 5q31.2  | TTID  | 10   | myotilin (structural, Z disc)                                        | Adulthood     | Slow        | Not observed   | 3-4X| Myopathy, myofibrillar, 3 (602600)                                                          |
|         |         |       |      |       |                                                                      |               |             |                |     | Myopathy, spheroid body (182920)                                                            |
| LGMD1B  | 1q22    | LMNA  | 12   | lamin A/C (structural, fibrous nuclear lamina)                       | Variable (4-38y) | Slow        | Frequent       | 1-6X| Cardiomyopathy, dilated, 1A(115200)                                                        |
|         |         |       |      |       |                                                                      |               |             |                |     | Charcot-Marie-Tooth disease, type 2B1(605588)                                               |
|         |         |       |      |       |                                                                      |               |             |                |     | Emery-Dreifuss muscular dystrophy 2, AD(181350)                                             |
|         |         |       |      |       |                                                                      |               |             |                |     | Emery-Dreifuss muscular dystrophy 3, AR(181350)                                             |
|         |         |       |      |       |                                                                      |               |             |                |     | Heart-hand syndrome, Slovenian type(610140)                                                |
|         |         |       |      |       |                                                                      |               |             |                |     | Hutchinson-Gilford progeria(176670)                                                        |
|         |         |       |      |       |                                                                      |               |             |                |     | Lipodystrophy, familial partial, 2(151660)                                                 |
|         |         |       |      |       |                                                                      |               |             |                |     | Malouf syndrome(212112)                                                                     |
|         |         |       |      |       |                                                                      |               |             |                |     | Mandibuloacral dysplasia(248370)                                                           |
|         |         |       |      |       |                                                                      |               |             |                |     | Muscular dystrophy, congenital(613205)                                                     |
|         |         |       |      |       |                                                                      |               |             |                |     | Restrictive dermopathy, lethal(275210)                                                     |
| LGMD1C  | 3p25.3  | CAV3  | 2    | caveolin 3 (scaffolding protein within caveolar membranes)          | Childhood     | Slow/ moderate | Frequent       | 10X | Cardiomyopathy, familial hypertrophic(192600)                                              |
|         |         |       |      |       |                                                                      |               |             |                |     | Creatine phosphokinase, elevated serum(123320)                                             |
|         |         |       |      |       |                                                                      |               |             |                |     | Long QT syndrome, 9(611818)                                                                |
|         |         |       |      |       |                                                                      |               |             |                |     | Myopathy, distal, Tateyama type(614321)                                                    |
|         |         |       |      |       |                                                                      |               |             |                |     | Rippling muscle disease(606072)                                                            |
| LGMD1D  | 7q36    | DNAJB6| 10   | DnaJ/Hsp40 homolog, subfamily B, member B (chaperone)               | Variable (25-50y) | Slow        | Not observed   | 1-10X| -                                                                                           |
| LGMD1E  | 2q35    | DES   | 9    | desmin (structural; intermediate filament)                           | Adulthood     | Slow        | Frequent       | 5-10X| Muscular dystrophy, limb-girdle, type 2R(61525)                                             |
|         |         |       |      |       |                                                                      |               |             |                |     | Cardiomyopathy, dilated, 11(604765)                                                        |
|         |         |       |      |       |                                                                      |               |             |                |     | Myopathy, myofibrillar, 1(601419)                                                          |
|         |         |       |      |       |                                                                      |               |             |                |     | Scapuloperoneal syndrome, neurogenic, Kaeser type(181400)                                  |
| LGMD1F  | 7q32    | TNPO3 | 23   | transportin 3 (nuclear importin)                                     | Variable (1-58y) | Slow/ moderate | Not observed   | 1-3X| -                                                                                           |
| LGMD1G  | 4q21    | HNRPDL| 9    | Heterogeneous nuclear ribonucleoprotein D-like protein (ribonucleoprotein, RNA-processing pathways) | Variable (13-53y) | Slow        | Not observed   | 1-9X| -                                                                                           |
| LGMD1H  | 3p23-p25|       |      | Variable (10-50y)                                                  | Slow         | Not observed  |                | 1-10X| -                                                                                           |
onset proximal weakness with a subsequent distal weakness. Some patients show nasal and dysarthric speech. Serum CK is normal or mildly elevated. Muscle pathology shows rimmed vacuoles with or without inclusions. Electron microscopy shows prominent Z-line streaming. Cardiac and respiratory involvement occasionally occurs.

**LGMD1B** - LGMD1B is also an occasional LGMD form caused by lamin A/C (LMNA) gene mutations at chr. 1q22 (7). The reference cDNA is of 3 kb and contains 12 exons. The LMNA gene gives rise to at least three splicing isoforms (lamin A, C, lamin AΔ10). The two main isoforms, lamin A and C, are constitutive components of the fibrous nuclear lamina and have different roles, ranging from mechanical nuclear membrane maintenance to gene regulation. The ‘laminopathies’ comprise different well-characterized phenotypes, some of which are confined to the skeletal muscles or skin, while others are multi-systemic, such as lipodystrophy, Charcot-Marie Tooth disease, progeroid syndromes, dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy (EDMD). The LGMD1B is characterized by a symmetric proximal weakness starting from the legs, associated with atrioventricular conduction disturbances and dysrhythmias. CK is normal to moderately elevated. Most patients develop proximal leg weakness, followed by cardiac arrhythmias and dilated cardiomyopathy, with sudden death 20-30 years later. However, there is a continuity between LGMD1B and EDMD (8). Usually the more severe forms of EDMD with a childhood onset have missense mutations, whereas the milder LGMD1B is associated with heterozygous truncating mutations: this may arise through a loss of LMNA function secondary to haploinsufficiency, whereas dominant-negative or toxic gain-of-function loss of LMNA function secondary to haploinsufficiency, whereas the milder LGMD1B is associated with heterozygous truncating mutations: this may arise through a loss of LMNA function secondary to haploinsufficiency, whereas dominant-negative or toxic gain-of-function mechanisms may underlie the EDMD phenotypes.

**LGMD1C** - LGMD1C is caused by mutations in the caveolin 3 gene (CAV3) at chr. 3p25.3. The CAV3 gene encodes a 1.4kb mRNA composed of only two exons. Caveolin-3 is a muscle-specific membrane protein and the principal component of caveolae membrane in muscle cells in vivo: at present this is the only gene in which mutations cause caveolinopathies (9). LGMD1C is characterized by an onset usually in the first decade, a mild-to-moderate proximal muscle weakness, calf hypertrophy, positive Gower sign, and variable muscle cramps after exercise.

