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Review

Where do we stand in trial readiness for autosomal recessive limb girdle muscular dystrophies?

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Abstract

Autosomal recessive limb girdle muscular dystrophies (LGMD2) are a group of genetically heterogeneous diseases that are typically characterised by progressive weakness and wasting of the shoulder and pelvic girdle muscles. Many of the more than 20 different conditions show overlapping clinical features with other forms of muscular dystrophy, congenital, myofibrillar or even distal myopathies and also with acquired muscle diseases. Although individually extremely rare, all types of LGMD2 together form an important differential diagnostic group among neuromuscular diseases. Despite improved diagnostics and pathomechanistic insight, a curative therapy is currently lacking for any of these diseases. Medical care consists of the symptomatic treatment of complications, aiming to improve life expectancy and quality of life. Besides well characterised pre-clinical tools like animal models and cell culture assays, the determinants of successful drug development programmes for rare diseases include a good understanding of the phenotype and natural history of the disease, the existence of clinically relevant outcome measures, guidance on care standards, up to date patient registries, and, ideally, biomarkers that can help assess disease severity or drug response. Strong patient organisations driving research and successful partnerships between academia, advocacy, industry and regulatory authorities can also help accelerate the elaboration of clinical trials. All these determinants constitute aspects of translational research efforts and influence patient access to therapies. Here we review the current status of determinants of successful drug development programmes for LGMD2, and the challenges of translating promising therapeutic strategies into effective and accessible treatments for patients.

Keywords: LGMD2; Translational research; Outcome measures; Clinical trials; Patient registries

1. Introduction

The term limb girdle muscular dystrophy (LGMD) includes a heterogeneous group of genetic disorders characterised by progressive muscle weakness and wasting involving mainly the pelvic, shoulder girdle and proximal limb muscles. LGMDs were first described as a distinct nosological entity in 1954 by John Walton and Frederick Nattrass [1]. The first responsible gene was identified in 1994 in the laboratory of Kevin Campbell [2]. Since then, at least 8 different forms of autosomal dominant LGMD (LGMD1) and more than 20 distinct forms of autosomal recessive LGMD (LGMD2) (Table 1) have been characterised that also encompass other allelic disorders.

The overall frequency of LGMD, autosomal dominant and autosomal recessive, has been shown to vary within different populations and has been estimated to be around 20–40/1.000.000 [18].

The most frequent presentation is proximal weakness with onset in the second decade of life. However, a broadening phenotypic spectrum highlights the importance of considering LGMD2 as a differential diagnosis in almost any patient presenting with primary muscle weakness. A precise genetic diagnosis is critical, as it allows more accurate follow-up, the prevention of known possible complications, and appropriate genetic counselling for family members. Although new therapeutic concepts are rapidly developing, there is currently no licenced treatment for any form of LGMD, except for LGMD2V, which is generally referred to as Pompe disease. It is therefore important to review the translational research pathway for LGMD2 in order to identify bottlenecks that may currently be hindering the development of promising treatments. The key stages of translational research are outlined in Fig. 1, demonstrating how basic science research, clinical research and clinical care are distinct but interdependent stages of translational research. The

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Table 1
Molecular genetics and main clinical features of LGMD2s [3–5]. DGC: Dystrophin glycoprotein complex. UL: upper limbs. LL: Lower limbs.

Disease name/gene	Protein function	Populations with founder mutations	CK	Onset	Progression (wheelchair bound)	Main clinical features	Complications		
							Contractures	Respiratory involvement	Cardiomyopathy (or other cardiac involvement)
LGMD2A – CAPN3	Calcium-sensitive protease involved in muscle re-modelling	Amish [6], Basque (Spain) [7], Northern Italy [8], Germany and eastern Europe [9]	5–80× but can be normal	2–40 yrs (8–15 yrs)	Moderate-rapid (11–28 years after onset)	Posterior thigh and scapular weakness common; often hip abductor sparing – calf atrophy	Yes	Rare	Rare
LGMD2B – DYSF	Structure and signalling function, involved in membrane repair	Libyan Jewish [10]	Often >100×	17–23 yrs	Slow	Proximal and/or distal weakness, little shoulder involvement – calf hypertrophy rare	Yes (ankles)	Rare	Not frequent
LGMD2C – SGCG	Structural components of the DGC	North Africans; Gypsies [11,12]	10–70×	3–15 yrs (8.5 yrs) Complete deficiency: difficulty run, walk	Rapid (~15 years after onset)	Proximal weakness – calf hypertrophy and scapular winging common	Yes	Yes (frequent)	Yes (rare in 2D)
LGMD2D – SGCA									
LGMD2E – SGCB		Amish [13]		Adolescent – young adulthood Partial deficiency: cramps, exercise intolerance					
LGMD2F – SGCD		Brazilian [7]							
LGMD2G -TCAP	Structural protein of the sarcomere		3–17×	9–15 yrs	Slow-moderate (~18 y after onset)	Prominent distal involvement (UL proximal, LL proximal and distal)			Yes
LGMD2H – TRIM32	E3 ubiquitin ligase involved in the differentiation of muscle stem cells and in muscle regeneration	Manitoba Hutterites [14]	4–30×	1–9 yrs	Slow (late in life)	Proximal LL, neck and facial weakness – muscle wasting			No
LGMD2I – FKRP	Involved in the glycosylation of alpha-dystroglycan		10–20×	1.5–27 yrs (11.5 yrs)	Slow-moderate (23–26 y after onset)	Mainly proximal weakness, muscle hypertrophy, BMD/DMD-like	Rare (scoliosis in childhood onset)	Yes	Yes
LGMD2J – TTN	Structural protein of the sarcomere.	Finland [15]	10–40×	5–25 yrs	Slow-moderate (20 y after onset)	Proximal weakness, ankle contractures	No	Yes	No
LGMD2K – POMT1	Involved in the glycosylation of alpha-dystroglycan	Turkish [16]	20–40×	1–3 yrs	Slow (1 patient – 17 y)	Mild weakness, proximal > distal – hypertrophy of calves and thighs	Yes (ankles, neck scoliosis)	Yes	Not frequent
LGMD2L – ANO5	Calcium activated chloride channel localised in the endoplasmic reticulum, function unknown	Northern European [17]	10–50×	3rd decade	Slow	Proximal pelvic – femoral or distal weakness in lower limbs, atrophy of quadriceps, hamstrings and biceps	Yes (wrist, fingers, ankles)	No	No
LGMD2M – FKTN	Involved in the glycosylation of alpha-dystroglycan		4–50×	Early childhood	Moderate	Proximal weakness, LL > UL – calves, thighs and triceps hypertrophy	Yes (scoliosis in childhood onset)	Yes	Not frequent
LGMD2N – POMT2	Involved in the glycosylation of alpha-dystroglycan		4–50×	Early childhood	Moderate (20 years – 1 patient)	No weakness – scapular winging and mild lordosis; intellectual disability – calf hypertrophy	Yes (scoliosis)	Yes	Not frequent
LGMD2O – POMGNT1	Involved in the glycosylation of alpha-dystroglycan		Normal to 2×	10–15 years	Moderate (19 y – 1 patient)	Weakness proximal > distal – hypertrophy of calves and quadriceps; wasting of hamstrings and deltoids	Yes (ankles)		No
LGMD2P – DAG1	Structural component of the DGC, basement membrane receptor		20×	Early childhood	Moderate (24 y – 1 patient)	Developmental delay without structural brain anomalies	Yes (ankles, lumbar lordosis)		No
LGMD2Q – PLEC	Component of intermediate filaments providing mechanical strength to cells		10–50×	2–3 yrs	Slow	Proximal weakness and generalised muscle atrophy	Yes		
LGMD2R – DES	Member of the intermediate filament protein family		Normal	Young adulthood	Slow	Proximal weakness and generalised muscle atrophy	No		Not frequent (conduction defects)
LGMD2S – TRAPPC11	Component of a protein complex involved in intracellular vesicle trafficking		9–16×	Childhood	?	Shoulder girdle more affected than hip girdle, possible hyperkinetic movement disorder, ataxia and developmental delay	Scoliosis	Possible restrictive	Not frequent
LGMD2T – GMPPB	Involved in the glycosylation of alpha-dystroglycan		Normal to 2×	Early childhood to young adulthood	Slow	Developmental delay, exercise intolerance, possible seizures	Ankles	Mild	
LGMD2U – ISPD	Involved in the glycosylation of alpha-dystroglycan		6–50×		?	Proximal weakness and muscle pseudo hypertrophy		Yes	Yes
LGMD2V – GAA	Lysosomal enzyme involved in the hydrolysation of glycogen		Normal to 20×	Variable	Variable	Proximal weakness, respiratory insufficiency	No	Yes	Not frequent in adults
LGMD2W – LIMS2	Mediates adhesion between cells and the extracellular matrix		10×	Childhood	?	Proximal weakness, macroglossia, calf hypertrophy			Yes

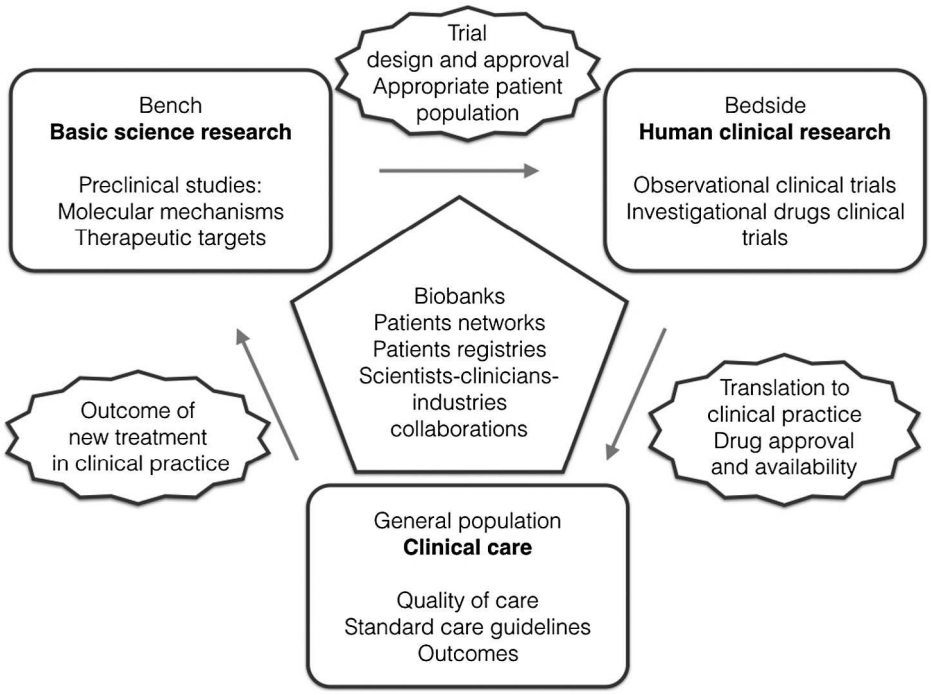

Fig. 1. Key Stages of Translational Research. This figure demonstrates how basic science research, clinical research and clinical care are distinct but interdependent stages of translational research. The main challenges involved in the progression from one stage to another as well as enabling pathways and networks are highlighted and discussed in this review.

main challenges involved in the progression from one stage to another, as well as enabling pathways and networks, are highlighted and discussed in this review.

