Emerging therapeutic strategies for sarcoglycanopathy

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Emerging therapeutic strategies for sarcoglycanopathy

Marcello Carotti, Chiara Fecchio and Dorianna Sandonà

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ABSTRACT

Introduction: Sarcoglycanopathy is the name shared by four rare autosomal recessive muscular dystrophies (LGMD2 C-F) that are usually characterized by early onset and rapid progression and an accompanying loss of independent walking since adolescence. Respiratory problems are frequent, and dilated cardiomyopathy may occur, although milder forms have also been described. However, sarcoglycanopathy is currently incurable, and we herein aim to describe the state of the art in the field of treatments for this disease. Areas covered: We summarize the pathogenesis of sarcoglycanopathy, with particular emphasis on the molecular mechanism(s) underlying the disease. We describe the very few published cases of symptomatic treatment with steroids and the gene therapy approaches that have entered phase II/III clinical trials. We then present emerging novel therapeutic strategies explored at the preclinical stage that are expected to replace the defective gene (cell therapy), address general effects of the disease, or address the primary events of the pathogenic mechanism (small molecule-based therapy). Expert opinion: Anti-inflammatory strategies, which are at present empirically applied, warrant further exploration. Although promising and currently being evaluated in clinical trials, gene therapy remains associated with concerns and requires additional confirmation. Thus, novel strategies targeting different aspects of the disease pathogenic mechanism are highly anticipated.

1. Sarcoglycanopathy phenotype, clinical features and epidemiology

Sarcoglycanopathy is the name given to four rare genetic diseases that affect striated muscle. These diseases are members of the autosomal recessive limb girdle muscular dystrophies (LGMD2) family because the most affected muscles are those of the pelvic and shoulder girdle [1,2]. Mutations in the SGCA gene, which encodes α-sarcoglycan [3], SGCB, which encodes β-sarcoglycan [4,5], SGCD, which encodes δ-sarcoglycan [6] and SGCG, which encodes γ-sarcoglycan [7], are responsible for LGMD2D, 2E, 2F, and 2C, respectively.

Sarcoglycanopathy is a heterogeneous disease, and most cases are characterized by early onset and rapid progression, with patients becoming wheelchair bound in adolescence. Nevertheless, milder phenotypes characterized by late onset and ambulation preserved until adulthood have been described. Interestingly, this variability has been reported among members of the same family and even between siblings [8,9]. However, the disease is invariably progressive, and muscle weakness eventually involves the respiratory muscle, with the patient’s need for respiratory support [10]. Cognitive impairment has never been reported, whereas cardiomyopathy may occur in all forms, although rarely in LGMD2D. Therefore, patients require constant medical surveillance [11,12]. This disease is also characterized by elevated levels of serum creatine kinase (more than 10 times the upper limit), calf hypertrophy, and scapular winging. Moreover, contractures and scoliosis commonly develop during the disease as a consequence of increasing weakness and the fibrotic degeneration of skeletal muscles [13].

Evaluating the worldwide prevalence of sarcoglycanopathy is difficult because this disease is rare and published papers have been regionally specific. For example, the combined LGMD2C–F prevalence in Northern England has been estimated to be approximately 0.27/100000 [14], whereas the prevalence is approximately 0.56/100000 in Northeastern Italy [15]. In outbred populations, LGMD2D seems to be the most frequent form of sarcoglycanopathy, followed by LGMD2C and 2E, whereas reports on LGMD2F have been very rare [16–18]. Moreover, a founder effect is observed in certain regions, such as a higher prevalence of LGMD2C in India and Maghreb, where the allele frequency of S2DF2-SGCC is high [19], and in the Gypsy population, who exhibit a high allele frequency of the p.C283T mutation in the SGCG gene [20]. In Europe, a founder effect has been observed for the p.R77C mutation of the SGCA gene, which is responsible for all LGMD2D cases reported in Finland [21] in both homozygous (10 cases) and heterozygous (1 case) carriers.

2. Sarcoglycans and the sarcoglycan complex

Sarcoglycans (SG) are glycosylated proteins with a large extracellular domain, a transmembrane helix and a short cytosolic tail. α-SG is a type I membrane protein, whereas β-, γ-, and δ-SG are type II membrane proteins that form a heterocomplex by unitary stoichiometry [3,4,7,22,23]. Two additional SG have been identified, ε-SG and ζ-SG. The former is homologous to α-SG (approximately

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Sarcoglycanopathy remains without a cure, and supportive interventions may only ameliorate deteriorations in the patient’s quality of life and delay disease progression.

Anti-inflammatory treatments have been adopted in patients, but they need to be tested in depth.

The first gene therapy clinical trials in LGMD2D are producing promising data.

The elucidation of the molecular pathogenesis of sarcoglycanopathy is essential for the identification of proper outcome measures and endpoints for clinical trials.

The study of the natural history of sarcoglycanopathy is essential for the identification of proper outcome measures and endpoints for clinical trials.

This box summarizes key points contained in the article.

Article highlights

- Sarcoglycanopathy remains without a cure, and supportive interventions may only ameliorate deteriorations in the patient’s quality of life and delay disease progression.
- Anti-inflammatory treatments have been adopted in patients, but they need to be tested in depth.
- The first gene therapy clinical trials in LGMD2D are producing promising data.
- The elucidation of the molecular pathogenesis of sarcoglycanopathy is essential for the identification of proper outcome measures and endpoints for clinical trials.
- The study of the natural history of sarcoglycanopathy is essential for the identification of proper outcome measures and endpoints for clinical trials.

In striated muscle, the SG complex is part of the dystrophin-associated protein complex (DAPC) and contributes to connect the intracellular cytoskeleton to the extracellular matrix. Overall, DAPC plays an essential role in transmitting the force generated by muscle contraction and protecting the sarcolemma during this process [28]. SG are cotranslationally translocated into the endoplasmic reticulum (ER), where they undergo folding and maturation, such as glycosylation and disulfide bond formation (Figure 1). Assembly also occurs in the ER [29]. Several studies, carried out with both myogenic cells endogenously expressing SGs or cellular models ectopically expressing SGs predicted a model in which β- and δ-SG first form a core complex to which γ-SG and α-SG subsequently bind. Once assembled, the tetramer leaves the ER and, moving through the Golgi apparatus toward the sarcolemma, interacts with the dystroglycan heterocomplex and sarcospan [30-32]. Both glycosylation and disulfide bridge formation seem to be essential to guarantee complex formation, traffic and interaction with the dystroglycan complex. The intracellular tails of β- and δ-SG are thought to be crucial for the interaction of the complex with dystrophin [33,34]. Moreover, α-SG, the less tightly bond subunit, seems to be able to traffic toward the plasma membrane alone when heterologously expressed, but it is unstable at the cell surface and quickly recycled in the absence of the other complex subunits [35].

Mutations in any SG often have deleterious consequence on the entire SG complex. In fact, not only the mutated protein but also wild-type SG are strongly reduced or even absent from the sarcolemma, strengthening the idea that SG operate as a single unit [16,23,36,37]. However, other studies suggest that not all SG play the same role in complex assembly and stability: mutations in SGCB or SGCD seem to be deleterious and result in total complex loss, whereas mutations in SGCG or SGCA may partially preserve the other three subunits [38,39].