**LGMD1D** - Autosomal dominant LGMD mapped to 7q36 has been classified as LGMD1E in OMIM, but as LGMD1D in the Human Gene Nomenclature Committee Database. In the literature there is another LGMD1D/E erroneously mapped to 6q, but we will use the acronym LGMD1D for the 7q-disease and LGMD1E for the 6q-form. LGMD1D is caused by heterozygous missense mutations in the DNAJB6 gene at chr. 7q36.3 (10). The reference cDNA sequence is 2.5kb-long, contains 10 exons and encodes DnaJ homolog, subfamily B, member 6. DNAJ family members are characterized by a highly conserved amino acid stretch (2) called the ‘J-domain’. They exemplify a molecular chaperone functioning in a wide range of cellular events, such as protein folding and oligomeric protein complex assembly (11). Missense heterozygous mutations of DNAJB6 (p.Phe89Ile, p.Phe93Leu and p.Pro96Arg) are all located in the Gly/Phe-rich domain of DNAJB6 leading to insufficient clearance of misfolded proteins. Functional testing in vivo have shown that the mutations have a dominant toxic effect mediated specifically by the cytoplasmic isoform of DNAJB6. In vitro studies have demonstrated that the mutations increase the half-life of DNAJB6, extending this effect to the wild-type protein, and reduce its protective anti-aggregation effect.

DNAJB6 is located in the Z line and interacts with BAG3. Mutations in BAG3 are known to cause myofibrillar myopathy (12). A characteristic pathological finding of LGMD1D is the presence of autophagic vacuoles and protein aggregation. These protein aggregations contain DNAJB6 together with its known ligands MLF1 and HSAP1, and also desmin, αB-crystallin, myotilin, and filamin C, which are known to aggregate in myofibrillar myopathy. These results suggest that the phenotype of LGMD1D also overlaps with that of myofibrillar myopathy.

LGMD1D patients show mildly elevated serum CK levels. The lower limbs are more affected, particularly the soleus, adductor magnus, semimembranosus and biceps femoris. In contrast, the rectus femoralis, gracilis and sartorius and the anterolateral lower leg muscles are mostly spared. DNAJB6 gene mutations may also be associated with distal-predominant myopathy. Symptoms in the upper limbs appear later. Some patients develop calf hypertrophy. Onset ranges from 25 to 50 years, with some patients maintaining ambulation throughout life. No cardiac or respiratory involvement has been reported so far. The pattern of differential involvement could be identified at different stages of the disease process.

**LGMD1E** - For the limb girdle muscular dystrophy originally linked to chr. 6q23 (13) we will use the name LGMD1E, even if it should be considered, more correctly, as a form of autosomal dominant desminopathy or myofibrillar myopathy. This form is also known as dilated cardiomyopathy type 1F (CMD1F). One family previously categorized as having LGMD and dilated cardiomyopathy was reported, indeed, to have the splice site mutation IVS3+3A>G in the desmin (DES) gene at 2q35 (14).
For desmin see also LGMD2R. As in the desminopathies, LGMD1E family members show dilated cardiomyopathy and conduction defects together with progressive proximal muscle weakness starting in the second or third decade. Some family members had a history of sudden death. Serum creatine kinase is mildly elevated (150-350U/l). Muscle pathology may show dystrophic changes, but later the presence of abundant perinuclear or subsarcolemmal granulofilamentous inclusions have been also observed. The study of these inclusions by laser capture microdissection followed by mass spectrometry analysis, led to the identification of the disease-causing mutations in desmin (14).

**LGMD1F** - LGMD1F was originally mapped to a 3.68-Mb interval on chromosome 7q32.1-7q32.2 in a very large Italo-Spanish family. We presented the identification of TNPO3 by whole exome sequencing of four affected family members and the complete refining of the region at the WMS 2012. Data were then published (15): a frame-shift mutation in the transportin 3 (TNPO3) gene is shared by all affected family members with 94% penetrance. The TNPO3 gene is composed of 23 exons and encodes a 923-amino acid protein, also expressed in skeletal muscle. The frame-shifted TNPO3 protein is larger than the wt, since it lacks the predicted stop codon and is found around the nucleus, but not inside. Patients with an onset in the early teens, show a more severe phenotype with a rapid disease course, while adult onset patients present a slower course. They have a prominent atrophy of lower limb muscles, involving especially the vastus lateralis and the ileopsoas muscle (16). Interestingly, some patients present with dysphagia, arachnodactyly and respiratory insufficiency. CK range is 1-3x. No cardiac involvement has been reported.

**LGMD1G** - LGMD1G has been mapped to chr. 4q21. Very recently, the defect in the RNA processing protein HNRPD1 has been identified (17) in two different families by whole exome sequencing. The HNRPD1 gene contains 8 exons and is ubiquitously expressed. The gene product is a heterogeneous ribonucleoprotein family member, which participates in mRNA biogenesis and metabolism. The reduced hnrpd1 in zebrafish produces a myopathic phenotype. Patients show late-onset LGMD associated with progressive fingers and toes flexion limitation (18).

**LGMD1H** - By studying a large pedigree from Southern Italy, a novel LGMD locus has been mapped on chromosome 3p23-p25.1 (19). Most of patients present with a slowly progressive proximal muscle weakness, in both upper and lower limbs, with onset during the fourth-fifth decade of life.

### Autosomal recessive LGMD

The autosomal recessive forms (LGMD2) are much more common, having a cumulative prevalence of 1:15,000 (2) with some differences among countries, depending on the carrier distribution and the degree of consanguinity.

There are recessive genes in which the loss-of-function mutations on both alleles typically result in a LGMD phenotype (ordinary LGMD genes): they correspond to the first 8 forms of LGMD2 (LGMD2A-2H) plus LGMD2L. On the contrary, other genes (occasional LGMD genes) show a phenotypic divergence with some mutations associated with LGMD and other ones determining a more complex disorder. Specific variations in occasional LGMD genes cause the other forms (LGMD2I-2U). The best examples come from dystroglycanopathies in which the LGMD presentation is associated with milder alleles of genes mutated in congenital forms with brain involvement (Table 2).

**LGMD2A** - LGMD2A is caused by Calpain 3 (CAPN3) gene mutations and represents the most frequent LGMD worldwide (20, 21). The CAPN3 gene spans 53kb of genomic sequence at chromosome 15q15.2 and the transcript is composed of 24 exons encoding a 94kDa muscle-specific protein. There is a number of heterozygotes (1:100), carrying many different CAPN3 pathogenic changes. Calpains are intracellular nonlysosomal cysteine proteases modulated by calcium ions. A typical calpain is a heterodimer composed of two distinct subunits, one large (> 80 kDa) and the other small (30 kDa). While only one gene encoding the small subunit has been demonstrated, there are many genes for the large one. CAPN3 is similar to ubiquitous Calpain 1 and 2 (m-calpain and micro-calpain), but contains specific insertion sequences (NS, IS1 and IS2). Calpains cleave target proteins to modify their properties, rather than “break down” the substrates.

The phenotypic spectrum of calpainopathies is very broad, but they are true LGMD. For the clinical course, see also (1).