Collaborative efforts between stakeholder groups involved in translational research in LGMD2 should aim to overcome existing bottlenecks. Strengthened collaboration between academic researchers, the pharmaceutical industry and patient advocacy groups is an essential prerequisite for successful drug development programmes for patients with LGMD2.

Basic research in the last 15–20 years not only improved our knowledge of the aetiology but also of the pathogenesis of a growing number of LGMD2s. Understanding the molecular mechanisms of disease is often the first step towards the identification of possible targets for drug development. Once a potential target has been identified, the next step is to demonstrate in pre-clinical *in vitro* and *in vivo* experiments the principal mechanism and efficacy of a candidate compound. Possible therapeutic strategies that are currently considered for LGMD2 include gene therapy using viral vectors, RNA modification through exon skipping and stop codon read-through, cell therapy, and pharmacological treatments. Once a potential compound or therapeutic strategy has been identified, safety needs to be demonstrated in toxicology studies in animals before a clinical trial can be initiated in humans. Available animal models for LGMD2s are listed in Table 2.

Designing a clinical trial is the next challenge in drug development programmes for rare diseases like LGMD2. Typical obstacles include the lack of natural history data due to the paucity of diagnosed patients, the absence of care standards, which again can affect the natural history of a disease, and the lack of validated and clinically meaningful outcome measures. For all these reasons, clinical trials in LGMD2 have been very limited (Table 3), even for the more common forms, as it has been difficult to establish homogeneous groups of patients to appropriately power clinical studies. International collaboration between physicians and patient groups, often on a global scale, is therefore required to diagnose and recruit sufficient numbers of patients with specific forms of LGMD2 for natural studies, as is currently happening for patients with dysferlinopathy (LGMD2B) (ClinicalTrials.gov NCT01676077). Natural history studies are a critical prerequisite for the development of interventional therapies, as they provide the relevant knowledge of the course of a disease without an intervention. They are also used to develop and apply standardised outcome measures for assessing changes over time, which form the basis for monitoring therapeutic efficacy in patients undergoing an experimental treatment. Few validated outcome measures are currently available for distinct forms of LGMD2 and several international efforts are trying to address this for the most common forms of LGMD2. In parallel to developing functional outcomes obtained by physical assessments, natural history studies are also aiming to characterise serum, urine and imaging biomarkers.

Because the recruitment of sufficient numbers of patients for both natural history studies and interventional trials is particularly challenging in the case of LGMD2, working with patient organisations and patient registries is essential for the collection of demographic and clinical data. Patient organisations can help disseminate accurate information about ongoing research projects and can reach out to patients who are potentially eligible for clinical trials.

Table 2
Available animal models for LGMD2s.

Disease – gene	Species	Genotype	Phenotype	Reference
LGMD2A – CAPN3	Mouse	*Capn3-/-*	Progressive mild muscular dystrophy. Dystrophic features at histologic analysis, with inflammatory infiltration	[19]
		Capn3-/- (C3KO)	Generalised atrophy. Muscle histology shows small foci of muscular necrosis	[20]
LGMD2B – DYSF	*D. melanogaster*	*mfr* mutation	Infertility	[21]
	C. elegans	*Fer-1* mutation	Infertility, altered gene expression of muscle enriched genes	[22]
	Zebrafish	Morpholino knockout of Dysf	Altered development and muscle disorganisation	[23]
		Morpholino knockout of Dysf and annexin A6	Myopathy due to altered sarcolemmal repair	[24]
	Mouse	SJL/J natural model	Proximal limb muscular dystrophy and trunk weakness, increased risk of sarcomas	[25]
		A/J natural model	Progressive mild muscular dystrophy and susceptibility to infections	[26]
		Dysf^{prmd}	A/J mice backcrossed on C57BL/6 background: progressive mild muscular dystrophy and no complement deficiency	[27]
LGMD2C – SGCG	Zebrafish	Morpholino knockout of delta-SG	Severe muscle and cardiac phenotype	[28]
	Mouse	*Sgcg-/-*	Pronounced dystrophic muscle changes in early life. Cardiomyopathy by the age of 20 weeks, shortened life span	[29]
LGMD2D – SGCA	Mouse	*Scga-/-*	Progressive muscular dystrophy and ongoing muscle necrosis with age, a hallmark of the human disease	[30]
LGMD2E – SGCB	Mouse	*Sgcb-/-*	Muscular dystrophy and cardiomyopathy	[31]
LGMD2F – SGCD	*D. melanogaster*	Three different δ-sarcoglycan deletion mutants	Reduced life span with heart and muscle dysfunction	[32]
	Mouse	*Scgcd-/-*	Muscular dystrophy and cardiomyopathy	[31]
		Scgcd-/-	Cardiomyopathy and muscular dystrophy	[33]
	Hamster	BIO14.6	Muscular dystrophy and cardiomyopathy	[34]
LGMD2G -TCAP	Zebrafish	Morpholino knockout of Tcap	Deformed muscle structure and impaired swimming ability	[35]
	Mouse	*Tcap-/-*	Abnormal myofibre size variation with central nucleation, decline in the ability to maintain balance, stiffness	[36]
LGMD2H – TRIM32	*D. melanogaster*	l(2)thin [l(2)tn]	tn mutant larvae show progressive muscular degeneration	[37]
	Mouse	*Trim32-/-* (T32KO)	Mixed myopathic and neurogenic phenotype, with dystrophic muscle and reduction of neurofilaments and myelination	[38]
		D489N KI	Phenotype overlapping Trim32-/- mouse	[39]
LGMD2I – FKRP	Zebrafish	Morpholino knockout of Fkrp	Reduced alpha-DG glycosylation, developmental defects in somitic structure, muscle fibre organisations and eye morphology	[40,41]
		Morpholino knockout of Fkrp	Altered muscle structure, altered laminin expression, notochord defect	[42]
	Mouse	Fkrp-/-	Embryonic lethal	[43]
		L276I KI	Late onset muscular dystrophy	[44]
		P448L KI	Reduced glycosylation of alpha-DG; severe phenotype with muscle, eye and brain developmental defects	[43]
LGMD2J – TTN	Zebrafish	*erzschlag* mutant	Abnormalities in both cardiac and skeletal muscles	[45]
	Mouse	IG KO mice (lacking Ig TTN domain)	Kyfosis, atrophic slow muscles	[46]
	Mouse	*Ttn*$^{tm1.1Isrd}$	Heterozygous mice: distal muscular dystrophy with onset at 9 months: few muscles involved. Homozygous mice: dystrophic muscle with onset at 1 month, generalised at 6 months	[47]
LGMD2K – POMT1	*D. melanogaster*	Pomt1 ko	Larvae mutant for either *Pomt1*, *Pomt2*, or double mutant for both: muscle attachment and muscle contraction phenotypes identical to those associated with reduced Dg function	[48]
		rotated abdomen (rt) ko	High myoblast density and position derangement, which result in apoptosis, muscle disorganisation, and muscle cell defects	[49]
	Mouse	*Pomt1-/-*	Embryonic lethal	[50,51]

(*continued on next page*)

Table 2 (*continued*)

Disease – gene	Species	Genotype	Phenotype	Reference
LGMD2M – FKTN	Zebrafish	Morpholino knockout of Fukutin	Altered muscle structure, altered laminin expression, notochord defect	[42]
	Mouse	*Fukutin-/-*	Embryonic lethal	[52]
LGMD2N – POMT2	*D. melanogaster*	Pomt2 ko	Larvae mutant for either *Pomt1*, *Pomt2*, or double mutant for both: muscle attachment and muscle contraction phenotypes identical to those associated with reduced DG function	[48]
	D. melanogaster	*twisted (tw) ko*	High myoblast density and position derangement, which result in apoptosis, muscle disorganisation, and muscle cell defects	[49]
	Mouse	*Pomt2-/-*	Embryonic lethal because of brain malformation	[53]
LGMD2O – POMGNT1	Mouse	*POMGnT1-/-*	Reduce glycosylation of alpha-DG, multiple developmental defects in muscle, eye, and brain	[54,55]
LGMD2P – DAG1	Zebrafish	V567D KI	Muscle shows disorganised terminal cisternae of sarcoplasmic reticulum, brain abnormalities, ocular defects	[56]
	Mouse	*Dg-/-*	Embryonic lethal	[57]
		T190M KI mice	Muscular dystrophy, centrally located nuclei, clasping phenotype	[58]
LGMD2Q – PLEC	Mouse	*Plec-/-*	Increased intermediate filament network and sarcomere dynamics, reduced myotube resilience following mechanical stretch	[59]
LGMD2R – DES	Zebrafish	Morpholino knockout of desmin	Smaller larvae, with diminished swimming activity	[60]
	Mouse	*Desmin-/-*	Disruption of skeletal muscle architecture and myocardial degeneration	[61,62]
LGMD2T – GMPPB	Zebrafish	*Gmppb-/-*	Structural muscle defects with decreased motility, eye abnormalities and reduced glycosylation of alpha-DG	[63]
LGMD2V – GAA	Mouse	*GAA -/-*	Dysregulation in glycogen metabolism	[64]

Here we review the challenges of translational research for LGMD2s, from basic research to drug development, with a focus on the current status of clinical trials and on clinical trial readiness for these rare diseases.

2. Effects of genetic and clinical variability on disease prognosis

2.1. Aetiology and epidemiology

The genetic classification of LGMD2 has become increasingly complex over the years and even more so since the application of next generation sequencing technologies. The current classification is shown in Table 1 [3].

To distinguish the autosomal recessive forms from the forms with dominant inheritance, the dominant forms have been named LGMD1 and the recessive forms LGMD2, followed by a letter assigned in alphabetical order based on the timeline of gene locus or gene identification. LGMD2A was accordingly the first recessive form of LGMD with an identified gene locus [74].

