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The sarcoglycan complex in skeletal muscle

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1. ABSTRACT

In skeletal muscle, the dystrophin-associated glycoprotein complex forms a link between the actin cytoskeleton and the extracellular matrix that is critical for muscle integrity. Within this complex resides the sarcoglycan subcomplex, which consists of four transmembrane glycoproteins (alpha-, beta-, gamma-, and delta-sarcoglycan). During assembly, beta-sarcoglycan tightly associates with delta-sarcoglycan to form a functional core that then recruits gamma- and alpha-sarcoglycan to form the sarcoglycan complex. Together with sarcospan, the sarcoglycan complex binds other components of the dystrophin-associated glycoprotein complex and integrates into the myofibre's membrane. Once integrated, the sarcoglycan complex plays a pivotal role in mechanically stabilising the sarcolemma as well as the dystrophin-associated glycoprotein complex. Additionally, the sarcoglycan complex undergoes chemical modifications in response to muscle contractions, thereby transducing mechanical information into a cellular signal. Mutations in the sarcoglycans induce limb girdle muscular dystrophy, and several animal models have been established to study the molecular biology and function of the sarcoglycan complex. This review discusses the role of the sarcoglycan complex in skeletal muscle and describes the functional deficiencies that lead to muscular dystrophies.

2. INTRODUCTION

Muscular dystrophies (MD) are muscle diseases that cause progressive muscle weakness as a result of degenerating myofibres and fibrosis (1). To date, over 40 genetically distinct muscular dystrophies have been identified (2). The most common and well-characterised MD is Duchenne MD, which is caused by mutations in the dystrophin gene (*DMD)* (3). Dystrophin is a large, rod-shaped intracellular protein that is associated with a large complex known as the dystrophin-associated glycoprotein complex (DGC) (4). The DGC is one of the linking complexes that anchors the cytoskeleton to the extracellular matrix and stabilises the sarcolemma in response to muscle contractions (5). In addition to providing a mechanical link, the DGC also plays a critical role in signal transduction to regulate interactions between the cytoskeleton, the membrane and the extracellular matrix. Within the DGC resides the sarcoglycan complex, which consists of four transmembrane glycoproteins: α-, β-, γ-, and δ-sarcoglycan. The sarcoglycan complex plays a pivotal role in mechanically stabilising the sarcolemma and the DGC (6). Moreover, the sarcoglycan complex is crucial for signal transduction of mechanical information and undergoes chemical modifications in response to muscle contractions (7).

The severe forms of MD induced by mutations in the sarcoglycans reflect the functional significance of the sarcoglycan complex. Limb girdle muscular dystrophy (LGMD) describes the broad group of genetically and clinically heterogeneous muscular dystrophies that primarily affect the muscles of the pelvic area and shoulders (8, 9). LGMD is categorised into two types: type 1 and type 2, which show an autosomal dominant and an autosomal recessive pattern of inheritance, respectively. Multiple studies have demonstrated that mutations in α-, β-, γ-, and δ-sarcoglycan cause limb girdle muscular dystrophy type 2D (LGMD2D) (10), LGMD2E (11, 12),