**LGMD2B** - It is caused by missense or null alleles of the dysferlin (DYSF) gene (22). The DYSF gene spans 233kb of genomic sequence at chr. 2p13.2 and the major transcript is composed of 6,911 nt containing 57 exons in the HGVS recommended cDNA Reference Sequence. Dysferlin is an ubiquitous 230-KDa transmembrane protein involved in calcium-mediated sarcolemma resealing. LGMD2B is the second most frequent LGMD2 form (15-25%) in numerous countries, but not everywhere (23). Muscle inflammation is recognized in dysferlinopathy and dysferlin is expressed in the immune cells.
Table 2. Autosomal recessive limb girdle muscular dystrophy.

| Gene | Clinical phenotype | Typical onset | Progression | Cardiomiopathy | sCK | Allelic disorders (OMIM, #) |
|------|-------------------|--------------|-------------|----------------|-----|--------------------------|
| LGMD2A | 15q15 | CAPN3 | 24 | Calpain 3 ordinary | Adolescence | Moderate/Rapid | Rarely observed | 3-20X |
| LGMD2B | 2p13.2 | DYSF | 56 | Dystrophlin ordinary | Young adulthood | Slow | Possible | 5-40X |
| LGMD2C | 13q12 | SGCG | 8 | γ-Sarcoglycan ordinary | Early childhood | Rapid | Often severe | 10-70X |
| LGMD2D | 17q21.33 | SGCA | 10 | α-Sarcoglycan ordinary | Early childhood | Rapid | Often severe | 10-70X |
| LGMD2E | 4q12 | SGCB | 6 | β-Sarcoglycan ordinary | Early childhood | Rapid | Often severe | 10-70X |
| LGMD2F | 5q33 | SGCD | 9 | δ-Sarcoglycan ordinary | Early childhood | Rapid | Rarely observed | 10-70X |
| LGMD2G | 17q12 | TCAP | 2 | Telethonin ordinary | Adolescence | Slow | Possible | 10X |
| LGMD2H | 9q33.1 | TRIM32 | 2 | Tripartite motif containing 32 ordinary | Adulthood | Slow | Not observed | 10X |
| LGMD2I | 19q13.3 | FKRP | 4 | Fukutin-related protein ordinary | Late childhood | Moderate | Possible | 10-20X |
| LGMD2J | 2q24.3 | TTN | 312 or more | Titin occasional | Young adulthood | Severe | Not observed | 10-40X |
| LGMD2K | 9q34.1 | POMT1 | 20 | Protein-O-mannosyl transferase 1 ordinary | Childhood | Slow | Not observed | 10-40X |
| LGMD2L | 11p13-p12 | ANOS5 | 22 | Anoctamin 5 ordinary | Variable (young to late adulthood) | Slow | Not observed | 1-15X |
| LGMD2M | 9q31 | FKTN | 11 | Fukutin occasional | Early childhood | Moderate | Possible | 10-70X |

(continues)
| Disease   | Locus   | Name   | Exons | Protein product                                      | LGMD phenotype | Typical onset | Progression | Cardiomiopathy | sCK | Allelic disorders                                      |
|-----------|---------|--------|-------|-----------------------------------------------------|----------------|---------------|-------------|----------------|-----|-------------------------------------------------------|
| LGMD2N    | 14q24   | POMT2  | 21    | Protein-O-mannosyl transferase 2                     | occasional     | Early childhood | Slow        | Rarely observed | 5-15X | Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 2 (613150) |
|           |         |        |       |                                                     |                |               |             |                |      | Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 2 (613156) |
| LGMD2O    | 1p34.1  | POMGnT1| 22    | Protein-O-linked mannose beta1,2-N-acetylglucosaminyl transferase | occasional     | Late childhood  | Moderate    | Not observed   | 2-10X | Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 3 (253280) |
|           |         |        |       |                                                     |                |               |             |                |      | Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 3 (613151) |
|           |         |        |       |                                                     |                |               |             |                |      | Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 3 (613157) |
| LGMD2P    | 3p21    | DAG1   | 3     | Dystroglycan                                      | singular       | Early childhood | Moderate    | Not observed   | 20X  |                                                        |
| LGMD2Q    | 8q24    | PLEC1  | 32    | Plectin                                            | singular       | Early childhood | Slow        | Not observed   | 10-50X | Epidermolysis bullosa simplex with pyloric atresia (612138) |
|           |         |        |       |                                                     |                |               |             |                |      | Epidermolysis bullosa simplex, Ogna type (131950)   |
|           |         |        |       |                                                     |                |               |             |                |      | Muscular dystrophy with epidermolysis bullosa simplex (226670) |
| LGMD2R    | 2q35    | DES    | 9     | Desmin (structural; intermediate filament)          | occasional     | Young adulthood | A-V conduction block | 1X    | Muscular dystrophy, limb-girdle, type 2R (615325) |
|           |         |        |       |                                                     |                |               |             |                |      | Cardiomyopathy, dilated, 1I (604765)               |
|           |         |        |       |                                                     |                |               |             |                |      | Myopathy, myofibrillar, 1 (607149)               |
|           |         |        |       |                                                     |                |               |             |                |      | Scapuloperoneal syndrome, neurogenic, Kaeser type (181400) |
| LGMD2S    | 4q35    | TRAPP1 | 30    | Transport protein particle complex 11               | occasional     | Young adulthood | Slow        | Not observed   | 9-16X | Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 14 (615350) |
|           |         |        |       |                                                     |                |               |             |                |      | Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 14 (615351) |
| LGMD2T    | 3p21    | GMPPB  | 8     | GDP-mannose pyrophosphorylase B                     | occasional     | Early childhood-young adulthood | Possible |                |      |                                                        |
| LGMD2U    | 7p21    | ISPD   | 10    | Isoprenoid synthase domain containing               | occasional     | Early / Late   | Rapid/Moderate | Possible | 6-50X | Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 7 (614643) |
| LGMD2V    | 17q25.3 | GAA    | 20    | Alpha-1,4-glucosidase                               | occasional     | Variable       | Variable (Rapid to slow) | Possible | 1-20X | Glycogen storage disease II (232300)               |
| LGMD2W    | 2q14    | LIMS2  | 7     | Lim and senescent cell antigen-like domains 2       | ?              | Childhood      | -           | Possible       | -    |                                                        |
The “dysferlinopathies” include limb-girdle muscular dystrophy type 2B (LGMD2B) and the allelic forms Miyoshi myopathy (MM), which is an adult-onset distal form, and distal myopathy with anterior tibialis onset (DMAT), but varied phenotypes are observed. LGMD2B affects earlier the proximal muscles of the arms whereas MM affects the posterior muscles of the leg.

DYSF gene mutations are associated with heterogeneous clinical pictures ranging from severe functional disability to mild late-onset forms (24). About 25% of cases are clinically misdiagnosed as having polymyositis (25). This classification into separate phenotypes does not reveal true disease differences (26) and the allelic forms are not due to different mutations. Additional factors (e.g., additional mutations in neuromuscular disease genes or sport activities that include maximal eccentric contractions) may worsen the disease expression of causative mutations in dysferlinopathies (27).