Patients with LGMD2 generally share weakness of the shoulder and pelvic girdle muscles as the main clinical symptom. Many forms show a wider spectrum of clinical symptoms that can include cardiac and respiratory problems, joint contractures, and, in some childhood onset forms, general developmental delay. The most frequent age of onset is in the second to third decade, but can vary from the first years of life in LGMD2K, LGMD2M or LGMD2N, up to the 5th decade of life in some cases of LGMD2L. Progression is generally slow, but in some forms, such as calpainopathy (LGMD2A) or sarcoglycanopathies (LGMD2C-F), it can lead to the loss of ambulation in less than 15 years from the presentation of first symptoms (Table 1).

The prevalence of each single form of LGMD2 is currently difficult to evaluate as all forms are individually very rare, and, for many subtypes, genetic diagnoses have only been recently established. The overall frequency of all forms of LGMD, autosomal recessive and autosomal dominant, is estimated to be around 20–40/1.000.000 [18], and has been shown to vary in different populations, from a frequency of 8/1.000.000 in the Netherlands [75] and 22/1.000.000 in Northern England [76], to 69/1.000.000 in the Basque country (Spain) [7].

In Northern England, all forms of LGMD together account for about 6% of patients who are diagnosed with genetic muscle diseases [76].

Single disease frequencies for the various forms of LGMD2 have only been estimated for a few subtypes in selected populations. Epidemiological data, in combination with a comprehensive clinical data set, are useful for diagnostic purposes, as the frequency of single forms of LGMD2 varies in different ethnic populations due to founder mutations. Sarcoglycanopathies as a group (LGMD2C-F) have, for example, been reported as one of the most common forms of LGMD2 after calpainopathy (LGMD2A) and dysferlinopathy (LGMD2B), representing up to 18% of LGMD2 in Italy [77] and even up to 68% of severe forms of all LGMD in Brazil [78]. Nevertheless, they seem to be extremely rare in Japan, where LGMD2C was diagnosed in less than 1% of all LGMD cases [79]. LGMD2C (SGCG) is more frequent in Tunisia and Roma populations [11,80–82], with a carrier frequency of 1/250 in Morocco [12] and up to 1/15 in some Roma groups [83]. LGMD2I (FKRP) has one of the highest prevalence rates of all

Table 3
Clinical research in LGMD2s: natural history clinical trials, investigational drug clinical trials and available registries.

	ClinicalTrials.gov ID/doi ref.	Trial name/article	Purpose	Interventional drug/natural history	State (last update on ClinicalTrials.gov)	LGMD2 A CAPN3	B DYSF	C SGCG	D SGCA	E SGCB	F SGCD	G TCAP	H TRIM32	I FKRP	J TTN	K POMT1	L ANO5	M FKTN	N POMT2	O POMGnT1	P DAG1	Q PLEC	R DES	S TRAPPC11	T GMPPB	U ISPD	V GAA	W LIMS2
Clinical trials	NCT00893334	Evaluation of Limb-Girdle Muscular Dystrophy	To understand the biochemistry of different types of LGMD – determine appropriate outcome measures	Natural history	Completed 2014 (March 2014)	X	X							X													
	NCT01403402	Congenital Muscle Disease Patient and Proxy Reported Outcome Study (CMDPROS)	To describe early sign and symptoms, and adverse events in CMDs	Natural history	Recruiting (February 2015)							X		X		X	X	X	X	X							
	NCT01676077	Clinical Outcome Study for Dysferlinopathy	To determine the clinical outcome measures – characterise the disease progression – collect biological samples for the identification of biomarkers	Natural history	Ongoing, recruitment closed March 2014 (April 2015)		x																				
	NCT02165358	Muscle MRI in Becker Muscular Dystrophy and in Limb-Girdle Muscular Dystrophy Type 2I	To investigate muscle hypertrophy in the calves and tongue seen in patients affected by Becker muscular dystrophy and LGMD2I	Natural history	Recruiting (June 2014)									X													
	NCT00313677	Clinical Trial Readiness for the Dystroglycanopathies	To describe the early signs and symptoms of the dystroglycanopathies, and to gather information that will be required for future clinical trials.	Natural history	Recruiting (May 2012)									X		X		X	X								
	NCT01126697	Clinical Trial of Coenzyme Q10 and Lisinopril in Muscular Dystrophies	To compare CoQ10/Lisinopril/ CoQ10+Lisinopril for CMP prevention	Interventional drug – Phase II/III	Ongoing, recruitment closed (October 2014)			X	X	X	X			X													
	NCT00527228	Deflazacort in Dysferlinopathies	To assess the natural history – evaluate therapeutic efficacy and side effects of deflazacort in LGMD2B	Interventional drug – Phase II/III	Completed 2008 (January 2009) [65]		x																				
	NCT01976091	Gene Transfer Clinical Trial for LGMD2D (Alpha-Sarcoglycan Deficiency) Using scAAVrh74.tMCK.hSGCA	To evaluate the clinical safety and efficacy of gene therapy in LGMD2D	Interventional drug – Phase I/IIa	Recruiting (February 2015)				X																		
	NCT00494195	Gene Transfer Therapy for Treating Children and Adults with Limb Girdle Muscular Dystrophy Type 2D (LGMD2D)	To evaluate the safety and effectiveness of gene therapy in treating children and adults with LGMD2D	Interventional drug – Phase I	Completed (February 2013) [66]				X																		
	NCT00104078	A Phase I/II Trial of MYO-029 in Adult Subjects with Muscular Dystrophy	To evaluate safety of MYO-029 in adult patients with muscular dystrophy	Interventional drug – Phase I/II	Completed (December 2007) [67]	X	X	X	X	X				X													
	NCT01898364	Safety and Efficacy Evaluation of Repeat neoGAA Dosing in Late Onset Pompe Disease Patients	To evaluate the safety and tolerability of neoGAA in treatment naïve and alglucosidase alfa treated late-onset Pompe disease patients	Interventional drug – Phase I	Completed (March 2015) (extension study NCT02032524 ongoing – phase 2/3)																					X	
	Not registered	1α,25(OH)(2)-Vitamin D3 Increases Dysferlin Expression in vitro and in a Human Clinical Trial	To evaluate effects of VitD in cell line and carriers subject (15 carriers, 12 months – increase in monocyte expression)	VitD	[68]		X																				
	Not registered	Effects of Rituximab in Two Patients with Dysferlin-Deficient Muscular Dystrophy	To evaluate effects of rituximab on muscle strength in 2 patients with Miyoshi myopathy	rituximab	[69]		X																				
	Not registered	Two Siblings with Limb-Girdle Muscular Dystrophy Type 2E Responsive to Deflazacort	To evaluate response to treatment with deflazacort in two patients with LGMD2E	Deflazacort	[70]					X																	
	Not registered	Inflammation and Response to Steroid Treatment in Limb-Girdle Muscular Dystrophy 2I	To evaluate response to treatment with prednisolone in 2 patients with LGMD2I	prednisolone	[71]									X													

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Table 3 (*continued*)

	ClinicalTrials.gov ID/doi ref.	Trial name/article	Purpose	Interventional drug/natural history	State (last update on ClinicalTrials.gov)	LGMD2 A CAPN3	B DYSF	C SGCG	D SGCA	E SGCB	F SGCD	G TCAP	H TRIM32	I FKRP	J TTN	K POMT1	L ANO5	M FKTN	N POMT2	O POMGnT1	P DAG1	Q PLEC	R DES	S TRAPPC11	T GMPPB	U ISPD	V GAA	W LIMS2
Available outcome measures		Quantitative Muscle MRI as an Assessment Tool for Monitoring Disease Progression in LGMD2I: A Multicentre Longitudinal Study	To evaluate quantitative muscle MRI as a possible longitudinal outcome measure to assess muscle pathology and monitor therapeutic efficacy		[72]									X													
		Cardiovascular Magnetic Resonance of Cardiomyopathy in Limb Girdle Muscular Dystrophy 2B and 2I	To evaluate cardiac function by MRI in monitoring cardiac pathology progression		[73]		X							X													
		http://www.researchrom.com/masterlist	Guidance, information and assistance for choosing the right outcome measures (OMs) for neuromuscular disease trials and studies																								
LGMD2 having available registries						X	X							X		X		X	X	X						X	

the limb girdle muscular dystrophies in Northern Europe and is more prevalent here than in the rest of the world, with an estimated carrier frequency of 1/116 in Norway [84] and about 1/240 in the North of England [76], while it appears to be considerably rarer in Australia, where it is the cause of only 3% of all forms of LGMD [85].

2.2. Complications and management

The LGMD2 genes encode for proteins with a variety of different functions in muscle. They are involved in maintaining muscle cell structure, in post-translational modification, in cell differentiation and in cell signalling [4,85–89]. The complexity of pathomechanisms renders the development of curative drug treatments considerably more challenging and adequate clinical care more relevant. Important aspects of clinical care include the management of symptoms and the early detection of anticipated disease complications [90,91].

The most common symptoms that need to be addressed in patients with LGMD2 are muscle and joint pain, joint contractures and scoliosis, cardiac and respiratory failure and gastrointestinal symptoms, with different risks among different subtypes (Table 1). An accurate multidisciplinary follow-up involving neurologists, neuro-paediatricians, geneticists, physiotherapists and occupational therapists, orthopaedic surgeons, cardiologists, respiratory physicians and gastroenterologists is essential to monitor potential complications, intervene in a timely manner when indicated, and coordinate care among different specialists.

Symptomatic treatments include regular physiotherapy and orthotic devices to manage contractures and pain, nocturnal non-invasive ventilation if weakness involves respiratory muscles, spinal surgery in cases of prominent scoliosis and pharmacological treatment at the first signs of impaired cardiac function. Regular follow-up and appropriate interventions can ameliorate quality of life of patients and prolong life expectancy.

3. Animal models

Therapeutic strategies will first be tested in a preclinical setting to establish proof of concept by improving a phenotype in a cellular or *in vivo* model, and by identifying possible side-effects and toxicities before designing a clinical trial involving patients.

Adequate models for investigating molecular mechanisms and testing potential therapeutic strategies need to be carefully selected. Most mouse models currently used for LGMD2 are, e.g., knockout models, while LGMD2 patients have missense mutations that might have a different molecular effect than haploinsufficiency. In addition, introducing a mutation known to be associated with a human disease into an animal model does not guarantee to result in a phenotype overlapping with the human disease. For example, introducing the common *SCGA* mutation p.R77C into a mouse did not produce an LGMD2D-like phenotype [92,93] and expressing mutations in the *Drosophila melanogaster* ferlin gene (mfr) did not show any motility problems [21].