Embedded in the DAPC, SG interact with many components of the macromolecular complex, such as biglycan, the membrane protein sarcospan and the dystroglycan heterocomplex. On the cytosolic side, the SG complex is in contact with the phosphoprotein α-dystrobrevin, which creates an indirect link with syntrophin and nNOS [28]. The main function of the SG complex is primarily structural, and it protects striated muscle from contraction-induced injury by stabilizing and strengthening the transmembrane link between dystrophin and the extracellular matrix. However, several lines of evidence suggest direct or indirect roles for the SG complex in signal transduction. For example, γ-SG seems to be involved in bidirectional signals with integrins [40,41], and it is thought to sense contractility upon becoming phosphorylated.

Figure 1. Sarcoglycan-complex biogenesis.

Sarcoglycans are co-translationally translocated into the endoplasmic reticulum (ER), where they undergo glycosylation and folding to acquire their native conformation. When properly folded, sarcoglycan complex formation starts with the establishment of a βδ-SG core with which γ-SG and α-SG subsequently interact to form a tetramer. Once assembled, the sarcoglycan complex leaves the ER and, passing through the Golgi apparatus, moves towards the sarcolemma [29].
after mechanical stimulation [42,43]. Furthermore, α-SG can bind to and hydrolyze extracellular ATP [44,45], suggesting a role in controlling the signaling initiated by this nucleotide via the purinergic receptors [46]. By participating in nNOS anchorage, the SG complex may play a role in NO signaling in both skeletal and smooth muscle cells [2]. Furthermore, α-SG seems to positively modulate FGF signaling by forming a stable complex with FGFR1 in muscle satellite cells under normal conditions [47].

3. Molecular pathogenesis of sarcoglycanopathy

The absence/strong reduction of SG in the sarcolemma reported in patients with sarcoglycanopathy, by altering the overall DAPC function, leads to the progressive degeneration of myofibers, which are consequently replaced with adipose and fibrotic tissue [2]. It has been reported that in LGMD2C, the absence of γ-SG induced subsarcolemmal lesions that were almost indistinguishable from those of Duchenne muscular dystrophy (DMD) [48]. Analyses of animal models of sarcoglycanopathy show the development of progressive muscular dystrophy, as evidenced by Evans blue Dye uptake in both the skeletal muscle and heart, which only occurs in damaged myofibers [49–51]. Moreover, an increase in cytosolic calcium has been observed in fibers isolated from these animals, which suggests that calcium overload is primarily responsible for the progressive degeneration of myofibers not only in DMD but also in sarcoglycanopathy. Furthermore, a membrane channel permeable to calcium was observed to be activated in the BIO 14.6 hamster, an animal model of LGMD2F [52]. The possible downstream effects of calcium dysregulation are not limited to calpain activation and the induction of myofiber necrosis. Indeed, calcium dysregulation may result in mitochondrial dysfunction, as observed in cells isolated from the cardiomyopathic BIO 14.6 hamster [53,54], and an increase in ROS production, as observed in the mdx mouse, the animal model of DMD [55]. DAPC disruption also results in the absence/strong reduction of nNOS, as observed in muscle biopsies from patients with sarcoglycanopathy. This phenomenon has always been associated with a severe disease phenotype, suggesting that changes in NO production may contribute to the progression and worsening of the disease [2].

Irrespective of the mechanism that results in the progressive myofiber degeneration, in sarcoglycanopathy the primary defect is the lack of or reduction in a mutated SG [16,36,37]. However, many different mutations can be responsible for the development of this disease, and all known variations in SG genes with pathogenic consequences listed in the Leiden database [http://www.dmd.nl] are reported in Table 1.

In SGCA and SGCD, null and frameshift mutations account for approximately 10% and 6% of defects, respectively, whereas they represent a consistent fraction of the total, approximately 22–23%, in SGCB and SGCG. Moreover, the majority of defects in all SG genes except SGCD are missense mutations that generate a protein with a single amino acid substitution.

At least 55, 29, 22, and 8 different missense mutations have been reported in SGCA, SGCB, SGCC, and SGCD, respectively (Table 2). The most frequently reported α-SG amino acid substitution is R77C, accounting for more than one-third of reported cases, followed by R284C and V247M. Of the 29 different known β-SG variants, the S114F amino acid substitution accounts for approximately 45% of all LGMD2E cases. Similarly, the mutation C283Y is responsible for half of all reported LGMD2C cases. Only eight different amino acid substitutions are known for δ-SG, with two variants (A31P and R97N) together accounting for more than half the reported cases of LGMD2F. Finally, many patients with sarcoglycanopathy are compound heterozygotes because of the presence of different mutations on the two alleles of the defective SG gene [http://www.dmd.nl].

The absence of the protein from the muscle of patients with sarcoglycanopathy can be easily explained when the mutation impairs either transcription or affects splicing sites, which results in the production of aberrant transcripts. On the other hand, both null, out-of-frame, in-frame insertions/deletions and missense mutations (see Table 1) are also responsible for the loss/strong reduction of the affected protein. While in some cases, the

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Protein</th>
<th>Length (aa)</th>
<th>Null</th>
<th>Frame shift</th>
<th>Missense</th>
<th>In-frame deletion, insertion &amp; duplication</th>
<th>N. different variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD2D</td>
<td>SGCA</td>
<td>α-SG</td>
<td>387</td>
<td>7.1</td>
<td>23.8</td>
<td>65.5</td>
<td>3</td>
<td>84</td>
</tr>
<tr>
<td>LGMD2E</td>
<td>SGCB</td>
<td>β-SG</td>
<td>318</td>
<td>13.7</td>
<td>25.5</td>
<td>56.9</td>
<td>4</td>
<td>51</td>
</tr>
<tr>
<td>LGMD2C</td>
<td>SGCG</td>
<td>γ-SG</td>
<td>291</td>
<td>14.5</td>
<td>30.8</td>
<td>40</td>
<td>14.5</td>
<td>55</td>
</tr>
<tr>
<td>LGMD2F</td>
<td>SGCD</td>
<td>δ-SG</td>
<td>290</td>
<td>21.4</td>
<td>7.1</td>
<td>57.2</td>
<td>14.3</td>
<td>14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Different missense mutations</th>
<th>Most frequently reported AA substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGCA</td>
<td>α-SG</td>
<td>55 R77C 36.30%</td>
<td>R284C 11.40% V247M 8.10%</td>
</tr>
<tr>
<td>SGCB</td>
<td>β-SG</td>
<td>29 S114F 45%</td>
<td>T151R 6.40% G167S 6.40%</td>
</tr>
<tr>
<td>SGCG</td>
<td>γ-SG</td>
<td>22 C283Y 50%</td>
<td>E263K 11.70% R116H 7%</td>
</tr>
<tr>
<td>SGCD</td>
<td>δ-SG</td>
<td>8 A31P 28%</td>
<td>R97N 25% S151A 1.40%</td>
</tr>
</tbody>
</table>
presence of a premature stop codon could activate the nonsense-mediated mRNA decay (NMD) resulting in the lack of polypeptide synthesis, in most cases, the defect leads to the production of a full-length protein with a single amino acid substitution. Therefore, a strong reduction in the amount of the mutated SG observed in these cases must be ascribed to a posttranslational mechanism.