WB analysis is very useful and specific (28) when $< 20\%$ level of Dysferlin has been identified, although Dysferlin can also be increased or secondarily reduced. NGS-based testing is preferred due to the huge number of exons to be screened and the lack of mutational hot-spots. mRNA analysis also works from blood, albeit with some splice differences (29).

**LGMD2C-D-E-F**

Loss-of-function mutations in any of the genes encoding the four members of the skeletal muscle sarcoglycan complex, alpha, beta, gamma and delta-sarcoglycan cause LGMD2D, 2E, 2C and 2F, respectively (30-33). Sarcoglycans are components of the dystrophin-complex. They are all N-glycosylated transmembrane proteins with a short intra-cellular domain, a single transmembrane region and a large extra-cellular domain containing a cluster of conserved cysteines.

Sarcoglycanopathies have a childhood onset, similar to intermediate form of Duchenne/Becker dystrophies, and involve both cardiac and respiratory functions. We consider the possibility to classify these forms apart from the other LGMD.

**LGMD2C** - The gamma-sarcoglycan gene spans 144kb of genomic sequence at chromosome 13q12.12 and the transcript is composed of 8 exons. LGMD2C is common in the Maghreb and India (34) for the high allele frequency of 525delT and in gypsies for the C283Y allele. LGMD2C patients may show the absence of $\gamma$-sarcoglycan together with traces of the other non-mutated sarcoglycans.

**LGMD2D** - The alpha-sarcoglycan gene spans 10kb of genomic sequence at chromosome 17q21.33 and the major transcript is composed of 10 exons. The protein product of 387 amino acids and 50kDa was originally named adhalin and contains a “dystroglycan-type” cadherin-like domain that is present in metazoan dystroglycans (35).

**LGMD2E** - The beta-sarcoglycan gene spans 15kb of genomic sequence at chromosome 4q11 and the major transcript is composed of 6 exons. The protein contains of 318 amino acids and weighs 43kDa.

**LGMD2F** - Delta-sarcoglycan is by far the largest LGMD gene, spanning 433kb of genomic sequence at chromosome 5q33.3 and the major transcript is composed of 9 exons. Intron 2 alone spans 164kb, one the largest of the human genome. Delta and gamma sarcoglycan are homologous and of identical size (35kDa).

**LGMD2G** - Mutations in titin cap (Tcap)/Telethonin cause LGMD2G, one of the rarest forms of LGMD (36). Tcap provides links to the N-terminus of titin and other Z-disc proteins. Patients show adolescence-onset weakness initially affecting the proximal pelvic muscles and then the distal legs with calf hypertrophy. A homozygous nonsense mutation in the TCAP gene has been described in a patient a congenital muscular dystrophy. The Tcap gene has also been associated with cardiomyopathy (37), while common variants may play a role in genetic susceptibility to dilated cardiomyopathy. Immunofluorescence and Western blot assays may show a Telethonin deficiency. Full sequencing testing may be cost-effective in all cases, because the gene is composed only of two small exons.

The telethonin gene (TCAP) spans 1.2kb of genomic sequence at chromosome 17q12 and the transcript is composed of 2 exons. The protein product is a 19kDa protein found in striated and cardiac muscle. It binds to the titin Z1-Z2 domains and is a substrate of titin kinase, interactions thought to be critical for sarcomere assembly. Only two different mutations have been described in the TCAP gene in Brazilian patients (36). A mutation (R87Q) was found in a patient with dilated cardiomyopathy (37). Moreover, a human muscle LIM protein (MLP) mutation (W4R) associated with dilated cardiomyopathy (DCM) results in a marked defect in Telethonin interaction/localization (38).

**LGMD2H** - The Tripartite-motif-containing gene 32 (TRIM32) gene spans 14kb of genomic sequence at chromosome 9q33.1 and the transcript is composed of 2 exons, with the first noncoding and the second encoding a 673 aa protein of 72kDa. TRIM32 is a ubiquitous E3 ubiquitin ligase that belongs to a protein family comprising at least 70 human members sharing the tripartite motif (TRIM). The TRIM motif includes three zinc-binding domains, a RING, a B-box type 1 and a B-box type 2, and a coiled-coil region. The protein localizes to cytoplasmic bodies. Although the function of TRIM32 is unknown, analysis of the domain structure of this protein suggests that it may be an E3-ubiquitin ligase (39).
LGMD2H is usually a late-onset condition characterized by proximal weakness, atrophy, and moderately raised levels of creatine kinase. Until 2008, the only LGMD2H mutation was Asp487Asn found in Hutterite families (40). Different TRIM32 mutations were then identified in Italian LGMD patients (41) that accounts for about 3% of LGMD2. The D487N mutation of TRIM32 causes the more severe saccotubular myopathy (STM). Recently, two other LGMD2H patients have been described associated with STM morphotype (42).

LGMD2I, LGMD2K, LGMD2M, LGMD2N, LGMD2O, and LGMD2P

The name dystroglycanopathy has been given to defects due to mutations in six genes (POMT1, POMT2, POMGnTI, FKTN, FKRP and DAG1) (43). These variations reduce dystroglycan glycosylation and cause a wide range of phenotypes ranging from mild congenital muscular dystrophies to dramatic conditions, including brain and eye anomalies (muscle–eye–brain disease or Walker–Warburg syndrome).

LGMD2I - The fukutin-related protein gene spans 12kb of genomic sequence at chromosome 19q13.32 and the transcript is composed of 4 exons, with the first three noncoding. The extracellular part of the dystrophin/utrophin-associated complex is also involved in congenital muscular dystrophies, as well as in LGMD2I. Fukuyama-type congenital muscular dystrophy (FCMD), is one of the most common autosomal recessive disorders in Japan characterized by a congenital muscular dystrophy associated with brain malformation (micropolygria) due to a defect in the migration of neurons caused by mutation in the fukutin gene at 9q31 (44). Mutations in the fukutin-related protein gene (FKRP) at 19q13 cause a form of congenital muscular dystrophy with secondary laminin alpha2 deficiency and abnormal glycosylation of alpha-dystroglycan (45). The same gene is also involved in LGMD2I (15).

All of these diseases are associated with changes in alpha-dystroglycan expression due to a glycosylation defect of alpha-dystroglycan. Dystroglycan is normally expressed and recognized by polyclonal antibodies, but it is abnormally glycosylated and not recognized by monoclonal antibodies directed against certain epitopes. FKRP is resident in the Golgi apparatus. The P448L mutation, that results in CMD1C, causes a complete mislocalization of the protein and the alpha-dystroglycan is not processed, while LGMD2I mutations affect the putative active site of the protein or cause inefficient Golgi localization (46).

LGMD2I mutations appear to be a relatively common cause of LGMD, accounting for at least 10% of all LGMD with either severe or mild phenotypes (47, 48).