The two most common organisms used for modelling muscle diseases are zebrafish and mice. Zebrafish models are frequently used in muscle diseases, being relatively easy and rapid to breed, translucent at an early developmental stage and having a simple embryonic organisation [94,95]. For most human genes involved in muscle structure and function, zebrafish have an orthologue [96–98] and a disease model can be generated by inducing the expression of a mutant gene or functionally knocking down the target gene by injecting selective anti-sense morpholino oligonucleotides (MOs). In LGMD2 this second approach has been successfully used for a number of diseases, such as LGMD2I, LGMD2G, LGMD2K and LGMD2N [35,40,41,99]. *Caenorhabditis elegans* and *D. melanogaster* are also simple organisms that can be genetically manipulated, as they are both small in size and genome and easy to observe for their motility [100,101]. Small animals can be helpful as they are cheaper models for unravelling molecular pathways. For this reason they

are used to introduce a mutation or to silence the orthologous gene that determines a muscular phenotype. They can also be used for primary drug screens complementary to *in vitro* systems [101,102].

The mouse genome is highly similar (99%) to the human genome and, for this reason, mice are the preferred animal for modelling human diseases and for testing the toxicity and safety of new drugs in preclinical studies [103].

Suitable animal models are available for most forms of LGMD2 (Table 3). Below, we briefly discuss models that have been used for drug development in the most common LGMD2 forms (LGMD2A, 2B, sarcoglycanopathies and LGMD2I).

3.1. LGMD2A – calpain-3

The *calpain-3* knockout (also named C3KO) mouse shows a mild phenotype. It is smaller than a wild type mouse, showing muscle atrophy, disruptions of myofibrillogenesis and sarcomere structure at muscle histology, and a partial force deficit [20].

3.2. LGMD2B – *dysferlin*

In developing animal models for dysferlinopathies, it has been shown that the main function of the dysferlin orthologue gene in *D. melanogaster* and *C. elegans* is related mainly to reproduction and not to muscle function [21,22], while silencing the dysf gene in zebrafish does result in altered development and muscle disorganisation [23]. Two independent spontaneous mouse models have been identified for dysferlinopathies that both manifest some additional features that are not observed in patients. The A/J mouse carries an altered Dysf gene that leads to aberrant splicing and to the absence of dysferlin expression. Dystrophic features appear at 4–5 months of age with a slow progression and a high susceptibility to infections due to a deficiency of the C5 complement component was also observed. The SJL/J mouse has an in-frame deletion in the dysferlin gene, producing a shorter and instable protein that determines a dystrophic phenotype similar to the A/J mouse. However, its high incidence of early onset reticulum cell sarcomas limits its possible use for the observation of the muscular phenotype. For this reason, both models have been backcrossed to more suitable genetic backgrounds in order to obtain a phenotype not affected by strain-specific factors [27]. For example, the Dysf^{prmd} mouse was obtained by backcrossing the A/J mice onto the C57BL/6 background. Homozygous mice show weakness from the age of 2 months and no complement 5 deficiency.

3.3. LGMD2C, 2D, 2E and 2F – sarcoglycans

The Bio 14.6 hamster, a spontaneous model for a recessive form of muscular dystrophy and cardiomyopathy, was first described in 1962 [104], but its underlying genetic defect was only identified 35 years later as being linked to the gene encoding for delta-sarcoglycan (SG) [105–107].

Gene transfer through the direct intramuscular injection of a vector (plasmid and adenovirus) containing the wild type delta-SG gene was shown to restore expression of delta-SG and the entire SG complex. Rescue of muscle pathology provided evidence that integrity of the SG complex was required for the maintenance of sarcolemmal stability [108].

The key role of the transmembrane and extracellular domains for delta-SG function has been confirmed through the generation of different mutants in *D. melanogaster*. The line with a large deletion demonstrated reduced motility, heart dysfunction and reduced life span, while the line carrying a small deletion affecting only the cytoplasmic domain showed a mildly reduced lifespan with normal locomotive activity and normal heart function [32]. The same group observed that beta and delta-SG null *D. melanogaster* show exercise-induced TGF beta signalling through the SMAD pathway, and that genetically decreasing SMAD signalling using haploinsufficient alleles was sufficient to rescue skeletal and cardiac muscle dysfunction in the SG null *D. melanogaster* [109]. The SMAD pathway could therefore be a target for reducing exercise-induced muscle damage and for slowing down disease progression. Knockout mice for the four sarcoglycan genes share similar features of muscular dystrophy and cardiomyopathy, with the exception of the *Sgca*-/- mouse, which only shows skeletal muscle involvement [29–31].

3.4. LGMD2I – FKRP

FKRP is a putative glycosyltransferase thought to be involved in glycosylation of alpha-dystroglycan (alpha-DG), but its exact role in the glycosylation pathway is still unclear. Down-regulating FKRP in zebrafish results in reduced alpha-DG glycosylation and developmental defects with an altered somitic structure, altered muscle fibre organisations and defects in eye morphology [40,41]. These features are present in patients with LGMD2I and other dystroglycanopathies. Defective glycosylation affecting dystroglycan complex interactions is considered to underlie the pathogenesis in this group of diseases. However, more recently it was suggested that Fukutin and FKRP could also have a different role, impairing a process that precedes the dystrophin-associated glycoprotein (DAG) complex interaction. By inhibiting Fukutin or FKRP in zebrafish, it was observed that muscle pathology in these models is different from the dystroglycan-deficient model, also showing a notochord defect and an altered laminin expression as a consequence of endoplasmic reticulum stress and unfolded protein response (UPR). The above suggests that both proteins could be involved in this process and that UPR could contribute to the phenotypic spectrum of dystroglycanopathies [42].

In mice, FKRP is essential for embryonic development, and homozygous null embryos die in utero [43]. Knock-in mice carrying the missense mutation P448L lack glycosylation of alpha-DG and show a severe phenotype with muscle, eye and brain developmental defects [43], while homozygous knock-in mice for the L276I mutation common in LGMD2I patients mimic the classic late onset phenotype of LGMD2I [44].

4. Therapeutical approaches in preclinical research

The identification of causative genes is essential to the understanding of molecular mechanisms affected in LGMD2. Delineating the molecular features of a disease is critical to evaluating potential therapeutic strategies that may involve

direct interventions at DNA, RNA and protein level or target downstream effects caused by the primary defect. Although efforts in all these areas have been made to address the therapeutic challenge, to date, most groups working on LGMD2 have focused on the correction of the primary genetic defect. Potential strategies depend on the molecular characteristics of the single condition. For example, the size of a specific gene influences an adeno-associated virus (AAV)-mediated gene delivery approach as the payload of DNA that an AAV can deliver is physically limited by the capacity of the viral particle (i.e., ~5 kb).

4.1. Gene transfer

Viral vector-mediated gene transfer aims to introduce a functional gene into the defective tissue to induce the expression of a functional protein. This approach has been successfully applied to more than one model for LGMD2.

Calpain-3 deficiency can be addressed through AAV-mediated gene transfer. The histological and functional phenotypes of the C3KO mouse have been rescued after injections of rAAV (recombinant adeno-associated virus) delivering wild type calpain-3 [110].

As the full-length dysferlin gene is too large to be transferred through a single viral vector, dysferlin-deficiency has been addressed through two different gene transfer strategies. *Minidysferlin* is a shorter gene, known to produce a milder phenotype in patients. rAAV-mediated *minidysferlin* transfer into the muscle of a *Dysf*$^{-/-}$ mouse led to protein expression and efficient repair of sarcolemmal lesions [111]. A dual AVV-mediated gene transfer in the *Dysf*^{prmd} mouse, splitting the dysferlin protein into two parts and injecting the muscle with two different vectors, also showed encouraging results, leading to the expression of a full length protein [112].

AAV-mediated gene transfer has successfully been applied through intramuscular injection in the Bio14.6 hamster [113], and in *Sgca*-/-, *Sgcb*-/- [114] and *Sgcg*-/- mice [115], showing efficient restoration of gene expression and muscle pathology. It has on the other hand failed in the *Sgca*-/- mouse model, where it only showed short-term expression due to transgene toxicity, limiting the possible application of this vector in patients. This issue has been addressed through a modified vector placing the gene under the control of an ubiquitously expressed cytomegalovirus (CMV) promoter or a muscle-specific promoter, thus obtaining sustained gene expression with a lack of cell toxicity in both cases [116]. The same approach has been shown to be effective also through the systemic injection of the vector [117].

AVV-mediated gene delivery was also shown to be effective in FKRP. After systemic delivery of the vector in L276I(KI) mice at birth or at 9 months, increased FKRP expression and restoration of alpha-DG glycosylation in skeletal and cardiac muscle was observed [44].

4.2. Transcriptional modification

Other possible therapeutic approaches that are considered for LGMD2 aim to modify the transcriptional process through exon skipping or through the induction of stop codon read-through. The approach used will depend on the type of the underlying mutation.

Exon skipping can be considered in the case of an out-of-frame mutation causing a premature stop codon with the production of a truncated protein. By skipping a specific exon, it is possible to restore the reading frame and to produce a shorter but possibly more functional protein.

One of the limits of exon skipping in recent clinical trials in Duchenne muscular dystrophy (DMD) was efficacy, as it failed to produce high levels of skipped protein [118]. In LGMD2, this could further reduce the number of eligible patients: targeting only one allele may not restore a sufficient amount of functional protein to rescue the phenotype. Only patients carrying homozygous mutations, or compound heterozygous mutations involving the same exon, would benefit from single exon skipping [119].

If the genetic defect is a nonsense mutation, a possible therapeutic approach is to induce stop codon read-through by PTC124®, a molecule that has been tested in Cystic Fibrosis and DMD [120,121]. In LGMD2, this strategy has already been applied in cultured myotubes from patients with stop mutations in the dysferlin gene [122].

4.3. Cell therapy

Cell therapy aims to increase the production of differentiated myotubes in dystrophic muscle through the transfer of stem cells. Different cell populations have been used in mouse models to test this therapeutic approach, with variable outcomes.

Mesoangioblasts, isolated from juvenile *Sgca*-/- mice and transduced with a lentiviral vector expressing alpha-SG, when injected into the femoral artery of dystrophic mice, reconstituted skeletal muscle [123]. Non-autologous hematopoietic stem cells, after transplantation into the cardiac and skeletal muscles of the *Sgcd*-/- mouse, have been shown to be incorporated into myofibres but failed to express delta-SG [124]. More recently, iPSC (induced pluripotent stem cells)-derived mesoangioblasts were transplanted into the skeletal muscle of *Sgca*-/- immunodeficient mice, generating muscle fibres that expressed alpha-SC and leading to an amelioration of the dystrophic phenotype [125].