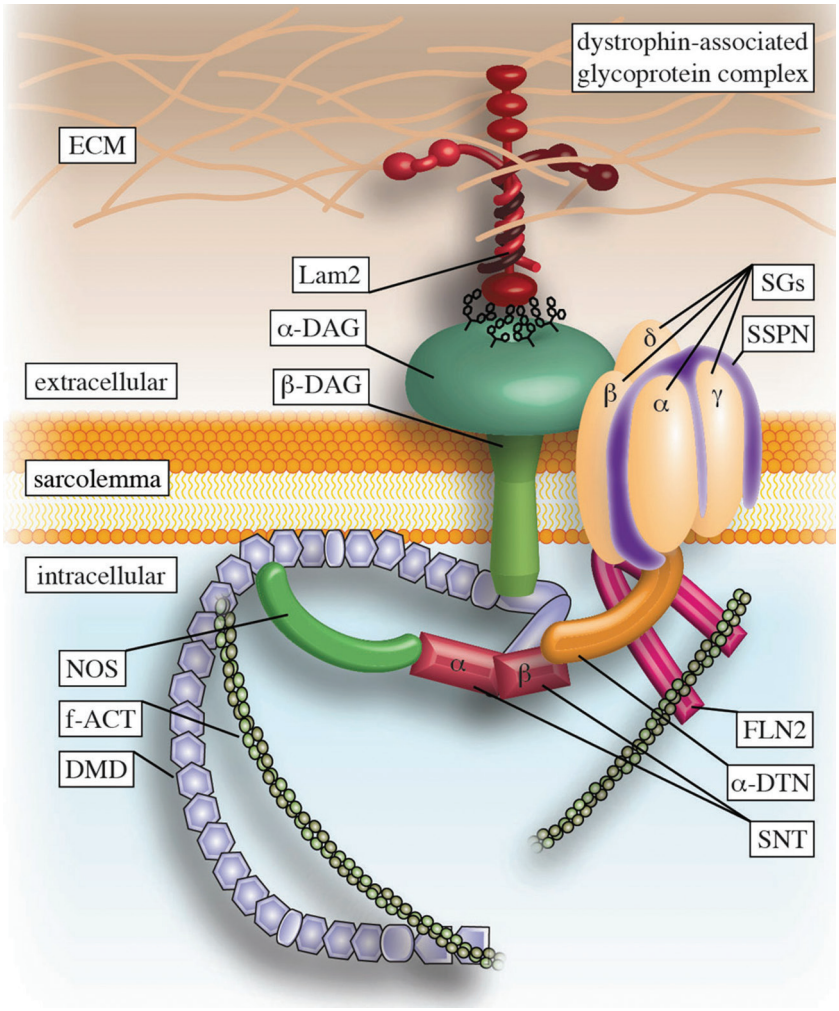

Figure 1. Molecular organisation of the dystrophin-associated glycoprotein complex (DGC) in skeletal muscle. Dystrophin binds to cytoskeletal F-actin and associates with several proteins of the DGC, which forms a mechanical link between the actin cytoskeleton and the extracellular matrix. The DGC thereby stabilises the cell membrane and plays a role in the detection and transmission of mechanical stress to the nucleus via signalling proteins.

LGMD2C (13), and LGMD2F respectively (14). These four distinct categories of LGMD type 2, collectively referred to as sarcoglycanopathies, show a high degree of variation in disease severity and progression (15).

3. THE DYSTROPHIN-ASSOCIATED GLYCOPROTEIN COMPLEX HAS THE SARCOGLYCAN COMPLEX EMBEDDED WITHIN

The dystrophin-associated glycoprotein complex (DGC) constitutes of a collection of integral and membrane-associated proteins that in skeletal muscle localise to the myofibre membrane (16), where it physically links the actin cytoskeleton to the extracellular matrix (Figure 1). As a result of this mechanical anchorage, the DGC effectively stabilises the sarcolemma in response to muscle contractions (5, 17) and is also involved in signal transduction to detect and transmit information regarding mechanical stress to the nucleus via signalling proteins (see (18) for a review). Several components of the DGC have been shown to be defective in various muscle disorders, demonstrating its significance for the integrity of skeletal muscle.

Dystrophin is a large rod-shaped protein that serves as a link between the actin cytoskeleton and other components of the DGC (19, 20). The C-terminal end of dystrophin binds and interacts with β-dystroglycan of the dystroglycan complex (21). Sugar molecules on the other subunit of the dystroglycan complex, α-dystroglycan, allows for direct binding to laminin-2 (22) that binds directly to the extracellular matrix (23). α1- and β1-syntrophin directly bind to dystrophin (24) and interact with sodium channels (25), kinases (26), and aquaporin-4 (27), thereby potentially functioning as