LGMD2J - TTN is one of the most complex human genes. The titin gene spans 294,442 bp of genomic sequence at chromosome 2q31 and the major transcript is composed of 363 exons. It encodes the largest protein of the human genome composed of 38,138 amino acids with a physical length of 2 microns. An 11-bp indel mutation in the last titin exon causes titibal muscular dystrophy and Gerull et al. (49) showed that a 2-bp insertion in exon 326 of the TTN gene causes autosomal dominant dilated cardiomyopathy (CMD1G; 604145). A homozygous mutation in the C terminus of titin (FINmaj 11bp deletion/insertion) causes LGMD2J (50). Titin is the giant sarcromeric protein that forms a continuous filament system in the myofibrils of striated muscle, with single molecules spanning from the sarcomeric Z-disc to the M-band (51). Other “titinopathic” clinical phenotypes are titbial muscular dystrophy (TMD, Udd myopathy) (52) or more severe cardiac and muscular phenotypes (53).

CAPN3 binds M-band titin at is7 within the region affected by the LGMD2J mutations and shows a secondary deficiency in the LGMD2J muscle (54). Interactions with titin may protect CAPN3 from autolytic activation and removal of the CAPN3 protease reverses the titin myopathy (55).

The French nonsense mutation (Q33396X) located in Mex6, seems to cause a milder phenotype than the typical FINmaj mutation (51). Due to the huge gene size, NGS sequencing is the only possible way to study this gene. However, the high number of variants and polymorphisms may have a confounding effect on the diagnosis.

LGMD2K - LGMD2K is caused by hypomorphic missense mutations in the POMT1 gene at 9q34, containing 20 exons and spanning about 20 kb. Mutations allowing a residual enzyme activity are linked to mild forms. Different POMT1 alleles, cause congenital muscular dystrophies due to defects of the dystroglycan glycosylation (MDDGC1) and including severe forms with brain and eye anomalies or mental retardation (56-58).

LGMD2L - LGMD2L is caused by mutations in the anoctamin-5 (AN05) gene at 11p14.3 (59). The AN05 gene spans 90,192 bp and contains 22 exons; the coding sequence is 2.7kb for 913 amino acids. Alternative gene names are TMEM16E and GDD1. Anoctamins are a family of calcium-activated chloride channels (60). This form of LGMD2 is one of the most frequent in Northern Europe encompassing 10%-20% of cases (61). The penetrance is probably incomplete, since females are less frequently affected than males. The most common mutation in Northern Europe is c.191dupA in exon 5 (62). Patients are usually ambulant and the onset is in adulthood. They show asymmetric muscle involvement with prevalent quadriiceps atrophy and pain following exercise. CK levels are 5-20x. There is no evidence for contractures,
cardiomyopathy or respiratory involvement. LGMD2L is allelic with the AD gnathodiaphyseal dysplasia (MMD3) (64).

LGMD2M - This is associated with mutations in the fukutin gene (FKTN) at chr. 9q31.2 (65). The FKTN gene spans 82,989 bp and contains 10 coding exons, the main transcript is 7.4kb encoding a protein of 413 amino acids. Also in this case LGMD2M is a milder form caused by at least one hypomorphic missense mutation in a gene that, with both non-functional alleles, is associated with more severe phenotypes (66): WWS, MEB or congenital muscular dystrophies (67). In LGMD2M the CNS is not affected and the intelligence is normal. Patients are hypotonic, may be ambulant and the onset is in early childhood. They show symmetric and diffuse muscle involvement that deteriorates with acute febrile illness. Improvement is seen with steroids. CK levels are 10-50x.

LGMD2N - Mutations in the POMT2 gene, containing 21 exons, at chr. 14q24 cause LGMD2N (68). POMT2 is a second O-mannosyltransferase overlapping with POMT1 expression. POMT2 mutations usually have a dramatic effect: they cause Walker-Warburg syndrome or muscle-eye-brain-like (69), but rarely are associated with LGMD (70). This may occur when the α-dystroglycan glycosylation is only slightly reduced. In these cases the muta-tions are usually missense and the phenotype is characterized by LGMD without brain involvement, very high serum CK.

LGMD2O - It is associated with milder mutations in the POMGnT1 gene at chr. 1p32 (71). Usually mutations in the POMGnT1 gene are associated with more severe phenotypes than LGMD, such as Walker-Warburg syndrome or MEB. A homozygous hypomorphic allele of the POMGnT1 gene was found as a 9-bp promoter duplication (72).

LGMD2P - LGMD2P is caused by specific changes in the dystroglycan (DAG1) gene itself. Recently, Campbell has reported a missense mutation in the dystroglycan gene in an LGMD patient with cognitive impairment (73). This substitution interferes with LARGE-dependent maturation of phosphorylated O-mannosyl glycans on α-dystroglycan affecting its binding to laminin. As a rule the dystroglycanopathies are due to mutations in genes involved in the glycosylation pathway of dystroglycan, but the dystroglycan gene is normal.

LGMD2Q - This form of LGMD is mutation-specific since other mutations in the Plectin (PLEC1) gene at chrom. 8q24.3 cause epidermolysis bullosa simplex (74). LGMD2Q has been identified as a homozygous 9-bp deletion in consanguineous Turkish families (75). The deletion affects an AUG that is only present in a muscle-specific transcript Plectin 1f), while there are many other alternative first exons that are spliced to a common exon 2. These patients produce normal skin plectin and do not show skin pathology. LGMD2Q patients show early-onset non-progressive or slowly progressive LGMD.

LGMD2R - Desmin is the muscle-specific member of the intermediate filament (IF) protein family (76). The desmin (DES) gene at 2q35 contains 9 exons and spans about 8.4 kb. It encodes a 468-amino acid protein. Autosomal dominant mutations in the DES gene are associated with myofibrillar myopathy (14). The overlap with the DES gene has also been claimed for LGMD1E (77). A homozygous splice site mutation has been identified in two Turkish sibs, born of consanguineous parents, in intron 7 of the DES gene (c.1289-2A>G), resulting in the addition of 16 amino acids from residue 428. Since then, other mutations have been identified. The patients have onset in their teens or twenties of progressive proximal muscle weakness and non-specific atrophy affecting both the upper and lower limbs. The serum CK is normal. LGMD2R patients usually show A-V conduction blocks but no cardiomyopathy.

LGMD2S - This is caused by mutation in the transport protein particle complex 11 (TRAPPC11) gene that spans 54,328 bp at chr. 4q35, the mRNA is 4.5kb and contains 30 exons.

Recently, mutations in TRAPPC11 have been identified in a consanguineous Syrian family with an uncharacterized form of LGMD and in five Hutterite individuals presenting with myopathy, ID, hyperkinetic movements and ataxia (78).

TRAPPC11 is a transport protein particle component involved in anterograde membrane transport from the endoplasmic reticulum (ER) to the ER-to-Golgi intermediate compartment (ERGIC) in mammals (79). Mutations identified so far (c.2938G>A/p.Gly980Arg and c.1287+5G>A) cause modifications in TRAPP complex composition, in Golgi morphology and in cell trafficking. The LGMD2S pathogenic mechanism is similar to that causing Danon disease, an X-linked myopathy due to LAMP2 mutations and affecting the secretory pathway (80).