Each newly synthesized protein is scrutinized by a quality control (QC) system that can discriminate between properly folded and folding-defective proteins. The former can proceed in the biosynthetic pathway, whereas the latter are discarded to avoid the accumulation of potentially harmful protein forms. The protein QC system operates in all cellular compartments and eliminates protein waste due to transcriptional/translational failures, genomic mutations or stress conditions [56]. SG, which are proteins destined for the plasma membrane, are thought to undergo QC surveillance inside the ER. In this organelle, a complex set of molecular chaperones, glycosylating and modifying enzymes helps proteins to properly fold. However, if the native conformation is not reached after repeated folding cycles, the defective polypeptides are recognized, delivered to ER exit sites and transferred into the cytosol via a macromolecular complex called dislocon. During this process, ubiquitination by ER membrane-E3 ligases marks the misfolded protein for subsequent degradation by the 26S proteasome. Overall, this pathway is known as the endoplasmic reticulum-associated protein degradation (ERAD) pathway [57–59]. In 2008, two papers for the first time showed that α-SG missense mutants are effective substrates of this degradative pathway. In heterologous cell models, proteins carrying different amino acid substitutions were polyubiquitinated and rapidly degraded by the proteasome [60,61]. A few years later, not only α-SG but all SG were shown to undergo the same fate when carrying amino acid substitutions leading to a minimal tertiary structure alteration [62]. Finally, many of the ERAD components responsible for α-SG mutant disposal were identified in 2014 via different and selective molecular approaches [63]. Figure 2 summarizes this process and highlights the involved enzymes. Specifically, α-mannosidase I ensures that α-SG is preferentially targeted for degradation if terminally misfolded; the E2 and E3 enzymes UbE2J1 and HRD1 are responsible for polyubiquitination; cofactors, such as Sel1L and Derlin1, help this process and retrotranslocation; the AAA-ATPase p97 provides the driving force for membrane eradication; and the 26S proteasome acts as the ultimate actual degradative machine (Figure 2). Interestingly, these studies showed that the SG mutants can be restored at the proper cellular location by preventing their disposal either acting at the step of recognition [60,62], ubiquitination [63] or proteasomal degradation [60,61]. This finding suggests that the primary defect in these forms of sarcoglycanopathy is the loss of function due to the premature disposal of a folding-defective but potentially functional SG. Moreover, these studies imply that all elements of the degradative pathway are potentially druggable targets. The latter is of special interest because effective therapies to treat sarcoglycanopathy are currently lacking.

4. Therapeutic interventions

The severe phenotype of sarcoglycanopathy is responsible for a fatal prognosis in most affected individuals. Though rare, the disease has a dramatic social impact, particularly concerning the management and assistance of patients. Unfortunately, despite extensive efforts, no efficacious therapy is currently in use to treat sarcoglycanopathy, and the paragraph related to investigated therapies examines supportive interventions that aim to preserve the quality of life as much as possible and prevent fatal consequences due to respiratory failure or sudden cardiac death, which are often associated with sarcoglycanopathy, particularly LGMD2E and 2F. The few reports related to the use of steroids for the treatment of sarcoglycanopathy and gene therapy approaches that are in the initial phases of clinical trials are also discussed below and summarized in Table 3. Finally, the paragraph dedicated to emerging therapeutic strategies analyzes approaches investigated at the preclinical stage, such as cell therapy or small-molecule-based strategies designed thanks to the knowledge of the molecular pathogenesis of this disease. These approaches are summarized in Table 4.

4.1. Investigated therapeutic strategies

4.1.1. Supportive interventions

Patients with sarcoglycanopathy require constant supportive physiotherapy, which should start immediately after diagnosis to prevent joint deformity and preserve ambulation as long as possible. The use of supportive tools, such as knee-ankle-foot orthoses at bedtime, is recommended to prevent contractures, but severe scoliosis and/or contracture deformities, especially of the Achilles tendons, may require surgical intervention for functional correction. In sarcoglycanopathy, factors triggering myoglobinuria, such as exercise-induced muscle damage, should be avoided because of overall muscle fragility. Nevertheless, moderate-strength training and aerobic exercise training may be beneficial for patients with sarcoglycanopathy [64]. Myalgia and arthralgia should be treated to improve mobility and quality of life, and the progressive wasting of respiratory muscles requires the frequent monitoring of patients and ventilatory assistance, especially at night, to avoid respiratory failure. The cardiac surveillance of patients with sarcoglycanopathy, especially LGMD2E and 2F, should be prescribed at least annually because most patients experience asymptomatic serious cardiac injuries prior to cardiac morbidity or fatal outcome. Cardiac symptoms should be treated as soon as the echocardiographic/electrocardiographic findings result abnormal, including the use of diuretics, angiotensin-converting enzyme inhibitors, and beta-blockers. Patients who do not respond to these interventions but whose respiratory function is conserved should be included in the lists for cardiac transplantation [2]. Another important aspect of management includes body weight control and emotional support to prepare patients and their families for the long-term consequences of muscular dystrophies.

4.1.2. Steroid treatments

At present, the sole pharmacological interventions for sarcoglycanopathy is based on the use of corticosteroids to alleviate symptoms and possibly delay the course of the disease. However, corticosteroids are adopted on an individual basis because of the presence of inflammatory infiltrate in biopsy
<table>
<thead>
<tr>
<th>Therapeutic intervention</th>
<th>Clinical trials</th>
<th>Disease</th>
<th>Mutation</th>
<th>N. of subjects</th>
<th>Duration/endpoint</th>
<th>Efficacy</th>
<th>Side effects</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steroid treatments</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Deflazacort</td>
<td>LGMD2D</td>
<td>p.R284C homo</td>
<td>1</td>
<td>6 months</td>
<td>Improved functional performance</td>
<td>Slight increase in blood pressure and irritability</td>
<td>[8]</td>
<td></td>
</tr>
<tr>
<td>Deflazazort</td>
<td>LGMD2E</td>
<td>p.S114F homo</td>
<td>2</td>
<td>22 and 30 months</td>
<td>Improved functional performance</td>
<td>No side effect</td>
<td>[68]</td>
<td></td>
</tr>
<tr>
<td>Prednisolone</td>
<td>LGMD2D (3)</td>
<td>N/A</td>
<td>1</td>
<td>1 to 4 years</td>
<td>Stable phenotype (3 subj.)</td>
<td>No adverse effects; slight increase in body weight (2 subj.)</td>
<td>[70]</td>
<td></td>
</tr>
<tr>
<td>Deflazacort</td>
<td>LGMD2C (2)</td>
<td>N/A</td>
<td>5</td>
<td>1 to 4 years</td>
<td>Worsened phenotype (3 subj.)</td>
<td>No adverse effects; slight increase in body weight (2 subj.)</td>
<td>[70]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LGMD2E (1)</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Gene replacement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rAAV1.tMCK.hSGCA</td>
<td>Phase I/II concluded</td>
<td>LGMD2D</td>
<td>Missense mutations</td>
<td>3</td>
<td>6 weeks-3 months</td>
<td>Full SG complex restored at the sarcolemma</td>
<td>No adverse effect; MHC1 overexpression; acquisition of AAV1 seropositivity</td>
<td>[81]</td>
</tr>
<tr>
<td>rAAV1.tMCK.hSGCA</td>
<td>Phase I/II concluded</td>
<td>LGMD2D</td>
<td>Missense mutations</td>
<td>3</td>
<td>6 months</td>
<td>Full SG complex restored at the sarcolemma (2/3 subj.)</td>
<td>No adverse effect; MHC1 overexpression; acquisition of AAV1 seropositivity</td>
<td>[82]</td>
</tr>
<tr>
<td>scAAVh74.tMCK.hSGCA</td>
<td>Phase I/II ongoing</td>
<td>LGMD2D</td>
<td>Missense mutations</td>
<td>6</td>
<td>30,60,90,180 days 1 and 2 years</td>
<td>N/A</td>
<td>N/A</td>
<td>NCT01976091</td>
</tr>
<tr>
<td>AAV1.des.hySGC (escalating doses)</td>
<td>Phase I/II concluded</td>
<td>LGMD2E</td>
<td>Missense mutations</td>
<td>9</td>
<td>6 months</td>
<td>Few fibers fully positive (3 subj. at higher dose)</td>
<td>No serious adverse effect; no MHC1 overexpression; acquisition of AAV1 seropositivity</td>
<td>[83]</td>
</tr>
</tbody>
</table>
### Table 4. Emerging therapeutic approaches.