Satellite cells (SC), mono-nucleated progenitor cells located between the basal lamina and sarcolemma of muscle fibres, mediate physiological muscle growth and regeneration [126] and are therefore considered a promising resource for the treatment of muscle wasting conditions [127]. Myosphere-derived progenitor cells (MDPCs), an SC population that differentiates into vascular smooth muscle cells and mesenchymal progeny, have been implanted into the cardiac muscle of *Sgcd*-/- mice, resulting in enhanced neo-angiogenesis, restored delta-SG expression and improved cardiac function [128].

Non-HLA-compatible cell transplantation into patients bears the high risk of an immune reaction, which could potentially be addressed with immunosuppressive treatments. Autologous transplantation would avoid the risk of an immune reaction, but patient derived cells carry the genetic defect and would first need to be genetically modified.

Therapeutic approaches that have been tested in patients are discussed in Section 6.2.

5. Outcome measures

Pre-clinical research leads to the identification of new drug targets that need to be tested in interventional clinical trials for their efficacy. In this context, the availability of standardised, validated and non-invasive outcome measures to evaluate disease progression becomes essential.

Muscle strength and function are typically assessed through manual muscle testing, hand-held myometry [129] and timed tests including the 10-metre run [130] and the 6-minute walk distance (6MWD) [131,132]. Even though evaluators are trained to be consistent in scoring and instructing patients, the results are still dependent on patient effort. Furthermore, lower limb function has been more thoroughly investigated than upper limb function and sensitivity in measuring disease progression over a short period is limited in slowly degenerating conditions like LGMD2. A tool evaluating performance of the upper limbs (PUL) has recently been developed for non-ambulant DMD patients [133], but could also be useful in clinical trials for LGMD2.

Quantitative muscle MRI is an objective and non-invasive tool with the potential to become a reliable measure of muscle pathology. Several studies have used MRI to assess skeletal muscle and cardiac pathology in patients with LGMD2. Willis et al. showed a significant increase in skeletal muscle fat fraction over 12 months in patients affected by LGMD2I, while no significant changes could be demonstrated over the same period by physical assessment measures [72]. Rosales et al. evaluated cardiac function in patients with LGMD2B and 2I by MRI to define which markers of cardiomyopathy could be useful in monitoring cardiac pathology. Only mild changes were identified in the scanned patients and further studies are needed to assess whether cardiac MRI could be effectively used for routine monitoring in LGMD2B and 2I [73]. Muscle MRI can also be a helpful tool in the differential diagnosis of some forms of LGMD2 [134–137] and more importantly to distinguish patients with LGMD2 from patients with myofibrillar myopathies, collagen VI related disorders or other myopathies [138–140]. More extensive studies are necessary to evaluate the usefulness of MRI as a diagnostic tool and as an outcome measure in the various forms of LGMD2.

A biomarker has been defined as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [141]. Except for elevated serum creatine kinase activity as an unspecific diagnostic biomarker, no specific biomarkers to monitor disease progression have yet been validated for LGMD2.

In a recent study, miR-1, miR-133a, and miR-206, previously reported as potential biomarkers for DMD, were evaluated as possible biomarkers for other muscle diseases but no significant change in their concentration was found in LGMD2A [142]. An observational clinical trial is currently recruiting patients with LGMD2B-F, 2I, 2L, myofibrillar myopathies, BMD and Miyoshi myopathy type 3, with the aim to identify biomarkers and clinical correlates of changes in cell membrane permeability before and after routine motor function testing and is expected to be completed in 2016 (ClinicalTrials.gov identifier: NCT01851447).

6. Patient registries and clinical trials

6.1. Patient registries

Patient registries are designed to collect demographic and epidemiological data or to gather information on natural history. Registries can also serve to keep patients informed about current research activities, to contact them when new clinical trials are about to start, and can help in collecting of pharmacovigilance data in phase IV studies. Patient registries can be created and coordinated by patient associations, by reference centres working in patient care, or by pharmaceutical companies with an interest in a specific disease.

Currently disease specific registries only exist for a few forms of LGMD2, namely LGMD2A, 2B, 2C, 2D, and 2I. The registry for patients with calpainopathy is held by the Coalition to Cure Calpain 3 (http://www.lgmd2a.org); patients with LGMD2B or Miyoshi myopathy can access the International Dysferlinopathy Registry (http://dysferlinregistry.org/) created by The Jain Foundation; patients with LGMD2C and 2D can register with the Kurt and Peter Foundation (www.lgmd2cregistry.org) or the registry held by the LGMD2D Foundation (www.lgmd2d.org/patient-resources); and patients with different forms of muscle diseases due to mutations in the *FKRP* gene (LGMD2I, MDC1C, MEB and WWS) can access the Global FKRP Registry (https://www.fkrp-registry.org). The Congenital Muscular Dystrophy International Registry was created for patients with congenital forms of muscle diseases, but is also open to patients with different forms of LGMD2 (https://www.cmdir.org/).

6.2. Clinical trials

Interventional clinical trials in LGMD2 have only been carried out for a handful of these conditions. For most forms of LGMD2 we are still lacking in-depth data on natural history, which would be helpful before moving into interventional drug trials. This will always present a challenge for the very rare forms of LGMD2 that have been described only in a few patients or in a single family, like LGMD2P (*DAG1*), LGMD2Q (*PLEC*) or LGMD2S (*TRAPPC11*). Completed or ongoing clinical trials in LGMD2 are summarised in Table 3, including natural history studies and interventional drug studies.

A multi-centric clinical outcome study for dysferlinopathy with over 200 patients is currently running in centres in the United States, Europe, Australia and Japan (NCT01676077). The aim is to obtain a better overview of the natural history and progression of the disease, as well as to identify biomarkers and outcome measures through standardised function and strength tests and through muscle MRI.

Other registered natural history studies of LGMD2 (NCT00893334, NCT00313677, NCT01403402) are summarised in Table 3.

The safety of gene therapy has been evaluated in sarcoglycanopathies (LGMD2C and 2D). A phase 1 study in LGMD2C patients assessed the safety and feasibility of AAV1-sarcoglycan gene transfer. The vector was injected for 30

consecutive days in the extensor carpi radialis muscle of nine non-ambulant adult patients, divided into three groups with each group receiving a different dose. Expression of gamma-SG was detected by immunohistochemical analysis in muscle biopsies of injected muscles from patients who received the highest dose. No serious adverse events were reported for the duration of the study and the six month follow-up [143].

Six patients with LGMD2D were included in a double-blind, randomised trial to evaluate the safety of AAV-mediated gene transfer through intramuscular injection in a selected site (extensor digitorum brevis muscle). All six patients, divided into two groups receiving two different doses, showed increased gene expression without adverse effects [144]. The phase I/II study, delivering the vector through intra-arterial injection, is currently ongoing and aims to recruit 8 patients (NCT01976091).

A recent clinical study using autologous bone marrow mononuclear cell therapy in 150 patients with muscular dystrophy, including 20 with a form of LGMD2, described a functional improvement after a 12 month follow-up. However, no controls were included in the study [145]. Long-term safety data still need to be collected and the evaluation of non-autologous stem cell transplantation in patients is currently limited by HLA compatibility and necessary immunosuppressive protocols with additional side effects that are an ethical concern.

No effective pharmacological therapy to improve muscle strength is currently available for patients with LGMD2 based on randomised controlled clinical trials. In DMD, it has been demonstrated that steroids improve strength and life span, and reduce the progression of motor deterioration [146].

Anecdotal reports suggest a positive response to steroids in a few forms of LGMD2. Deflazacort was associated with an increase in muscle strength and function in a patient with LGMD2D treated for 6 months [147] and in two siblings with LGMD2E treated for 22 months [70]. Two unrelated patients affected by LGMD2I showed a positive response to prednisolone [71]. However, these constitute reports of single patients and there is no evidence of steroid efficacy from placebo-controlled trials.

A randomised placebo-controlled clinical trial with deflazacort conducted in 25 patients with LGMD2B did not show any significant clinical difference between treated and non-treated patients [65]. Moreover, deteriorating muscle strength was observed in the deflazacort group, accompanied by known steroid side-effects. It is still not clear if steroids are effective in some patients with LGMD2, however, based on the experience in DMD, patients with LGMD2 associated with the dystrophin-glycoprotein complex and a DMD-like phenotype may more likely than others benefit from steroids.

Other pharmacological treatments evaluated in patients with LGMD2 in registered clinical trials are Coenzyme Q10, Lisinopril and MYO-029, a neutralising antibody against myostatin. The randomised, open label, phase 2/3 trial of Coenzyme Q10 and Lisinopril (NCT01126697) is evaluating the safety and efficacy of these two compounds, as well as of a combined treatment, in patients with dystrophinopathies or LGMD2C-2F and 2I that have no clinical cardiac symptoms. The trial is expected to be completed by the end of 2016. A dose-escalation, randomised, double-blind, placebo-controlled study of intravenous administration of MYO-029, an antibody aiming to induce muscle growth through the inhibition of myostatin, has been conducted in 116 subjects including patients with LGMD2A and LGMD2I. The trial confirmed the safety of the compound but failed to show clear efficacy [67].

7. Discussion

LGMD2 is a genetically heterogeneous group of rare diseases that generally share proximal weakness as a presenting clinical sign. Since they were first described as a distinct clinical entity in 1954 significant advances in the understanding of their underlying genetic causes have been achieved. More than 20 disease causing genes have been identified over the past 21 years and pre-clinical work on gene and protein function has contributed immensely to our understanding of disease mechanisms and muscle function in general. At the same time detailed studies on the clinical spectrum of the more common forms of LGMD2 have helped to improve the clinical diagnosis in affected patients and to establish risk factors like cardiac and respiratory involvement for which patients can be effectively monitored. The application of diagnostic tools like muscle MRI, muscle biopsies and more recently exome sequencing and other omics technologies has contributed to the phenotypic characterisation of several of the larger cohorts of LGMD2 patients. Better access to diagnostic services and technologies has helped increase the number of patients with a confirmed diagnosis and through the support of patient advocacy groups, patient registries have now been established for several of the LGMD2 subtypes. Patient involvement is absolutely crucial for the development of a translational research pathway and supportive infrastructure in rare diseases like LGMD2 and a lot of progress has been made over the past few years in this respect.