The LGMD2S phenotype ranges from a slowly progressive LGMD with childhood onset and high CK to a syndrome characterized by myopathy but also neurological involvement (ID and ataxia).

LGMD2T - LGMD2T is caused by milder mutations in the GDP-mannose pyrophosphorylase B (GMPPB) gene (81). The GMPPB gene is a small gene of 2,453bp at chr. 3p21. The mRNA is 1.7kb and contains 8 exons. Mutations in the GMPPB gene have been associated with congenital muscular dystrophies with hypoglycosylation of α-dystroglycan and also with LGMD only in three un-
related patients so far reported. The patients from Indian and Egyptian descent presented with microcephaly and intellectual delay. All 3 patients had increased serum creatine kinase and dystrophic findings on muscle biopsy. Muscle biopsy showed hypoglycosylation of DAG1. The English LGMD patient was a 6-year-old boy with exercise intolerance and CK = 3,000 UI. Two missense mutations were identified: p.Asp27His and p.Val330Ile.

**LGMD2U** - This is the form caused by some particular alleles of the isoprenoid synthase domain containing (ISPD) gene. The ISPD gene spans 333kb at chromosome 7p21 and contains 10 exons. ISPD mutations disrupt dystroglycan mannosylation and cause of Walker-Warburg syndrome (82, 83). Mutations in ISPD as well as TMEM5 genes have been associated with severe cobblestone lissencephaly (84). Null alleles of ISPD produce Walker Warburg or cobblestone lissencephaly with brain vascular anomalies, but at least one milder mutation in one allele has been found in LGMD (68 69). We named this forms as LGMD2U. The association between mutations in the ISPD gene and LGMD was, however, older than that of forms 2P-2T, but to avoid discordant definitions among the LGMD2U should be considered as that caused by some alleles of ISPD. LGMD2U is progressive, with most cases with LGMD losing ambulation in their early teenage years, thus following a DMD-like path. In several patients, there is muscle pseudohypertrophy, including the tongue. Respiratory and cardiac functions also decline, resembling other dystroglycanopathies.

**LGMD2V** - This is a proposal to name as LGMD2V an occasional LGMD form that derives from mild mutations of the acid alpha-glucosidase (GAA) gene (85). The GAA gene maps at chr 17q25.3 and comprises 20 exons with a protein product of 953 aa. Defects in GAA are the cause of glycogen storage disease type 2 (GSD2, MIM: 232300). GSD2 is a metabolic disorder with a broad clinical spectrum. The severe infantile form, or Pompe disease, presents at birth with massive accumulation of glycogen in muscle, heart and liver. 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. Final outcome depends on respiratory muscle failure.

**LGMD2W** - This caused by mutations in the LIM and senescent cell antigen-like-containing domain protein 2 (LIMS2/PINCH2) gene at chromosome 2q14. The gene comprises 7 coding exons. It encodes a 341-aa member of a small family of focal adhesion proteins. The encoded protein has five LIM domains, each domain forming two zinc fingers, which permit interactions which regulate cell shape and migration. Patients show a childhood onset LGMD with macroglossia and calf enlargement. They also developed decreased ejection fraction with global left ventricular dysfunction in their 3rd decade, severe quadripareisis and relative sparing of the face, and characteristically a broad based triangular tongue. This form has been presented in a poster session at the ASHG 2013.

The classification of LGMD is becoming too complex. We tried to reorganize the different genes so far described following the traditional nomenclature. However for the autosomal recessive forms there are few letters available. The next forms will be LGMD2X, LGMD2Y and LGMD2Z. We propose, after the LGMD2Z form, the acronyms LGMD2AA, LGMD2AB, LGMD2AC, etc. to avoid renaming consolidated definitions thereby generating even higher confusion.

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**References**

1. Nigro V, Aurino S, Piluso G. Limb girdle muscular dystrophies: update on genetic diagnosis and therapeutic approaches. Curr Opin Neurol 2011;24:429-36.
2. Nigro V. Molecular bases of autosomal recessive limb-girdle muscular dystrophies. Acta Myol 2003;22:35-42.
3. Nigro V, Piluso G. Next generation sequencing (NGS) strategies for the genetic testing of myopathies. Acta Myol 2012;31:196-200.
4. Reilich P, Krause S, Schramm N, et al. A novel mutation in the myotilin gene (MYOT) causes a severe form of limb girdle muscular dystrophy 1A (LGMD1A). J Neurol 2011;258:1437-44.
5. Yamaoka LH, Westbrook CA, Speer MC, et al. Development of a microsatellite genetic map spanning 5q31-q33 and subsequent placement of the LGMD1A locus between D5S178 and II.9. Neuromuscul Disord 1994;4:471-5.
6. Hauser MA, Horrigan SK, Salmikangas P, et al. Myotilin is mutated in limb girdle muscular dystrophy 1A. Hum Mol Genet 2000;9:2141-7.
7. Muchir A, Bonne G, van der Kooi AJ, et al. Identification of mutations in the gene encoding lamin A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). Hum Mol Genet 2000:9:1453-9.
8. Politano L, Carboni N, Madej-Pilarczyk A, et al. Advances in basic and clinical research in laminopathies. Acta Myol 2013;32:18-22.
9. Gazzero E, Bonetto A, Minetti C. Caveolinopathies: translational implications of caveolin-3 in skeletal and cardiac muscle disorders. Handb Clin Neurol 2011;101:135-42.
10. Sarparanta J, Jonson PH, Golzio C, et al. Mutations affecting the cytoplasmic functions of the co-chaperone DNAJB6 cause limb-girdle muscular dystrophy. Nat Genet;44:450-5, S1-2.
11. Chuang JZ, Zhou H, Zhu M, et al. Characterization of a brain-en-

10
riched chaperone, MRJ, that inhibits Huntingtin aggregation and toxicity independently. J Biol Chem 2002;277:19831-8.

12. Lee HC, Cherk SW, Chan SK, et al. BAG3-related myofibrillar myopathy in a Chinese family. Clin Genet 2012;81:394-8.

13. Messina DN, Speer MC, Pericak-Vance MA, et al. Linkage of familial dilated cardiomyopathy with conduction defect and muscular dystrophy to chromosome 6q23. Am J Hum Genet 1997;61:909-17.

14. Greenberg SA, Ralser M, Merchant N, et al. Etiology of limb girdle muscular dystrophy 1D/1E determined by laser capture microdissection proteomics. Ann Neurol 2012;71:141-5.

15. Torella A, Fanin M, Mutarelli M, et al. Next-generation sequencing identifies transportin 3 as the causative gene for LGMD1F. PLoS One 2013;8:e63536.