<table>
<thead>
<tr>
<th>Therapeutic strategy</th>
<th>Gene</th>
<th>Mutation</th>
<th>Drug target/compound</th>
<th>in vitro</th>
<th>in vivo</th>
<th>Way of administration</th>
<th>Efficacy</th>
<th>Ref.</th>
</tr>
</thead>
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<tr>
<td><strong>Cell therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Adult muscle mononuclear cells (AMMCS)</td>
<td>Sgcd</td>
<td>Sgcd-null</td>
<td></td>
<td></td>
<td></td>
<td>Intramuscular injection</td>
<td>Regeneration of dystrophic muscle in aged mice</td>
<td>[85]</td>
</tr>
<tr>
<td>Genetically corrected human IPS derived</td>
<td>Sgca</td>
<td>Sgca-null</td>
<td></td>
<td></td>
<td></td>
<td>Intramuscular, vein injection</td>
<td>Amelioration of dystrophic phenotype, restoration of depleted progenitors</td>
<td>[86]</td>
</tr>
</tbody>
</table>
| **Exon skipping**                         | SGC
      | 521-ΔT      | Exon 6 of human SGCD/antisense oligonucleotide          | Fibroblast from LGMD2C patient                              |                           |              |                        | Expression of Mini-Gamma SG                                                                 | [91] |
| **ERAD targeting**                        | SGCA       | p.R77C            | α-Mannosidase 1/kifunensine proteasome/MEG132           | HER-911 cells expressing R77C-α-SG                               | α-SG null mice transduced with AAV expressing R77C-α-SG | Intraperitoneal injection | Restoration of proper membrane localization of mutated α-SG                               | [60] |
|                                           | SGCA, SGCB, SGCC, SGCD | Different pathogenic missense variants | α-Mannosidase 1/kifunensine                               | HeLa and HER-911 cells expressing different SG mutants |                           |                        | Restoration of proper membrane localization of mutated α-SG                               | [62] |
| **Muscle mass manipulation**              | Sgcd       | Sgcd-null         | Recombinant human IGF-1                                 | Hamster CHF-147 (δ-SG null)                                      |                           | Under-skin osmotic pump | Preservation of cardiac function, increased survival                                        | [94] |
|                                           | Sgca       | Sgca-null         | Histone deacetylase (HDAC)/trichostatin                  | Satellite cells from muscle of α-SG null mice                   | α-SG null mice transduced with AAV expressing R77C-α-SG | Intraperitoneal injection | Improved muscle mass, regeneration, reduced fibrosis and cellular infiltrate                | [95] |
|                                           | SGC
<pre><code>  | N/A            | Myostatin/MYO-029 (anti myostatin monoclonal antibody)   |                                                        |                           |              | Intravenous infusion | No improvement of muscle strength or function at end point                                  | [99] |
</code></pre>
<p>| <strong>Modification of calcium homeostasis</strong>   | Sgcd       | Sgcd-null         | L-type calcium channels/tranilast, diltiazem, FK506 (antagonists) |                                                        |                           | Oral administration | Protective effect for muscle degeneration                                                  | [100]|</p>
<table>
<thead>
<tr>
<th>Therapeutic strategy</th>
<th>Gene</th>
<th>Mutation</th>
<th>Drug target/compound</th>
<th>in vitro</th>
<th>in vivo</th>
<th>Way of administration</th>
<th>Efficacy</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sgcd</td>
<td>Sgcd-null</td>
<td>Calcineurin gene knock-down</td>
<td></td>
<td>6-5G null mice</td>
<td>Gene targeting in ES cells</td>
<td>Reduction in fibrosis in skeletal and cardiac muscle</td>
<td>[101]</td>
</tr>
<tr>
<td></td>
<td>Sgcd</td>
<td>Sgcd-null</td>
<td>Overexpression of SERCA1 and SERCA2</td>
<td></td>
<td>6-5G null mice</td>
<td>AAV injection in gastrocnemious</td>
<td>Reduction in fibrosis and CK release, improvement in exercise capacity</td>
<td>[102]</td>
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<td>Muscle membrane repair</td>
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<td>Overexpression of MG53</td>
<td>6-5G deficient TO-2 hamster</td>
<td>Intraperitoneal injection of AAV</td>
<td>Improved muscle and heart functions</td>
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<td>Normalization of NO production</td>
<td>Sgca</td>
<td>Sgca-null</td>
<td>HCT1026 (NO donor)</td>
<td>α-5G null mice</td>
<td>Drug incorporated in diet</td>
<td>Slowed disease progression</td>
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<td>Sgca</td>
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<td>Isosorbide dinitrate (NO donor) + ibuprofen</td>
<td>α-5G null mice</td>
<td>Drug incorporated in diet</td>
<td>Reduced muscle necrotic damage and inflammation</td>
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<td>Sgca</td>
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<td>Drug incorporated in diet</td>
<td>Improvement in oxidative metabolism</td>
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N/A: not available; subj: subject.
samples and/or based on the beneficial effects of similar treatments in DMD [65,66]. In 1998, Corrado Angelini’s group published the first report in which a 40-year-old female affected by a mild form of LGMD2D and homozygous for the R284C amino acid substitution in α-SG was treated with deflazacort for 6 months. For the first month, the dose was 60 mg/day (1 mg/kg), and this dose was subsequently reduced to 60 mg/alternate day (a.d.) because of an erythematous rash on the trunk and arms. The muscle strength and functional performance of the patient improved, as scored by a protocol that included five functional tests and eight graded muscles (MRC index) (the score increased from 86.25% before treatment to 93.75% after 6 months of treatment). The only side effects were a slight increase in blood pressure and irritability [8]. However, no data of longer treatment has been subsequently published. The same year, another paper described the effect of prolonged treatment with prednisone (up to 3 years) on an 8-year-old girl. A muscle biopsy clearly indicated dystrophy, as evidenced by the infiltration of inflammatory cells, and the genetic analysis identified a mutation in the SGCA gene. Furthermore, proximal muscle strength improved during the first 2 months of treatment, and this improvement persisted throughout the treatment period and was not associated with side effects [67]. Deflazacort also seems to be effective in some patients with LGMD2E. Specifically, two siblings who are homozygous for the amino acid substitution S114F in β-SG were treated with 0.9 mg/kg/day deflazacort, which improved or stabilized muscle strength and maintained pulmonary function after 22 and 30 months of treatment without steroid-associated side effects [68]. In this study, pentoxifylline, a nonspecific phosphodiesterases inhibitor with anti-inflammatory properties, was added 4 months after initiating deflazacort administration and maintained throughout the treatment. Although pentoxifylline did not improve muscle strength and function in a DMD clinical trial [69], its presence during the treatment precluded evaluating the effective contribute of deflazacort in the study [68]. A retrospectively study, performed by Albuquerque et al. in 2014 [70], analyzed 6 patients diagnosed with differential sarcoglycanopathy (3 LGMD2D, 2 LGMD2C and 1 LGMD2E) who were treated with steroids, that is, prednisolone (1 subject) or deflazacort (5 subjects) for a period ranging from 1 to 4 years, but failed to show an overt clinical improvement. In fact, three patients presented a stable phenotype, but three worsened. The authors argue that the response to corticosteroid can vary by individual and may depend on the type of mutation [70]. Considering these few reports and the small number of patients treated, the regimen diversity, and the short evaluation period, conclusions regarding the efficacy of corticosteroids in sarcoglycanopathy cannot be drawn. However, based on the positive effect in patients with DMD and patients affected by other forms of LGMD [71,72], studies of larger cohorts with defined subgroups and control groups are warranted to clearly identify the real benefits of steroids for sarcoglycanopathy.

