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INVITED REVIEW

Genetic basis of limb-girdle muscular dystrophies: the 2014 update

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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. Thity-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 (plectin), 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

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Table 1. Autosomal dominant limb girdle muscular dystrophy.

Gene					Clinical phenotype				
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 (609200)
									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 6 (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(615325)
									Cardiomyopathy, dilated, 1I(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 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 HNRPDL has been identified (17) in two different families by whole exome sequencing. The *HNRPDL* gene contains 8 exons and is ubiquiously expressed. The gene product is a heterogeneous ribonucleoprotein family member, which participates in mRNA biogenesis and metabolism. The reduced hnrpdl in zebrafish prodeces 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 tipically 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					
Disease	Locus	Name	Exons	Protein product	LGMD 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	Dysferlin	ordinary	Young adulthood	Slow	Possible	5-40X	Miyoshi muscular dystrophy 1 (254130) Myopathy, distal, with anterior tibial onset (606768)
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	Cardiomyopathy, dilated, 1L (606685)
LGMD2G	17q12	TCAP	2	Telethonin	ordinary	Adolescence	Slow	Possible	10X	Cardiomyopathy, dilated, 1N (607487)
LGMD2H	9q33.1	TRIM32	2	Tripartite motif containing 32	ordinary	Adulthood	Slow	Not observed	10X	Bardet-Biedl syndrome 11 (209900)
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	Cardiomyopathy, dilated, 1G (604145) Cardiomyopathy, familial hypertrophic, 9 (613765) Myopathy, early-onset, with fatal cardiomyopathy (611705) Myopathy, proximal, with early respiratory muscle involvement (603689) Tibial muscular dystrophy, tardive (600334)
LGMD2K	9q34.1	POMT1	20	Protein-O-mannosyl transferase 1	occasional	Childhood	Slow	Not observed	10-40X	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 1 (236670) Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 1 (613155) Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 1 (609308)
LGMD2L	11p13-p12	ANO5	22	Anoctamin 5	ordinary	Variable (young to late adulthood)	Slow	Not observed	1-15X	Gnathodiaphyseal dysplasia (166260) Miyoshi muscular dystrophy 3 (613319)
LGMD2M	9q31	FKTN	11	Fukutin	occasional	Early childhood	Moderate	Possible	10-70X	Cardiomyopathy, dilated, 1X (611615) Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 4 (253800) Muscular dystrophy-dystroglycanopathy (congenital without mental retardation), type B, 4 (613152)

(*continues*)

Table 2. (*follows*).

Gene					Clinical phenotype					
Disease	Locus	Name	Exons	Protein product	LGMD phenotype	Typical onset	Progression	Cardiomiopathy	sCK	Allelic disorders (OMIM, #)
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(601419)
										Scapuloperoneal syndrome, neurogenic, Kaeser type(181400)
LGMD2S	4q35	TRAPPC11	30	Transport protein particle complex 11	occasional	Young adulthood	Slow	Not observed	9-16X	
LGMD2T	3p21	GMPPB	8	GDP-mannose pyrophosphorylase B	occasional	Early childhood-Young adulthood		Possible		Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 14 (615350)
										Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 14 (615351)
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 ψ-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 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 sarcotubular 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, POMGnT1, 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 tibial 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 sarcomeric 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 tibial 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 myopathology (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 (*ANO5*) gene at 11p14.3 (59). The *ANO5* 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.191 dupA in exon 5 (62). Patients are usually ambulant and the onset is in adulthood. They show asymmetric muscle involvement with prevalent quadriceps 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 dysphasia (63) and with AR distal myopathy (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. There is also evidence for spinal rigidity, contractures and cardiomyopathy and respiratory involvement.

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 mutations 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 promotor duplication (72).

LGMD2P - LGMD2P is caused by specific changes of 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: pAsp27His 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 quadriparesis 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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