Nevertheless, there is currently no curative treatment for any form of LGMD2 and clinical care is based on the prevention and management of known complications. Despite all the pre-clinical and clinical advancements and the efforts to improve the translational research pathway in LGMD2, the lack of therapeutic options targeting the underlying cause of the various forms of LGMD2 is based on the rarity of the diseases, our patchy understanding of their pathophysiology, the sparsity of completed natural history studies and finally the lack of standardised patient cohorts. As a consequence the field is also missing a set of well-established and validated outcome measures and biomarkers for patients with LGMD2 that could be used as clinically meaningful endpoints or surrogate markers in clinical trials, respectively. These known deficits are now addressed at different levels. Pre-clinical work in animal models, which have been characterised for all forms of LGMD2, will help to understand underlying molecular mechanisms and to identify possible therapeutic targets. Mouse models for LGMD2 in particular will be and have been very helpful to test advanced therapy medicinal products. Once pre-clinical proof of concept is obtained and sufficient safety data are collected, a robust strategy needs to be developed to move promising compounds into the clinic. High quality pre-clinical work and properly conducted treatment studies in LGMD2

mouse models will provide important information for clinical trial design, safety monitoring and efficacy measures in patients. For now the translation of pre-clinical therapeutic concepts to the clinical stage is still hampered by the lack of natural history data due to the paucity of patients and the absence of validated and clinically meaningful outcome measures.

Successful recruitment of LGMD2B and 2I patients into ongoing natural history studies has shown that patients with LGMD2 seem generally very keen to participate in clinical trials. There is a real need to carry out additional multi-centric natural history studies in other cohorts of LGMD2 patients and to make use of existing international clinical networks like TREAT-NMD (http://www.treat-nmd.org) and the Cooperative International Neuromuscular Research Group (CINRG, http://www.cinrgresearch.org/) to reach a meaningful number of patients. Networking is also important for the generation, dissemination and implementation of care standards and for the identification and validation of outcome measures. Worldwide clinical and scientific collaboration, essential to achieve these aims in rare and complex conditions like LGMD2, is already a reality and needs to be supported and enhanced. Patient organisations are important stakeholders and play a central role in promoting translational research. They can encourage and empower collaboration among scientists and clinicians, advocate for research and care funding, provide advice to patients and families and keep them informed about current research achievements and standards of care.

Today and future challenges in LGMD2 consist of translating promising therapeutic strategies into effective and accessible treatment for patients, reducing the burden of disease and improving their quality of life.

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References

[1] Walton JN, Nattrass FJ. On the classification, natural history and treatment of the myopathies. Brain 1954;77:169–231.
[2] 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.
[3] Nigro V, Savarese M. Genetic basis of limb-girdle muscular dystrophies: the 2014 update. Acta Myol 2014;33:1–12.
[4] Straub V, Bushby K. Therapeutic possibilities in the autosomal recessive limb-girdle muscular dystrophies. Neurother 2008;5:619–26.
[5] Mitsuhashi S, Kang PB. Update on the genetics of limb girdle muscular dystrophy. Semin Pediatr Neurol 2012;19:211–18.
[6] Allamand V, Broux O, Bourg N, et al. Genetic heterogeneity of autosomal recessive limb-girdle muscular dystrophy in a genetic isolate (Amish) and evidence for a new locus. Hum Mol Genet 1995;4:459–63.
[7] Urtasun M, Sáenz A, Roudaut C, et al. Limb-girdle muscular dystrophy in Guipúzcoa (Basque Country, Spain). Brain 1998;121 (Pt 9):1735–47.
[8] Fanin M, Nascimbeni AC, Aurino S, et al. Frequency of LGMD gene mutations in Italian patients with distinct clinical phenotypes. Neurology 2009;72:1432–5.
[9] Hanisch F, Müller CR, Grimm D, et al. Frequency of calpain-3 c.550delA mutation in limb girdle muscular dystrophy type 2 and isolated hyperCKemia in German patients. Clin Neuropathol 2007;26:157–63.
[10] Leshinsky-Silver E, Argov Z, Rozenboim L, et al. Dysferlinopathy in the Jews of the Caucasus: a frequent mutation in the dysferlin gene. Neuromuscul Disord 2007;17:950–4.
[11] Piccolo F, Jeanpierre M, Leturcq F, et al. A founder mutation in the gamma-sarcoglycan gene of gypsies possibly predating their migration out of India. Hum Mol Genet 1996;5:2019–22.
[12] Kerch El F, Ratbi I, Sbiti A, Laarabi F-Z, Barkat A, Sefiani A. Carrier frequency of the c.525delT mutation in the SGCG gene and estimated prevalence of limb girdle muscular dystrophy type 2C among the Moroccan population. Genet Test Mol Biomarkers 2014;18:253–6.
[13] Duclos F, Broux O, Bourg N, et al. Beta-sarcoglycan: genomic analysis and identification of a novel missense mutation in the LGMD2E Amish isolate. Neuromuscul Disord 1998;8:30–8.
[14] Frosk P, Greenberg CR, Tennese AAP, et al. The most common mutation in FKRP causing limb girdle muscular dystrophy type 2I (LGMD2I) may have occurred only once and is present in Hutterites and other populations. Hum Mutat 2005;25:38–44.
[15] Udd B. Limb-girdle type muscular dystrophy in a large family with distal myopathy: homozygous manifestation of a dominant gene? J Med Genet 1992;29:383–9.
[16] 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.
[17] Sarkozy A, Hicks D, Hudson J, et al. ANO5 gene analysis in a large cohort of patients with anoctaminopathy: confirmation of male prevalence and high occurrence of the common exon 5 gene mutation. Hum Mutat 2013;34:1111–18.
[18] Emery AE. Population frequencies of inherited neuromuscular diseases–a world survey. Neuromuscul Disord 1991;1:19–29.
[19] Richard I, Roudaut C, Marchand S, et al. Loss of calpain 3 proteolytic activity leads to muscular dystrophy and to apoptosis-associated IkappaBalpha/nuclear factor kappaB pathway perturbation in mice. J Cell Biol 2000;151:1583–90.
[20] Kramerova I, Kudryashova E, Tidball JG, Spencer MJ. Null mutation of calpain 3 (p94) in mice causes abnormal sarcomere formation in vivo and in vitro. Hum Mol Genet 2004;13:1373–88.
[21] Smith MK, Wakimoto BT. Complex regulation and multiple developmental functions of misfire, the Drosophila melanogaster ferlin gene. BMC Dev Biol 2007;7:21.
[22] Krajacic P, Hermanowski J, Lozynska O, Khurana TS, Lamitina TC. elegans dysferlin homolog fer-1 is expressed in muscle, and fer-1 mutations initiate altered gene expression of muscle enriched genes. Physiol Genomics 2009;40:8–14.
[23] Kawahara G, Serafini PR, Myers JA, Alexander MS, Kunkel LM. Characterization of zebrafish dysferlin by morpholino knockdown. Biochem Biophys Res Commun 2011;413:358–63.
[24] Roostalu U, Strähle U. In vivo imaging of molecular interactions at damaged sarcolemma. Dev Cell 2012;22:515–29.
[25] Bittner RE, Anderson LV, Burkhardt E, et al. Dysferlin deletion in SJL mice (SJL-Dysf) defines a natural model for limb girdle muscular dystrophy 2B. Nat Genet 1999;23:141–2.
[26] Ho M, Post CM, Donahue LR, et al. Disruption of muscle membrane and phenotype divergence in two novel mouse models of dysferlin deficiency. Hum Mol Genet 2004;13:1999–2010.
[27] Hornsey MA, Laval SH, Barresi R, Lochmüller H, Bushby K. Muscular dystrophy in dysferlin-deficient mouse models. Neuromuscul Disord 2013;23:377–87.