4.1.3. Gene replacement
Because sarcoglycanopathy is characterized by the loss or the strong reduction of the protein encoded by the mutated SG gene, the most convenient and potentially effective strategy to recover the disease phenotype likely is the virus-mediated transfer of a healthy copy of the gene. An additional advantage of applying this strategy in sarcoglycanopathy is the small dimension of the SG coding sequences, which allows them to be packaged into many different types of viral vectors. The successful re-expression of any SG and concomitant recovery of the dystrophin glycoprotein complex, the reduction in muscle damage [73–75] and the prevention of cardiac

Figure 2. ER associated degradation (ERAD) of folding-defective α-sarcoglycan mutants.

During the biosynthetic process, SG undergo careful screening by the quality control system of the ER. When a mutation (here exemplified by a red star) prevents α-SG from reaching the native conformation despite repeated folding attempts, the α-mannosidase I enzyme terminates the maturation process and delivers the terminally misfolded α-SG to ER exit sites (dislocan). Dislocation into the cytosol occurs via a multimeric complex that consists of, among others, E2-E3 enzymes (Ube2J1 and HRD1), which are responsible for the poly-ubiquitination of the mutant, co-factors that help both ubiquitination and retrotranslocation, such as Sel1 L and Derlin1, and the AAA ATPase p97 on the cytosolic side, which provides the driving force for the membrane eradication of α-SG. Once in the cytosol, the α-SG mutant is disintegrated into small peptides by the activity of the 26S proteasome. Sarcoglycan partners that become orphan subunits, are thought to be also rapidly degraded, with the consequent impairment of complex assembly and traffic towards the sarcolemma [63].
impairment in LGMD2F animal models [76,77] fostered research attempting to translate these approaches into human applications. Indeed, preclinical studies in murine models allowed to compare the efficacy of adenovirus (AD)-versus adeno-associated virus (AAV)-mediated transduction to identify the serotype of AAV that better targets novel gene into skeletal and cardiac muscle and assess the potential of using muscle-specific promoters, the duration of the transgene expression and toxicity related to overexpression [78–80]. Based on the successful conclusion of these studies Jerry Mendell’s group obtained authorization to start the first SGCA gene transfer phase I/II clinical trial for LGMD2D (NCT00494195). The study involved three patients who were 12–14-years-old, non-ambulatory, and homozygous or heterozygous for SGCA missense mutations. The patients were injected with $3.25 \times 10^{10}$ vector genomes of replication-defective AAV1.hSGCA in the extensor digitorum brevis (EDB) muscle on one side, whereas the contralateral muscle received saline. An immunsuppressive regimen with methylprednisolone was adopted for three days after virus injection, and the truncated muscle-specific MCK (creatine kinase) promoter was utilized to express α-SG. This treatment assured the sustained expression of α-SG in treated muscle fibers (4/5-fold over the control site) after 6 weeks for up to 3 months in all patients. The full SG complex was restored at the sarcolemma, and no adverse events were observed. All subjects exhibited MHCI overexpression on the treated side, suggesting the antigen presentation of a novel acquired protein, that is, wild-type α-SG or viral protein. Although AAV1-neutralizing antibodies and scattered foci of inflammation were visible two weeks after injection, an interferon-γ response to α-SG or AAV1 capsid peptide pools was not observed [81]. A second phase I/II clinical trial (NCT01344798) employing a similar intramuscular injection of rAAV1.tMCK.hSGCA evaluated prolonged α-SG expression in three patients with LGMD2D. In this study, the persistent expression of α-SG and relocalization of the entire SG complex were observed for up to 6 months in two of the three subjects in the absence of adverse effects. The subject failing gene transfer demonstrated an early increase in the neutralizing antibody titers and T cell immunity against AAV, suggesting a preexisting immunity to this specific virus serotype. All subjects showing exogenous α-SG expression also exhibited MHCI overexpression. Furthermore, this study also reported a rapid increase in neutralizing antibody against the AAV serotype-1 and inflammatory cell infiltration [82]. Moreover, a novel phase I/II clinical trial started in February 2015 and is currently underway. Specifically, it consists of a dose escalation study of self-complementary scAAVrh74.tMCK.hSGCA delivered via an indwelling catheter in the femoral artery of each leg by isolated limb perfusion (ILP) to eight subjects with LGMD2D. Efficacy will be measured using the 6-minute walk test and direct testing for the strength (MVICT) of lower limb muscles. The patients will be assessed at time 0 and on days 30, 60, 90, and 180 and at the end of 1st and 2nd years (NCT01976091); results are expected to be released in 2017.

Herson and collaborators published the results of a phase I clinical trial for LGMD2C in 2012. Specifically, this study entailed the intramuscular delivery of escalating doses (from 0.3 to $4.5 \times 10^{10}$ viral genomes) of replication-defective AAV expressing γ-SG under the control of the muscle-specific desmin promoter (AAV1.des.hγSGC) into the extensor carpi radialis muscle. Six months after injection, the highest doses of virus induced exogenous γ-SG expression, although only 4.7–10.5% of fibers were fully positive, with no serious adverse effects in all three treated subjects. Conversely, the six patients receiving lower doses exhibited no or very few positive fibers, as indicated by an immunofluorescence analysis. All patients became AAV serotype-1 seropositive, and one developed a cytotoxic response to the AAV capsid. No significant variation in MHCI staining was detected 30 days after AAV injection compared to the level measured 30 days before injection, and none of the subjects produced antibodies against γ-SG [83]. Overall, the results from these first clinical trials seem promising because wild-type SG was successfully expressed in the absence of serious adverse effects. However, the evaluation of sustained gene expression was relatively short (6 months), and the effect may have been compromised by immune responses to AAV or exogenous wild-type SG, as suggested by the overexpression of MHCI protein and the presence of cell infiltrate at the site of injection. Finally, these first studies, which targeted a single small muscle, did not evaluate functional recovery. Moreover, although promising, gene replacement must overcome additional challenges to become a productive strategy, such as the delivery of a gene into the most abundant tissue of the human body (approximately 50%), difficulty in accessing some muscles and the need for enormous amounts of high-purity virus. Hopefully, the outcome of the undergoing clinical trial for LGMD2D (NCT01976091) will address at least some of these difficulties.