16. Peterle E, Fanin M, Semplicini C, et al. Clinical phenotype, muscle MRI and muscle pathology of LGMD1F. J Neurol 2013;260:2033-41.

17. Vieira NM, Naslavsky MS, Liciño L, et al. A defect in the RNA-processing protein HNRNPD causes limb-girdle muscular dystrophy 1G (LGMD1G). Hum Mol Genet 2014. [Epub ahead of print]

18. Starling A, Kok F, Passos-Bueno MR, et al. A new form of autosomal dominant limb-girdle muscular dystrophy (LGMD1G) with progressive fingers and toes flexion limitation maps to chromosome 4p21. Eur J Hum Genet 2004;12:1033-40.

19. Bisciglia L, Zuccolotta S, Torraco A, et al. A new locus on 3p23-p25 for an autosomal-dominant limb-girdle muscular dystrophy, LGMD1H. Eur J Neurol 2010;17:636-41.

20. Fanin M, Nascimbeni AC, Fulizio L, et al. The frequency of limb girdle muscular dystrophy 2A in northeastern Italy. Neuromuscul Disord 2005;15:218-24.

21. Pathak P, Sharma MC, Sarkar C, et al. Limb girdle muscular dystrophy type 2A in India: a study based on semi-quantitative protein analysis, with clinical and histopathological correlation. Neurol India 2010;58:549-54.

22. Bashir R, Britton S, Strachan T, et al. A gene related to Caenorhabditis elegans spermatogenesis factor fer-1 is mutated in limb-girdle muscular dystrophy type 2B. Nat Genet 1998;20:37-42.

23. van der Kooi AJ, Frankhuizen WS, Barth PG, et al. Limb-girdle muscular dystrophy 1G (LGMD1G). Hum Mol Genet 2010;19:471-5.

24. Locke M, Tinsley CL, Benson MA, et al. TRIM32 is an E3 ubiquitin ligase for dysbindin. Hum Mol Genet 2009;18:2344-58.

25. Frook P, Weiler T, Nylen E, et al. Limb-girdle muscular dystrophy type 2H associated with mutation in TRIM32, a putative E3-ubiquitin-ligase gene. Am J Hum Genet 2002;70:663-72.

26. Sacco V, Palmieri M, Passamano L, et al. Mutations that impair interaction properties of TRIM32 associated with limb-girdle muscular dystrophy 2H. Hum Mutat 2008;29:240-7.

27. Borg K, Stucka R, Locke M, et al. Intragenic deletion of TRIM32 in compound heterozygotes with sarcotubular myopathy/LGMD2H. Hum Mutat 2009;30:E831-44.

28. Muntoni F, Torelli S, Wells DJ, et al. Muscular dystrophies due to glycosylation defects: diagnosis and therapeutic strategies. Curr Opin Neurol 2014;24:437-42.

29. Kobayashi K, Nakahori Y, Miyake M, et al. An ancient retrotransposon insertion causes Fukuyama-type congenital muscular dystrophy. Hum Mutat 2003;22:1043-6.

30. Nigro V, Piluso G, Belsito A, et al. Identification of a novel sarcoglycan gene at 5p33 encoding a sarcolemmal 35 kDa glycoprotein. Hum Mol Genet 1996;5:1179-86.

31. Noguchi S, McNally EM, Ben Othmane K, et al. Mutations in the dystrophin-associated protein gamma-sarcoglycan in chromosome 13 muscular dystrophy. Science 1995;270:819-22.

32. Lim LE, Duclos F, Broux O, et al. Beta-sarcoglycan: characterization and role in limb-girdle muscular dystrophy linked to 4q12. Nat Genet 1995;11:257-65.

33. Roberds SL, Leturcq F, Allamand V, et al. Missense mutations in the adhalin gene linked to autosomal recessive muscular dystrophy. Cell 1994;78:625-33.

34. Moreira ES, Wiltshire TJ, Faulkner G, et al. Limb-girdle muscular dystrophy type 2G is caused by mutations in the gene encoding the sarcomeric protein telethonin. Nat Genet 2000;24:163-6.

35. Knoll R, Hoshijima M, Hoffman HM, et al. The cardiac mechanical stretch sensor machinery involves a Z-disc complex that is defective in a subset of human dilated cardiomyopathy. Cell 2002;111:943-55.

36. Knoll R, Kostin S, Klee S, et al. A common MLP (muscle LIM protein) variant is associated with cardiomyopathy. Circ Res 2010;106:695-704.

37. Locke M, Tinsley CL, Benson MA, et al. TRIM32 is an E3 ubiquitin ligase for dysbindin. Hum Mol Genet 2009;18:2344-58.

38. Frook P, Weiler T, Nylen E, et al. Limb-girdle muscular dystrophy type 2H associated with mutation in TRIM32, a putative E3-ubiquitin-ligase gene. Am J Hum Genet 2002;70:663-72.

39. Sacco V, Palmieri M, Passamano L, et al. Mutations that impair interaction properties of TRIM32 associated with limb-girdle muscular dystrophy 2H. Hum Mutat 2008;29:240-7.

40. Borg K, Stucka R, Locke M, et al. Intragenic deletion of TRIM32 in compound heterozygotes with sarcotubular myopathy/LGMD2H. Hum Mutat 2009;30:E831-44.

41. Muntoni F, Torelli S, Wells DJ, et al. Muscular dystrophies due to glycosylation defects: diagnosis and therapeutic strategies. Curr Opin Neurol 2014;24:437-42.

42. Kobayashi K, Nakahori Y, Miyake M, et al. An ancient retrotransposon insertion causes Fukuyama-type congenital muscular dystrophy. Nature 1998;394:388-92.

43. Brockington M, Blake DJ, Prandini P, et al. Mutations in the fukutin-related protein gene (FKRP) cause a form of congenital muscular dystrophy with secondary laminin alpha2 deficiency and abnormal glycosylation of alpha-dystroglycan. Am J Hum Genet 2001;69:1198-209.

44. Esapa CT, Benson MA, Schroder JE, et al. Functional requirements for fukutin-related protein in the Golgi apparatus. Hum Mol Genet 2002;11:3319-31.

45. Stensland E, Lindal S, Jonsrud C, et al. Prevalence, mutation spectrum and phenotypic variability in Norwegian patients with Limb Girdle Muscular Dystrophy 2I. Neuromuscul Disord 2011;21:41-6.

46. Mercuri E, Brockington M, Straub V, et al. Phenotypic spectrum associated with mutations in the fukutin-related protein gene. Ann Neurol 2003;53:537-42.

47. Gerull B, Gramlich M, Atherton J, et al. Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. Nat Genet 2002;30:201-4.

48. Udd B, Vihola A, Sarpalanta J, et al. Titinopathies and extension of the M-line mutation phenotype beyond distal myopathy and LGMD2J. Neurology 2005;64:636-42.
51. Penisson-Besnier I, Hackman P, Suominen T, et al. Myopathies caused by homozygous titin mutations: limb-girdle muscular dystrophy 2J and variations of phenotype. J Neurol Neurosurg Psychiatry 2010;81:1200-2.