[28] Cheng L, Guo X-F, Yang X-Y, et al. Delta-sarcoglycan is necessary for early heart and muscle development in zebrafish. Biochem Biophys Res Commun 2006;344:1290–9.
[29] Hack AA, Ly CT, Jiang F, et al. Gamma-sarcoglycan deficiency leads to muscle membrane defects and apoptosis independent of dystrophin. J Cell Biol 1998;142:1279–87.
[30] Duclos F, Straub V, Moore SA, et al. Progressive muscular dystrophy in alpha-sarcoglycan-deficient mice. J Cell Biol 1998;142:1461–71.
[31] Durbeej M, Campbell KP. Muscular dystrophies involving the dystrophin-glycoprotein complex: an overview of current mouse models. Curr Opin Genet Dev 2002;12:349–61.
[32] Allikian MJ, Bhabha G, Dospoy P, et al. Reduced life span with heart and muscle dysfunction in Drosophila sarcoglycan mutants. Hum Mol Genet 2007;16:2933–43.
[33] Coral-Vázquez R, Cohn RD, Moore SA, et al. Disruption of the sarcoglycan-sarcospan complex in vascular smooth muscle: a novel mechanism for cardiomyopathy and muscular dystrophy. Cell 1999; 98:465–74.
[34] Straub V, Duclos F, Venzke DP, et al. Molecular pathogenesis of muscle degeneration in the delta-sarcoglycan-deficient hamster. Am J Pathol 1998;153:1623–30.
[35] Zhang R, Yang J, Zhu J, Xu X. Depletion of zebrafish Tcap leads to muscular dystrophy via disrupting sarcomere–membrane interaction, not sarcomere assembly. Hum Mol Genet 2009;18:4130–40.
[36] Markert CD, Meaney MP, Voelker KA, et al. Functional muscle analysis of the Tcap knockout mouse. Hum Mol Genet 2010;19:2268–83.
[37] LaBeau-DiMenna EM, Clark KA, Bauman KD, Parker DS, Cripps RM, Geisbrecht ER. Thin, a Trim32 ortholog, is essential for myofibril stability and is required for the integrity of the costamere in Drosophila. Proc Natl Acad Sci U S A 2012;109:17983–8.
[38] Kudryashova E, Wu J, Havton LA, Spencer MJ. Deficiency of the E3 ubiquitin ligase TRIM32 in mice leads to a myopathy with a neurogenic component. Hum Mol Genet 2009;18:1353–67.
[39] Kudryashova E, Struyk A, Mokhonova E, Cannon SC, Spencer MJ. The common missense mutation D489N in TRIM32 causing limb girdle muscular dystrophy 2H leads to loss of the mutated protein in knock-in mice resulting in a Trim32-null phenotype. Hum Mol Genet 2011; 20:3925–32.
[40] Thornhill P, Bassett D, Lochmüller H, Bushby K, Straub V. Developmental defects in a zebrafish model for muscular dystrophies associated with the loss of fukutin-related protein (FKRP). Brain 2008;131:1551–61.
[41] Kawahara G, Guyon JR, Nakamura Y, Kunkel LM. Zebrafish models for human FKRP muscular dystrophies. Hum Mol Genet 2010;19:623–33.
[42] Lin Y-Y, White RJ, Torelli S, Cirak S, Muntoni F, Stemple DL. Zebrafish Fukutin family proteins link the unfolded protein response with dystroglycanopathies dystroglycanopathies. Hum Mol Genet 2011; 20:1763–75.
[43] Chan YM, Keramaris-Vrantsis E, Lidov HG, et al. Fukutin-related protein is essential for mouse muscle, brain and eye development and mutation recapitulates the wide clinical spectrums of dystroglycanopathies. Hum Mol Genet 2010;19:3995–4006.
[44] Qiao C, Wang C-H, Zhao C, et al. Muscle and heart function restoration in a limb girdle muscular dystrophy 2I (LGMD2I) mouse model by systemic FKRP gene delivery. Mol Ther 2014;22:1890–9.
[45] Myhre JL, Hills JA, Prill K, Wohlgemuth SL, Pilgrim DB. The titin A-band rod domain is dispensable for initial thick filament assembly in zebrafish. Dev Biol 2014;387:93–108.
[46] Buck D, Smith JE 3rd, Chung CS, et al. Removal of immunoglobulin-like domains from titin's spring segment alters titin splicing in mouse skeletal muscle and causes myopathy. J Gen Physiol 2014;143:215–30.
[47] Charton K, Danièle N, Vihola A, et al. Removal of the calpain 3 protease reverses the myopathology in a mouse model for titinopathies. Hum Mol Genet 2010;19:4608–24.
[48] Haines N, Seabrooke S, Stewart BA. Dystroglycan and protein O-mannosyltransferases 1 and 2 are required to maintain integrity of Drosophila larval muscles. Mol Biol Cell 2007;18:4721–30.
[49] Ueyama M, Akimoto Y, Ichimiya T, et al. Increased apoptosis of myoblasts in Drosophila model for the Walker-Warburg syndrome. PLoS ONE 2010;5:e11557.
[50] Lommel M, Willer T, Cruces J, Strahl S. POMT1 is essential for protein O-mannosylation in mammals. Funct Glycom 2010;479:323–42.
[51] Willer T, Prados B, Falcón-Pérez JM, et al. Targeted disruption of the Walker-Warburg syndrome gene Pomt1 in mouse results in embryonic lethality. PNAS 2004;101:14126–31.
[52] Kurahashi H, Taniguchi M, Meno C, et al. Basement membrane fragility underlies embryonic lethality in fukutin-null mice. Neurobiol Dis 2005; 19:208–17.
[53] Hu H, Li J, Gagen CS, et al. Conditional knockout of protein O-mannosyltransferase 2 reveals tissue-specific roles of O-mannosyl glycosylation in brain development. J Comp Neurol 2011;519:1320–37.
[54] Liu J, Ball SL, Yang Y, et al. A genetic model for muscle-eye-brain disease in mice lacking protein O-mannose 1,2-N-acetylglucosaminyltransferase (POMGnT1). Mech Dev 2006;123: 228–40.
[55] Zhang P, Yang Y, Candiello J, et al. Biochemical and biophysical changes underlie the mechanisms of basement membrane disruptions in a mouse model of dystroglycanopathy. Matrix Biol 2013;32:196–207.
[56] Gupta V, Kawahara G, Gundry SR, et al. The zebrafish dag1 mutant: a novel genetic model for dystroglycanopathies. Hum Mol Genet 2011;20:1712–25.
[57] Williamson RA, Henry MD, Daniels KJ, et al. Dystroglycan is essential for early embryonic development: disruption of Reichert's membrane in Dag1-null mice. Hum Mol Genet 1997;6:831–41.
[58] Hara Y, Balci-Hayta B, Yoshida-Moriguchi T, et al. A dystroglycan mutation associated with limb-girdle muscular dystrophy. N Engl J Med 2011;364:939–46.
[59] Winter L, Staszewska I, Mihailovska E, et al. Chemical chaperone ameliorates pathological protein aggregation in plectin-deficient muscle. J Clin Invest 2014;124:1144–57.
[60] Li M, Andersson-Lendahl M, Sejersen T, Arner A. Knockdown of desmin in zebrafish larvae affects interfilament spacing and mechanical properties of skeletal muscle. J Gen Physiol 2013;141:335–45.
[61] Capetanaki Y, Milner DJ, Weitzer G. Desmin in muscle formation and maintenance: knockouts and consequences. Cell Struct Funct 1997;22:103–16.
[62] Milner DJ, Weitzer G, Tran D, Bradley A, Capetanaki Y. Disruption of muscle architecture and myocardial degeneration in mice lacking desmin. J Cell Biol 1996;134:1255–70.
[63] Carss KJ, Stevens E, Foley AR, et al. Mutations in GDP-mannose pyrophosphorylase B cause congenital and limb-girdle muscular dystrophies associated with hypoglycosylation of α-dystroglycan. Am J Hum Genet 2013;93:29–41.
[64] Taylor KM, Meyers E, Phipps M, et al. Dysregulation of multiple facets of glycogen metabolism in a murine model of Pompe disease. PLoS ONE 2013;8:e56181.
[65] Walter MC, Reilich P, Thiele S, et al. Treatment of dysferlinopathy with deflazacort: a double-blind, placebo-controlled clinical trial. Orphanet J Rare Dis 2013;8:26.
[66] Mendell JR, Rodino-Klapac LR, Rosales XQ, et al. Sustained alpha-sarcoglycan gene expression after gene transfer in limb-girdle muscular dystrophy, type 2D. Ann Neurol 2010;68:629–38.
[67] Wagner KR, Fleckenstein JL, Amato AA, et al. A phase I/II trial of MYO-029 in adult subjects with muscular dystrophy. Ann Neurol 2008;63:561–71.
[68] Luna ND, Díaz-Manera J, Paradas C, et al. 1α,25(OH)2-Vitamin D3 increases dysferlin expression *in vitro* and in a human clinical trial. Mol Ther 2012;20:1988–97.
[69] Lerario A, Cogiamanian F, Marchesi C, et al. Effects of rituximab in two patients with dysferlin-deficient muscular dystrophy. BMC Musculoskelet Disord 2010;11:157.
[70] Wong-Kisiel LC, Kuntz NL. Two siblings with limb-girdle muscular dystrophy type 2E responsive to deflazacort. Neuromuscul Disord 2010; 20:122–4.

[71] Darin N, Kroksmark A-K, Ahlander A-C, Moslemi A-R, Oldfors A, Tulinius M. Inflammation and response to steroid treatment in limb-girdle muscular dystrophy 2I. Eur J Paediatr Neurol 2007; 11:353–7.
[72] Willis TA, Hollingsworth KG, Coombs A, et al. Quantitative muscle MRI as an assessment tool for monitoring disease progression in LGMD2I: a multicentre longitudinal study. PLoS ONE 2013;8:e70993.
[73] Rosales XQ, Moser SJ, Tran T, et al. Cardiovascular magnetic resonance of cardiomyopathy in limb girdle muscular dystrophy 2B and 2I. J Cardiovasc Magn Reson 2011;13:39.
[74] Beckmann JS, Richard I, Hillaire D, et al. A gene for limb-girdle muscular dystrophy maps to chromosome 15 by linkage. C R Acad Sci III 1991;312:141–8.
[75] van der Kooi AJ, Barth PG, Busch HF, et al. The clinical spectrum of limb girdle muscular dystrophy. Brain 1996;119 (Pt 5):1471–80.
[76] Norwood FLM, Harling C, Chinnery PF, Eagle M, Bushby K, Straub V. Prevalence of genetic muscle disease in Northern England: in-depth analysis of a muscle clinic population. Brain 2009;132:3175–86.
[77] Guglieri M, Magri F, D'Angelo MG, et al. Clinical, molecular, and protein correlations in a large sample of genetically diagnosed Italian limb girdle muscular dystrophy patients. Hum Mutat 2008;29:258–66.
[78] Vainzof M, Passos-Bueno MR, Pavanello RC, Marie SK, Oliveira AS, Zatz M. Sarcoglycanopathies are responsible for 68% of severe autosomal recessive limb-girdle muscular dystrophy in the Brazilian population. J Neurol Sci 1999;164:44–9.
[79] Okizuka Y, Takeshima Y, Itoh K, et al. Low incidence of limb-girdle muscular dystrophy type 2C revealed by a mutation study in Japanese patients clinically diagnosed with DMD. BMC Med Genet 2010;11: 49.
[80] Georgieva B, Todorova A, Tournev I, Mitev V, Kremensky I. C283Y gamma-sarcoglycan gene mutation in the Bulgarian Roma (Gypsy) population: prevalence study and carrier screening in a high-risk community. Clin Genet 2004;66:467–72.
[81] Kefi M, Amouri R, Driss A, et al. Phenotype and sarcoglycan expression in Tunisian LGMD 2C patients sharing the same del521-T mutation. Neuromuscul Disord 2003;13:779–87.
[82] Spengos K, Walter MC, Dekomien G, Papadopoulos K, Lochmüller H, Manta P. C283Y mutation in the gamma-sarcoglycan gene in Greek Gypsies with severe limb girdle muscular dystrophy. Eur J Neurol 2010;17:e41–e42.
[83] Todorova A, Tournev I, Ninova N, Georgieva V, Kremensky I. Screening for C283Y gamma-sarcoglycan mutation in a high-risk group of Bulgarian Gypsies: evidence for a geographical localization and a non-random distribution among Gypsy subgroups. Community Genet 2002;5:217–21.
[84] 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.
[85] Lo HP, Cooper ST, Evesson FJ, et al. Limb-girdle muscular dystrophy: diagnostic evaluation, frequency and clues to pathogenesis. Neuromuscul Disord 2008;18:34–44.
[86] Laval SH, Bushby KMD. Limb-girdle muscular dystrophies–from genetics to molecular pathology. Neuropathol Appl Neurobiol 2004;30:91–105.
[87] Beckmann JS, Bushby KM. Advances in the molecular genetics of the limb-girdle type of autosomal recessive progressive muscular dystrophy. Curr Opin Neurol 1996;9:389–93.
[88] Bushby KM. The limb-girdle muscular dystrophies-multiple genes, multiple mechanisms. Hum Mol Genet 1999;8:1875–82.
[89] Bushby KM. Making sense of the limb-girdle muscular dystrophies. Brain 1999;122 (Pt 8):1403–20.
[90] Narayanaswami P, Weiss M, Selcen D, et al. Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Neurology 2014;83:1453–63.
[91] Norwood F, de Visser M, Eymard B, Lochmüller H, Bushby K. EFNS guideline task force. EFNS guideline on diagnosis and management of limb girdle muscular dystrophies. Eur J Neurol 2007;14:1305–12.
[92] Kobuke K, Piccolo F, Garringer KW, et al. A common disease-associated missense mutation in alpha-sarcoglycan fails to cause muscular dystrophy in mice. Hum Mol Genet 2008;17:1201–13.
[93] Bartoli M, Gicquel E, Barrault L, et al. Mannosidase I inhibition rescues the human alpha-sarcoglycan R77C recurrent mutation. Hum Mol Genet 2008;17:1214–21.
[94] Santoriello C, Zon LI. Hooked! Modeling human disease in zebrafish. J Clin Invest 2012;122:2337–43.
[95] Pappalardo A, Pitto L, Fiorillo C, Alice Donati M, Bruno C, Santorelli FM. Neuromuscular disorders in zebrafish: state of the art and future perspectives. Neuromolecular Med 2013;15:405–19.
[96] Steffen LS, Guyon JR, Vogel ED, et al. Zebrafish orthologs of human muscular dystrophy genes. BMC Genomics 2007;8:79.
[97] Moore CJ, Goh HT, Hewitt JE. Genes required for functional glycosylation of dystroglycan are conserved in zebrafish. Genomics 2008;92:159–67.
[98] Sato T, Mishina M. Representational difference analysis, high-resolution physical mapping, and transcript identification of the zebrafish genomic region for a motor behaviour. Genomics 2003;82:218–29.
[99] Avsar-Ban E, Ishikawa H, Manya H, et al. Protein O-mannosylation is necessary for normal embryonic development in zebrafish. Glycobiology 2010;20:1089–102.
[100] Chamberlain JS, Benian GM. Muscular dystrophy: the worm turns to genetic disease. Curr Biol 2000;10:R795–R797.
[101] Plantié E, Migocka-Patrzałek M, Daczewska M, Jagla K. Model organisms in the fight against muscular dystrophy: lessons from drosophila and zebrafish. Molecules 2015;20:6237–53.
[102] Giacomotto J, Ségalat L. High-throughput screening and small animal models, where are we? Br J Pharmacol 2010;160:204–16.
[103] Vandamme TF. Use of rodents as models of human diseases. J Pharm Bioall Sci 2014;6:2–9.
[104] Homburger F, Baker JR, Nixon CW Wilgram G. New hereditary disease of Syrian hamsters. Primary, generalized polymyopathy and cardiac necrosis. Arch Intern Med 1962;110:660–2.
[105] Sakamoto A, Ono K, Abe M, et al. Both hypertrophic and dilated cardiomyopathies are caused by mutation of the same gene, delta-sarcoglycan, in hamster: an animal model of disrupted dystrophin-associated glycoprotein complex. PNAS 1997;94:13873–8.
[106] Nigro V, Okazaki Y, Belsito A, et al. Identification of the Syrian hamster cardiomyopathy gene. Hum Mol Genet 1997;6:601–7.
[107] Okazaki Y, Okuizumi H, Ohsumi T, et al. A genetic linkage map of the Syrian hamster and localization of cardiomyopathy locus on chromosome 9qa2.1-b1 using RLGS spot-mapping. Nat Genet 1996;13:87–90.
[108] Holt KH, Lim LE, Straub V, et al. Functional rescue of the sarcoglycan complex in the BIO 14.6 hamster using delta-sarcoglycan gene transfer. Mol Cell 1998;1:841–8.
[109] Goldstein JA, Kelly SM, LoPresti PP, et al. SMAD signaling drives heart and muscle dysfunction in a Drosophila model of muscular dystrophy. Hum Mol Genet 2011;20:894–904.
[110] Bartoli M, Roudaut C, Martin S, et al. Safety and efficacy of AAV-mediated calpain 3 gene transfer in a mouse model of limb-girdle muscular dystrophy type 2A. Mol Ther 2006;13:250–9.
[111] Krahn M, Wein N, Bartoli M, et al. A naturally occurring human minidysferlin protein repairs sarcolemmal lesions in a mouse model of dysferlinopathy. Sci Transl Med 2010;2:50ra69–50ra69.
[112] Lostal W, Bartoli M, Bourg N, et al. Efficient recovery of dysferlin deficiency by dual adeno-associated vector-mediated gene transfer. Hum Mol Genet 2010;19:1897–907.
[113] Li J, Dressman D, Tsao YP, Sakamoto A, Hoffman EP, Xiao X. rAAV vector-mediated sarcogylcan gene transfer in a hamster model for limb girdle muscular dystrophy. Gene Ther 1999;6:74–82.
[114] Dressman D, Araishi K, Imamura M, et al. Delivery of alpha- and beta-sarcoglycan by recombinant adeno-associated virus: efficient rescue of muscle, but differential toxicity. Hum Gene Ther 2002; 13:1631–46.