4.2. Emerging therapeutic strategies

4.2.1. Cell therapy

Cell grafting is another innovative therapeutic approach for gene delivery into dystrophic muscle. Specifically, the introduction of genetically ‘cured’ host satellite cells or healthy cells from donors has the potential to correct the gene deficiency and remodel the dystrophic tissue. This point is of importance at the late stage of the disease, when fibrotic and fat tissue may replace muscle fibers. A number of studies, which mainly aimed to treat DMD, allowed researchers to test a repertoire of cells, such as muscle satellite cells, bone marrow-derived mesenchymal stem cells, pericytes or mesangio blasts derived from blood vessel walls, in animal models in an attempt to identify the ideal stem cell for this application [84]. Indeed, adult muscle mononuclear cells (AMMCs) isolated from normal, strain-matched muscle successfully restored SG expression in δ-SG null mice 1 month after injection, and multiple injections increased the efficiency of treatment. AMMCs were not only able to migrate inside the injected muscle, as indicated by positive fibers throughout the length of transplanted muscles, but they were also able to self-renew because the number of δ-SG-positive fibers almost doubled three months after a single injection. Interestingly, AMMCs were able to regenerate dystrophic muscle in aged mice with advanced disease (7- to 10-month-old mice), indicating that the environment of severely dystrophic muscle continues...
to support regeneration [85]. Another interesting study examined the transplantation of genetically corrected, human-induced pluripotent stem cell (iPSC)-derived mesoangiolasts into a mouse model of LGMD2D [86]. In this study, the authors isolated fibroblasts and myoblasts from patients with LGMD2D and converted them into iPSCs using reprogramming factors (Oct3/4, Sox2, c-Myc, and Klf4). A specific protocol then ensured mesodermal commitment and differentiation into mesangiolast (MAB)-like cells. After correcting the gene defect and activating the differentiation program by lentiviral vector transduction, these cells were reimplanted (either by intramuscular or vein injection) into immune-deficient SGCG-null mice. Using this complex but elegant protocol, the authors demonstrated that these cells reached the damaged skeletal muscle, where they proliferated and differentiated into muscle fibers that expressed α-SG and ultimately resulted in functional improvement [86]. This strategy may be able to produce unlimited numbers of genetically corrected progenitor cells, which may overcome the problem of limited stem cell availability for transplantation. However, the long-term safety of therapies involving iPSCs needs to be meticulously tested due to their potential oncogenic features [87]. Moreover, although successful in animal models of mice and dogs, the few published cell-therapy clinical trials in muscular dystrophy, such as DMD, have not demonstrated substantial recovery [88].

4.2.2. Exon skipping

The use of antisense oligonucleotides (AONs) to remove either a premature stop codon or out-of-frame mutations by skipping one or a few exons is emerging as an effective strategy for DMD. In dystrophin, the loss of some exons that code for the spectrin-like repeats of the rod domain, may restore the reading frame by expressing Becker muscular dystrophy (BMD)-like truncated protein [89,90]. Last year, the FDA authorized the use of etepliren (from Serepta Therapeutics) for the treatment of DMD in patients who harbor a confirmed mutation of the dystrophin gene amenable to exon 51 skipping. Recently, an approach aiming to rearrange the γ-SG protein by skipping several exons has been proposed for LGMD2C [91]. In LGMD2C, the most common mutation is the deletion of a single T in exon 6 of the SGCG gene. Referred to as 521-ΔT, this mutation shifts the reading frame and results in the absence of γ-SG and the secondary reduction of β- and δ-SG [7]. Exons 4–7 need to be removed to restore the reading frame of this γ-SG mutant. An engineered γ-SG cDNA that consists only of exons 1, 2, 3, and 8, was shown to generate a Mini-Gamma protein retaining the intracellular, transmembrane, and extreme carboxy-terminus domains. This internally truncated form of γ-SG was incorporated into the SG complex when expressed in Drosophila or mice deficient for γ-SG and improved both muscle and cardiac function [91]. In LGMD2C, the most common mutation is the deletion of a single T in exon 6 of the SGCG gene. Referred to as 521-ΔT, this mutation shifts the reading frame and results in the absence of γ-SG and the secondary reduction of β- and δ-SG [7]. Exons 4–7 need to be removed to restore the reading frame of this γ-SG mutant. An engineered γ-SG cDNA that consists only of exons 1, 2, 3, and 8, was shown to generate a Mini-Gamma protein retaining the intracellular, transmembrane, and extreme carboxy-terminus domains. This internally truncated form of γ-SG was incorporated into the SG complex when expressed in Drosophila or mice deficient for γ-SG and improved both muscle and cardiac function [91]. Subsequently, AONs were shown to effectively skip the desired exons in primary fibroblast cells from patients with LGMD2C induced to differentiate into myogenic cells by the exogenous expression of MyoD [91]. However, additional work is required to demonstrate that the shorter version of γ-SG protein is also effectively produced in human cells upon AON administration and leads to functional muscle recovery. Nevertheless, this work is the first attempt to demonstrate that exon skipping could also be useful in sarcoglycanopathy or, at least, in most LGMD2C cases. Indeed, the effect of internal deletions in other SG has not yet been explored, leaving still far the possibility to a broad application of this strategy.

4.2.3. Small molecule-based pharmacological approaches

The use of small molecules, such as PTC 124 (PTC Therapeutics) to induce stop codon read-through recently received EMA authorization for the treatment of DMD when a nonsense mutation is the cause of the disease [90]. In sarcoglycanopathy, null mutations comprise a small fraction of genetic defects but cause up to 20% of LGMD2E cases (see Table 1) and may benefit from stop codon read-through. Nevertheless, preclinical studies of the application of such therapeutic strategy in sarcoglycanopathy (in vitro or in vivo) have not been published yet.