Table 1. Summary of human sarcoglycans

Gene	Protein	N-Gly	Cys	Expression pattern	Disease	References
SGCA	α-sarcoglycan	2	5	Striated muscle	LGMD2D	35, 43
SGCB	β-sarcoglycan	3	5	Predominantly skeletal muscle, also present in other tissues	LGMD2E	11, 12, 66
SGCG	γ-sarcoglycan	1	4	Striated muscle	LGMD2C	13, 44
SGCD	δ-sarcoglycan	3	4	Striated and smooth muscle	LGMD2F	14, 81, 86
SGCE	ε-sarcoglycan	1	4	Widely expressed (high in embryo)	Myoclonus-dystonia syndrome	40, 58, 87, 88
SGCZ	ζ-sarcoglycan	1	4	Highly expressed in brain	Not implicated in disease	41, 89

N-Gly: Number of extracellular N-linked (or asparagine-linked) glycosylation sites; Cys: Number of extracellular cysteine residues

adaptor proteins that recruit signalling proteins to the sarcolemma. Additionally, neuronal nitric-oxide synthase (nNOS) associates with α-syntrophin (28) to regulate cell signalling and modulate contractile force (29). Thereby, the DGC has been implicated to have a critical signalling role.

Embedded within the DGC is the sarcoglycan complex, a tetrameric complex of transmembrane glycoproteins that is essential for the stability of the sarcolemma and the DGC. Thereby, the sarcoglycan complex maintains the link between the cytoskeleton and extracellular matrix (6, 30). Once integrated, the sarcoglycan complex interacts with key members of the DGC. α-dystrobrevin is a cytoplasmic phosphoprotein that interacts with dystrophin and the sarcoglycan complex (6, 31). Sarcospan contains four transmembrane domains and is tightly associated with the sarcoglycan complex (32, 33). Collectively, the sarcoglycans and sarcospan (or sarcoglycan-sarcospan complex) acts to stabilise α-dystroglycan to the sarcolemma, potentially strengthening the bond between dystrophin and α-dystroglycan with β-dystroglycan (33). In addition, the sarcoglycan complex binds to the N-terminus of α-dystrobrevin, thereby forming a link with nNOS via α-syntrophin (6).

4. THE SARCOGLYCAN COMPLEX

The sarcoglycans are a group of transmembrane glycoproteins with a large extracellular and a short intracellular domain (12, 13, 34, 35). In skeletal and cardiac muscle, the sarcoglycan complex is comprised of four subunits at an equal stoichiometric ratio: α-, β-, γ-, and δ-sarcoglycan (34). In general, loss of one of the sarcoglycan subunits results in a complete loss or reduction of all sarcoglycan subunits from the sarcolemma (11, 13, 14, 36, 37). Protein denaturing detergents such as SDS (38) or octyl glucoside (39) are ineffective at disrupting the sarcoglycan complex, suggesting the sarcoglycans are tightly associated with one another. In smooth muscle, ε- and ζ-sarcoglycan are incorporated into the sarcoglycan complex and replace α- and γ-sarcoglycan, respectively (40, 41). A summary of the sarcoglycan subunits can be found in Table 1.

The sarcoglycan complex acts to stabilise the DGC by strengthening the interaction between dystrophin and dystroglycan (6). This current notion is based on the loss of DGC components secondary to deficiencies in the sarcoglycan complex in the muscle of both patients and animal models. However, the exact cause of the reduction or loss of DGC components as a result of defects in the sarcoglycan complex still remains elusive.

A possible mechanism that explains the molecular defect in sarcoglycanopathies is an increased concentration of cytoplasmic calcium. Deficiencies in the assembly and localisation of the sarcoglycan complex cause a reduction or complete loss of the sarcoglycan complex at the sarcolemma (2). This in turn destabalises the DGC and makes sarcolemmas more susceptible to damage following muscular contractions, as demonstrated by Evans blue dye uptake in several animal models of sarcoglycanopathies (42-45). Sarcolemmal damage or defects in calcium channels can cause an increase in intracellular calcium, ultimately resulting in cell death.