52. Udd B, Partanen J, Halonen P, et al. Tibial muscular dystrophy. Late adult-onset distal myopathy in 66 Finnish patients. Arch Neurol 1993;50:604-8.

53. Carmignac V, Salih MA, Quijano-Roy S, et al. C-terminal titin deletions cause a novel early-onset myopathy with fatal cardiomyopathy. Ann Neurol 2007;61:340-51.

54. Sarpantara J, Blandin G, Charton K, et al. Interactions with M-band titin and calpain 3 link myosyn (CMYA5) to tibal and limb-girdle muscular dystrophies. J Biol Chem 2010;285:30304-15.

55. Charton K, Daniele N, Vihola A, et al. Removal of the calpain 3 protease reverses the myopathy in a mouse model for titinopathies. Hum Mol Genet 2010;19:4608-24.

56. Beltran-Valero de Bernabe D, Currier S, Steinbrecher A, et al. Mutations in the O-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg syndrome. Am J Hum Genet 2002;71:1033-43.

57. Balci B, Uyanik G, Dincer P, et al. An autosomal recessive limb girdle muscular dystrophy (LGMD2) with mild mental retardation is allelic to Walker-Warburg syndrome (WWS) caused by a mutation in the POMT1 gene. Neuromuscul Disord 2005;15:271-5.

58. Mercure E, Messina S, Bruno C, et al. Congenital muscular dystrophies with defective glycosylation of dystroglycan: a population study. Neurology 2009;72:1802-9.

59. Bolduc V, Marlow G, Boycott KM, et al. Recessive mutations in the putative calcium-activated chloride channel Anoctamin 5 cause proximal LGMD2L and distal MMD3 muscular dystrophies. Am J Hum Genet;86:213-21.

60. Tian Y, Schreiber R, Kunzelmann K. Anoctamins are a family of Ca2+-activated Cl- channels. J Cell Sci;125:4991-8.

61. Witting N, Duno M, Petri H, et al. Anoctamin 5 muscular dystrophy in Denmark: prevalence, genotypes, phenotypes, cardiac findings, and muscle protein expression. J Neurol 2013;260:2084-93.

62. Hicks D, Sarkozy A, Muelas N, et al. A founder mutation in Anoctamin 5 is a major cause of limb-girdle muscular dystrophy. Brain;134:171-82.

63. Tsutsumi S, Kamata N, Vihola A, et al. The novel gene encoding a putative transmembrane protein is mutated in gnathodiaphyseal dysplasia (GDD). Am J Hum Genet 2004;74:1255-61.

64. Penttila S, Palmio J, Suominen T, et al. Eight new mutations and the expanding phenotype variability in muscular dystrophy caused by the putative calcium-activated chloride channel ANO5. J Biol Chem 2007;282:35211-21.

65. Godfrey C, Escolar D, Brockington M, et al. Fukutin gene mutations associated with limb-girdle muscular dystrophy. J Med Genet 2003;40:845-8.

66. Puckett RL, Moore SA, Winder TL, et al. Further evidence of Fukutin mutations as a cause of childhood onset limb-girdle muscular dystrophy without mental retardation. Neuromuscul Disord 2009;19:352-6.

67. de Bernabe DB, van Bokhoven H, van Beusekom E, et al. A homozygous nonsense mutation in the fukutin gene causes a Walker-Warburg syndrome phenotype. J Med Genet 2003;40:845-8.

68. Biancheri R, Falace A, Tessa A, et al. POMT2 gene mutation in limb-girdle muscular dystrophy with inflammatory changes. Biochem Biophys Res Commun 2007;363:1033-7.

69. Godfrey C, Clement E, Mein R, et al. Refining genotype phenotype correlations in muscular dystrophies with defective glycosylation of dystroglycan. Brain 2007;130:2725-35.

70. Saredi S, Gibertini S, Ardissonne A, et al. A fourth case of POMT2-related limb girdle muscle dystrophy with mild reduction of alpha-dystroglycan glycosylation. Eur J Paediatr Neuro 2013. [Epub ahead of print]

71. Clement EM, Godfrey C, Tan J, et al. Mild POMGnT1 mutations underlie a novel limb-girdle muscular dystrophy variant. Arch Neurol 2008;65:137-41.

72. Raduco U, Baets J, Fano O, et al. Promoter alteration causes transcriptional repression of the POMGnT1 gene in limb-girdle muscular dystrophy type 2O. Eur J Hum Genet 2012;20:945-52.

73. Hara Y, Balci-Hayta B, Yoshida-Moriguchi T, et al. A dystroglycan mutation associated with limb-girdle muscular dystrophy. N Engl J Med;364:939-46.

74. Smith FJ, Eady RA, Leigh IM, et al. Plectin deficiency results in muscular dystrophy with epidermolysis bullosa. Nat Genet 1996;13:450-7.

75. Gundesli H, Talim B, Korkusuz P, et al. Mutation in exon 1f of PLEC, leading to disruption of plectin isoform 1f, causes autosomal-recessive limb-girdle muscular dystrophy. Am J Hum Genet;87:834-41.

76. Kouloumenta A, Mavroidis M, Capetanaki Y. Proper perinuclear localization of the TRIM-like protein myospryn requires its binding partner desmin. J Biol Chem 2007;282:5211-21.

77. Cetin N, Balci-Hayta B, Gundesli H, et al. A novel desmin mutation leading to autosomal recessive limb-girdle muscular dystrophy: distinct histopathological outcomes compared with desminopathies. J Med Genet 2013;50:437-43.

78. Bogershausen N, Shahzad N, Chong JX, et al. Recessive TRAPPC11 mutations cause a disease spectrum of limb girdle muscular dystrophy and myopathy with movement disorder and intellectual disability. Am J Hum Genet;93:181-90.

79. Scrivens PJ, Shahzad N, Moores A, et al. TRAPPC2L is a novel, high-confidence conserved TRAPP-interacting protein. Traffic 2009;10:724-36.

80. Nishino I, Fu J, Tanji K, et al. ISPD loss-of-function mutations disrupt dystroglycan glycosylation and cause Walker-Warburg syndrome. Nature genetics 2012;44:575-80.

81. Roscioli T, Kamsteeg EJ, Buyse K, et al. Mutations in ISPD cause Walker-Warburg syndrome and defective glycosylation of alpha-dystroglycan. Nat Genet 2012;44:581-5.

82. Vuillaume-Barrot S, Bouchet-Seraphin C, Chelbi M, et al. Identification of Mutations in TMEMS and ISPD as a Cause of Severe Cobblestone Lissencephaly. Am J Hum Genet 2012;91:1135-43.

83. Preisler N, Lukacs Z, Vinge L, et al. Late-onset Pompe disease is prevalent in unclassified limb-girdle muscular dystrophies. Molecular genetics and metabolism 2013;110:287-9.