The recent elucidation of the pathogenic mechanism underpinning the forms of sarcoglycanopathy due to the presence of a missense mutation and the identification of many ERAD elements involved in the degradative route of mutated SG have opened new avenues of therapeutic intervention [60–63]. For example, inhibiting the 26S proteasome with either MG132, which is a widely used tool to study the ubiquitin-proteasome system, or bortezomib, an FDA-approved drug for multiple myeloma, preserved different SGCA mutants (p.D97G, p.R98H, p.V247M, and p.P228Q) from degradation and recovered these proteins at the plasma membrane in an intact SG complex in vitro. Specifically, blocking the last step of the degradative pathway was hypothesized to slow the retro-translocations of the folding defective proteins from the ER, allowing them to carry out additional rounds of folding. As a consequence, the equilibrium of the QC system shifted to increase the availability of mutant proteins for the assembly and trafficking processes. Importantly, bortezomib also effectively rescued the SG complex in small-muscle explants from a patient with LGMD2D, validating the data obtained with cell models [61]. A few months prior, another paper [60] showed that the most commonly recurrent mutant (p.R77C) in LGMD2D, which is characterized by a high propensity to aggregate and be trapped in the ER [35], could be recovered at the plasma membrane both in vitro and in vivo by blocking the first step of the degradative pathway. In fact, treatment with kifunensine, a specific inhibitor of α-mannosidase I, induced the recovery of the protein in both a cell model and in mice transduced with AAVs expressing the human R77C-α-SG sequence [60]. Importantly, kifunensine, which was well tolerated by animals, stabilized the SG complex at the sarcolemma, suggesting its potential as a therapeutic agent in sarcoglycanopathy. A few years later, a second paper by Isabelle Richard’s group showed that not only α-SG but also β-, γ-, and δ-SG bearing amino acid substitutions, moderately affecting protein structure, could be rescued at the plasma membrane by kifunensine treatment [62]. Although only a fraction of the tested mutants (7/23) were successfully recovered, they comprised the most commonly reported defects of SGCA (p.R77C) and SGCG (p.C283Y). Therefore, at least one-third of sarcoglycanopathy patients might be candidates for a therapeutic strategy targeting the first step of the ERAD pathway, provided the rescued mutant at least partially retains its functionality [62].
several ERAD components involved in the disposal of V247M-α-SG mutants in sarcoglycanopathy [63]. Specifically, attention was focused on E3 ubiquitin ligases, which are key enzymes of the degradative pathway and responsible for the poly-ubiquitination of α-SG mutants. Two small molecules (LS101 and LS102) were identified as selective inhibitors of the E3 ligase HRD1 [92] and utilized in a cell model expressing human V247M-α-SG and in myogenic cells derived from a patient with LGMD2D carrying the p.L31P and p.V247M mutations on the two SGCA alleles. In both cases, a brief incubation (from 8 to 48 hours) with these small molecules induced the recovery of the mutated protein and the reconstitution of the entire SG complex in the patient’s myotubes, confirming the efficacy of this approach in pathologic samples and the potency of these compounds [63]. Interestingly, V247M-α-SG is among the mutants not recoverable by blocking α-mannosidase I [62]. Data collected from human primary cells could not conclusively identify whether one or both the allelic forms of SGCA expressed by this patient were recovered. However, based on the rescue of both mutated and wild-type SG, the authors suggested that the amount of the saved mutant was sufficient to allow assembly with the otherwise orphan subunits, preventing their degradation. Overall, the pharmacological strategies targeting ERAD are very promising in vitro, even in human specimens. However, additional work is needed to verify the stability of the complex containing a mutated subunit and, especially, the functional recovery of dystrophic muscle at both the beginning and advanced stage of the disease. To this end, animal models carrying missense mutations in SG genes are mandatory. However, the only α-SG<sup>H77C/H77C</sup> KI mouse model currently available, which was independently generated in two distinct laboratories, lacks a phenotype [60,93]. Indeed, the SG complex containing the mutated protein localized at the sarcolemma, and no dystrophic signs developed over the lifetime of the animals. This lack of phenotype may signify that α-SG remains functional despite its mutation, strengthening the suitability of therapeutic approaches aimed at protecting mutant SG from degradation. Conversely, these data may suggest differences in the processing pathway of mutated SG between human and murine species [60,93] and imply a need for suitable animal models of sarcoglycanopathy to carry out pharmacological tests.

4.2.4. Other therapeutic approaches

Alternative approaches that aim to compensate for muscle mass wasting, by increasing positive or blocking negative regulators of muscle growth have been proposed, although these approaches have yielded conflicting results. Specifically, the administration of insulin-like growth factor (IGF-1) increased the life expectancy of Δ-SG deficient hamsters, which are a natural animal model of LGMD2F [94], and treating Sgca-null mice with deacetylase inhibitors to re-express the myostatin inhibitor follistatin also had positive effects [95]. The early loss of myostatin activity, either by gene KO or the use of monoclonal anti-myostatin antibody, improved muscle mass and regeneration and reduced fibrosis in young Sgcd-null mice, but this effect was not recapitulated in mice with late-stage disease [96]. Similarly, the administration of an anti-myostatin antibody in an LGMD2C mouse model increased fiber size, muscle mass, and absolute force but did not improve muscle histopathology [97]. Moreover, injecting recombinant AAVs expressing an inactive form of myostatin into the tibialis anterior muscle of 2-month-old Sgca-null mice did not ameliorate the dystrophic phenotype [98], which suggests that myostatin inhibition may only be beneficial in early-stage muscular dystrophy or when a mild phenotype is present. A phase I/II randomized trial evaluating the safety of a neutralizing antibody (MYO-029) against myostatin was concluded and published in 2008. The study, which enrolled 116 patients with different forms of muscular dystrophy, including sarcoglycanopathy, showed that the antibody is likely safe and tolerable but lacked positive effects at the end point [99].

Because calcium dysregulation was proposed as one of the main features responsible for the progressive degeneration of myofibers in sarcoglycanopathy [2], several studies tested compounds that can modify calcium homeostasis or the expression of key elements of calcium handling. For example, several compounds, such as diltiazem and nifedipine (L-type Ca<sup>2+</sup> channel antagonists), FK506 (calcineurin inhibitor), or E64 (calpain inhibitor), prevented CK release and significantly protected muscle from degeneration in BIO 14.6 hamsters [100]. Furthermore, the overexpression of calcineurin in Sgcd-null mice worsened the pathological phenotype, whereas its depletion substantially ameliorated both skeletal and cardiac symptoms [101]. In addition, the overexpression of the sarco/endoplasmic reticulum calcium ATPase 1 or 2 (SERCA1 or SERCA2) by AAV transduction dramatically improved the dystrophic phenotype of both mdx- and Sgcd-null mice, as evidenced by reductions in myofiber central nucleation, fibrosis and CK release and an improvement in exercise capacity [102]. Taken together, these data suggest that targeting changes in the cytosolic calcium level may serve as a therapeutic option in sarcoglycanopathy and many other muscular dystrophies if calcium overload is the final common pathway for myofiber necrosis.

The loss of the SG complex is thought to induce sarcolemma instability and fragility. Therefore, studies aiming to enhance the muscle membrane-repair machinery by overexpressing the MG53 protein have to be mentioned. MG53 is a muscle-specific TRIM-family protein and an essential component of the membrane repair system necessary for vesicle trafficking to sites of injury. The systemic delivery and muscle-specific overexpression of human MG53 cDNA by recombinant AAVs enhanced membrane repair, ameliorated pathology, and improved muscle and heart functions in Δ-SG-deficient TO-2 hamsters, another animal model of LGMD2 F [103].

Because NO dysregulation seems to be involved in the pathogenesis of sarcoglycanopathy [2,104], an approach aiming to normalize NO production could be conceived as a therapeutic option to treat the disease. Treating Sgca-null mice with the flurbiprofen derivative HCT 1026, a potent nonsteroidal anti-inflammatory drug (NSAID) that can release NO, yielded promising results. Specifically, this drug, which is already approved for use in humans, dramatically slowed disease progression and maintained muscle integrity and functionality in animals [105]. A few years later, the same group demonstrated the synergistic and prolonged beneficial effects of the combined administration of ibuprofen, an NSAID, and the NO donor compound isosorbide...
dinitrate (ISDN) in Sgca-null mice [106]. Subsequently, an open-label, single-center pilot study was conducted to evaluate the safety of the prolonged (1 year) coadministration of ibuprofen and ISDN in a cohort of adult patients with DMD (21 subjects), BMD (16 subjects) and LGMD (34 subjects) [107]. This study, although limited to a small cohort of patients, has demonstrated a good safety profile for the coadministration of the two drugs, but further studies assessing functional recovery of the disease in response to the same or similar treatment have not been reported and are not currently planned.

Finally, a recent paper [108] showing impaired mitochondrial biogenesis and consequent compromised oxidative capacity in both Sgca-null mice and patients with LGMD2D must be mentioned. The authors showed that this defect is linked to the condensed chromatin state of the promoter of PGC-1α, a key element that regulates mitochondrial biogenesis. By applying two different approaches in mice, the authors restored mitochondrial functionality and dramatically ameliorated the dystrophic phenotype. Specifically, treating mice with the pan-HDAC inhibitor (HDACi) Trichostatin A (TSA) reactivated the gene expression program leading to mitochondrial biogenesis. Moreover, treating mice with the NO donor molsidomine significantly improved the oxidative metabolism and energy expenditure without modifying the acetylation status of the PGC-1α promoter [108]. Because data on the dystrophic phenotype were not provided, additional work is needed to confirm these findings in the setting of dystrophy. Nevertheless, these results shed light on the novel pathogenic mechanisms in sarcoglycaneopathy and suggest the possibility of novel therapeutic strategies that are applicable irrespective of the type of mutation responsible for the disease.