[115] Cordier L. Rescue of skeletal muscles of gamma-sarcoglycan-deficient mice with adeno-associated virus-mediated gene transfer. Mol Ther 2000;1:119–29.
[116] Rodino-Klapac LR, Lee J-S, Mulligan RC, Clark KR, Mendell JR. Lack of toxicity of alpha-sarcoglycan overexpression supports clinical gene transfer trial in LGMD2D. Neurology 2008;71:240–7.
[117] Fougerousse F, Bartoli M, Poupiot J, et al. Phenotypic correction of alpha-sarcoglycan deficiency by intra-arterial injection of a muscle-specific serotype 1 rAAV vector. Mol Ther 2007;15:53–61.
[118] Mendell JR, Rodino-Klapac LR, Sahenk Z, et al. Eteplirsen for the treatment of Duchenne muscular dystrophy. Ann Neurol 2013;74:637–47.
[119] Aartsma-Rus A, Singh KHK, Fokkema IFAC, et al. Therapeutic exon skipping for dysferlinopathies? Eur J Hum Genet 2010;18:889–94.
[120] Pettit RS, Fellner C. CFTR modulators for the treatment of cystic fibrosis. P T 2014;39:500–11.
[121] Bushby K, Finkel R, Wong B, et al. Ataluren treatment of patients with nonsense mutation dystrophinopathy. Muscle Nerve 2014;50:477–87.
[122] Wang B, Yang Z, Brisson BK, et al. Membrane blebbing as an assessment of functional rescue of dysferlin-deficient human myotubes via nonsense suppression. J Appl Physiol 2010;109:901–5.
[123] Sampaolesi M, Torrente Y, Innocenzi A, et al. Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. Science 2003;301:487–92.
[124] Lapidos KA, Chen YE, Earley JU, et al. Transplanted hematopoietic stem cells demonstrate impaired sarcoglycan expression after engraftment into cardiac and skeletal muscle. J Clin Invest 2004; 114:1577–85.
[125] Tedesco FS, Gerli MFM, Perani L, et al. Transplantation of genetically corrected human iPSC-derived progenitors in mice with limb-girdle muscular dystrophy. Sci Transl Med 2012;4:140ra89.
[126] Kuang S, Rudnicki MA. The emerging biology of satellite cells and their therapeutic potential. Trends Mol Med 2008;14:82–91.
[127] de Souza GT, Zanette Rde S, do Amaral DL, et al. Satellite cells: regenerative mechanisms and applicability in muscular dystrophy. Stem Cells Int 2015;2015:1–12.
[128] Nomura T, Ashihara E, Tateishi K, et al. Skeletal myosphere-derived progenitor cell transplantation promotes neovascularization in delta-sarcoglycan knockdown cardiomyopathy. Biochem Biophys Res Commun 2007;352:668–74.
[129] van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. J Neurol Neurosurg Psychiatry 1991;54:244–7.
[130] Mazzone ES, Messina S, Vasco G, et al. Reliability of the North Star Ambulatory Assessment in a multicentric setting. Neuromuscul Disord 2009;19:458–61.
[131] ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111–17.
[132] Florence JM, van der Ploeg A, Clemens PR, et al. Use of the 6 min walk test as an endpoint in clinical trials for neuromuscular diseases. Neuromuscul Disord 2008;18:738–9.
[133] Mayhew A, Mazzone ES, Eagle M, et al. Development of the Performance of the Upper Limb module for Duchenne muscular dystrophy. Dev Med Child Neurol 2013;55:1038–45.
[134] Sarkozy A, Deschauer M, Carlier R-Y, et al. Muscle MRI findings in limb girdle muscular dystrophy type 2L. Neuromuscul Disord 2012;22(Suppl. 2):S122–S129.
[135] Hafner P, Bonati U, Fischmann A, et al. Skeletal muscle MRI of the lower limbs in congenital muscular dystrophy patients with novel POMT1 and POMT2 mutations. Neuromuscul Disord 2014;24:321–4.
[136] Mercuri E, Bushby K, Ricci E, et al. Muscle MRI findings in patients with limb girdle muscular dystrophy with calpain 3 deficiency (LGMD2A) and early contractures. Neuromuscul Disord 2005;15:164–71.
[137] Fischer D, Walter MC, Kesper K, et al. Diagnostic value of muscle MRI in differentiating LGMD2I from other LGMDs. J Neurol 2005; 252:538–47.
[138] Wattjes MP, Kley RA, Fischer D. Neuromuscular imaging in inherited muscle diseases. Eur Radiol 2010;20:2447–60.
[139] Fischer D, Kley RA, Strach K, et al. Distinct muscle imaging patterns in myofibrillar myopathies. Neurology 2008;71:758–65.
[140] Quijano-Roy S, Avila-Smirnow D, Carlier RY. WB-MRI muscle study group. Whole body muscle MRI protocol: pattern recognition in early onset NM disorders. Neuromuscul Disord 2012;22(Suppl. 2):S68–S84.
[141] Biomarkers Definitions Working Group Bethesda. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89–95.
[142] Matsuzaka Y, Kishi S, Aoki Y, et al. Three novel serum biomarkers, miR-1, miR-133a, and miR-206 for Limb-girdle muscular dystrophy, facioscapulohumeral muscular dystrophy, and Becker muscular dystrophy. Environ Health Prev Med 2014;19:452–8.
[143] Herson S, Hentati F, Rigolet A, et al. A phase I trial of adeno-associated virus serotype 1-γ-sarcoglycan gene therapy for limb girdle muscular dystrophy type 2C. Brain 2012;135:483–92.
[144] Mendell JR, Rodino-Klapac L, Sahenk Z, et al. Gene therapy for muscular dystrophy: lessons learned and path forward. Neurosci Lett 2012;527:90–9.
[145] Sharma A, Sane H, Badhe P, et al. A clinical study shows safety and efficacy of autologous bone marrow mononuclear cell therapy to improve quality of life in muscular dystrophy patients. Cell Transplant 2013;22(Suppl. 1):S127–S138.
[146] Manzur AY, Kuntzer T, Pike M, Swan A. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. Cochrane Database Syst Rev 2008;(1):CD003725.
[147] Angelini C, Fanin M, Menegazzo E, Freda MP, Duggan DJ, Hoffman EP. Homozygous alpha-sarcoglycan mutation in two siblings: one asymptomatic and one steroid-responsive mild limb-girdle muscular dystrophy patient. Muscle Nerve 1998;21:769–75.