5. Expert opinion

Although characterized by variable age of onset and severity, sarcoglycaneopathy is an invariably progressive muscle-wasting disease that primarily compromises the proximal musculature. Moreover, this disease involves the respiratory muscles and is associated with the development of dilated cardiomyopathy, especially when mutations are present in the SGCD and SGCB genes. This disease interferes with the patient’s daily functions, which rapidly and profoundly reduces quality of life. Moreover, cardiac and respiratory worsening can be fatal if not continuously monitored. Therefore, the emotional and financial burden on patients and their families is extremely high. Unfortunately, sarcoglycaneopathy lacks a cure and most therapeutic approaches described in this paper are currently in the early preclinical stages, likely because this disease is rare.

Symptomatic treatments with steroids to reduce inflammation have been empirically applied in some patients with LGMD2D, 2E, and 2C and somewhat improved both muscle strength and cardiac function. However, the actual efficacy of this pharmacological approach for sarcoglycaneopathy cannot be defined due to a lack of randomized and systemic studies. Therefore, an in-depth investigation of the inflammatory environment in sarcoglycaneopathy muscles with a special attention to the different mononucleated cells that play a role in muscle waste disposal, induction of regeneration and fibrosis is warranted. On the other hand, planning specific clinical trials that address the long-term efficacy and safety of this pharmacological approach for sarcoglycaneopathy is also important considering the positive effect of steroid treatments in other patients with muscular dystrophy.

Approaches that do not directly address the pathogenic mechanism but aim to reduce the loss of muscle mass consequent to disease progression are also notable. However, the results of these strategies, particularly those based on the use of the monoclonal antibody against myostatin, remain contradictory and warrant further investigation, especially studies that focus on muscles that exhibit different grades of fibrosis. Strategies that act on the downstream steps of the pathogenic mechanism of the disease, such as the manipulation of intracellular calcium concentrations and the membrane repair system, have also yielded conflicting results.

At present, only gene therapy has been examined in clinical trials. Potentially helpful for all sarcoglycaneopathy cases, gene therapy could be the solution when mutations impair SG protein production. The first two clinical trials (phase I/II studies) that injected recombinant AAVs expressing α-SG into a specific small muscle were promising, and the third study, which is examining the systemic delivery of α-SG-AAVs into the lower limbs, is ongoing. This study is expected to address several questions related mainly to the functional outcome and the long-term effects of gene replacement. As we await these data, gene replacement approaches remain associated with concerns. First, gene replacement must occur in the most abundant tissue of the body in sarcoglycaneopathy, which consequently requires a large amount of high-purity virus. Moreover, considering the cardiac involvement, especially in LGMD2E and 2F, the AAV serotype must also target cardiac cells. A second concern is related to the possible immune response against the virus if a patient has already encountered the same AAV serotype. Moreover, such a response may also be provoked upon the first AAV injection. Therefore, the virus serotype may need to be changed, especially in cases of subsequent injections, which are expected for the treatment of a chronic disease. Alternatively, an immunosuppressive regime can be adopted during the phase of virus delivery. No evidence of a specific immune response against the newly synthesized SG was observed in the first clinical trials. Nonetheless, the presence of the transgene in patients who do not express the endogenous SG protein may elicit neutralizing antibodies or cell-mediated immune responses. Thus, an immunosuppressive/immunomodulating regimen should be chronically adopted in these cases. On the other hand, the sarcoglycaneopathy population primarily consists of patients who express missense mutants of SG and very often are compound heterozygotes. Although it cannot be ruled out that the small difference between wild-type and missense mutant may be sufficient to induce an immune response, the patient’s immune system likely recognizes the transgene as self, even when, for example, one deletion is present at one allele.

Elucidating the pathogenic mechanism of sarcoglycaneopathy forms linked to missense mutations has yielded novel avenues of therapeutic interventions. Very promising data have been collected in vitro and in primary myogenic cells from a patient with LGMD2D, which demonstrated that small molecules targeting the degradative pathway of SG mutants could recover the SG...
complex at the sarcolemma. However, novel animal models carrying a SG with a single amino acid substitution are necessary to confirm these data in vivo, evaluate functional rescue and assess the stability of the complex containing a mutated subunit. Because the only currently available mouse model, that is, the SGCA KI mouse model, is not suitable for this purpose, developing different vertebrate models would be useful. For example, zebrafish (D. rerio) is garnering significant attention as animal model of many human diseases because of the high genome sequence similarity, conservation of cell signaling and concordance in the developmental phenotype with mammals. Zebrafish muscle tissue is almost indistinguishable from that of mammals, and this fish exhibits reproducible and easily measurable motor behaviors starting on the first day of life. Moreover, generating KO and KI zebrafish mutants has become easy due to genome-editing technologies. Therefore, considering the advantages of this vertebrate over mammals in terms of size, time of development, and number of offspring, the availability of sarcoglycanopathy zebrafish models would help to not only test already identified small molecules but also to screen for novel compounds and orphan drugs.

Because the high number of cases reporting a missense mutation in one of the SG genes, most patients with sarcoglycanopathy will likely benefit from a therapeutic approach that targets the degradative pathway of SG mutants. Moreover, the advantages of using small molecules over other approaches must be highlighted, such as the ease of administration, whole-body distribution (skeletal muscle is the most abundant human tissue), the opportunity for drug optimization and the possible combined administration of different compounds. Using pharmacological strategies that prevent the degradation allowing the rescue of endogenous mutated protein also avoids immunological responses against the virus and/or the transgene that may be associated with gene therapy.

To properly develop effective therapeutic treatments for sarcoglycanopathy, the natural history of the disease needs to be described in detail, especially given the phenotypic variability and the number of different mutations. Furthermore, the identification of proper outcome measures for follow-up evaluation and the establishment of appropriate end points are also essential for the proper management of any novel clinical trials.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

References

Papers of special note have been highlighted as either of interest (-) or of considerable interest (**) to readers.


** A recent description of the pathogenesis and clinical features of sarcoglycanopathies


** This paper highlights the phenotypic variability observable among sarcoglycanopathy patients, even siblings, carrying the same mutation


**Study showing as missense mutants of any sarcoglycan may be recovered by inhibiting α-mannosidase I (first step of ERAD pathway)**


**This paper proposes E3 ligase inhibition as potential therapeutic approach in LGMD2D**


**Study adopting corticosteroid in the treatment of sarcoglycanopathy.**

68. Wong-Kisiel LC, Kuntz NL. Two siblings with limb-girdle muscular dystrophy type 2E responsive to deflazacort. Neuromuscul Disord. 2010;20:122–124. DOI: 10.1016/j.nmd.2009.11.005


**Interesting review highlighting potential and drawbacks of cell therapy in muscular dystrophies.**


**Preliminary study suggesting high potential of exon skipping for sarcoglycanopathies.**


**Study demonstrating that α-SG<sup>777G<sup>C</sup></sup> KI mouse is phenotype less.**


- Paper showing NO dysregulation in sarcoglycanopathies.


- Paper showing dysregulation of mitochondrial biogenesis in LGMD2D and potential therapeutic interventions.