The sarcoglycan complex is formed in the ER following co-translation of all sarcoglycan subunits (46). Correct trafficking of the sarcoglycan complex from the ER to the plasma membrane requires simultaneous expression of all four sarcoglycans (2). Co-transfection experiments suggest that β-sarcoglycan functions as the initiation factor for assembly of the sarcoglycan complex (47). Through their strong affinity, β- and δ-sarcoglycan are tightly associated and effectively form the functional core of the sarcoglycan complex (46, 48, 49). Following formation of the β-δ-sarcoglycan core, γ-sarcoglycan binds to the δ-subunit (47). Finally, α-sarcoglycan binds to γ-sarcoglycan, potentially through the interaction of cysteine residues in the intracellular domain (47). Once assembled, the sarcoglycan complex interacts with the dystroglycan complex and sarcospan during transport from the Golgi apparatus before integrating into the sarcolemma (46).

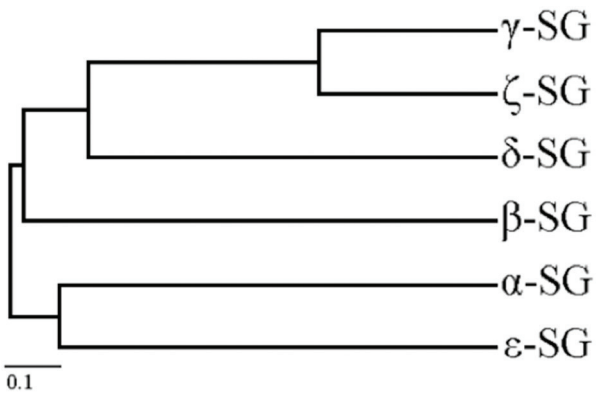

Figure 2. Phylogenetic tree illustrating the relationships among the sarcoglycan subunits. The phylogenetic tree was constructed based on the human amino acid sequence of the sarcoglycans.

4.1. α-Sarcoglycan

α-sarcoglycan was initially described as a 50 kDa dystrophin-associated glycoprotein (50-DAG) with an extracellular N-terminus (4). After the cDNA was cloned from a rabbit skeletal muscle cDNA library (35), the gene product was termed adhalin (from the Arabic word 'adhal' for muscle), as it was deficient in children with severe congenital autosomal recessive muscular dystrophy (SCARMD) (50). After the discovery of additional sarcoglycan subunits, adhalin was then renamed to α-sarcoglycan. Northern blot analyses showed that α-sarcoglycan expression was highest in skeletal muscle and cardiac muscle (35).

An extracellular ATP-binding domain has been identified in α-sarcoglycan (51). The ATPase activity of α-sarcoglycan is dependant on the presence of both Ca^{2+} and Mg^{2+} ions (52). α-sarcoglycan was observed to be one of the major contributing factors responsible for the increase in ATPase activity of cultured C2C12 mouse myoblast cells during differentiation (52). Since ATP is a regulator of skeletal muscle differentiation (53), α-sarcoglycan may therefore be an important factor for the differentiation of myofibres (52).

Similar to all other sarcoglycan subunits, the extracellular domain of α-sarcoglycan contains closely spaced cysteine residues that resemble laminin-type epidermal growth factor (EGF)-like repeats (10, 48, 54). Proteins that contain laminin-type EGF-like repeats have been shown to form a complex and function as receptors. Based on this observation, the sarcoglycans have been suggested to function as receptors for a yet to be identified extracellular ligand (10, 48). The extracellular domain of α-sarcoglycan also contains a cadherin domain and Ca^{2+}-binding pockets, homologous to those observed in α-dystroglycan (55). The presence of these cadherin domains suggests calcium-dependent binding of α-sarcoglycan to the dystroglycan complex (55).

Mutations in α-sarcoglycan are associated with limb girdle muscular dystrophy type 2D (LGMD2D) (10). Consistent with the disease, α-sarcoglycan is primarily expressed in skeletal muscle and at lower levels in cardiac muscle, which explains the rare cardiac involvement observed in patients with LGMD2D (56). The severity of the disease is correlated with the type of mutation, such that nonsense mutations resulting in a complete loss of the sarcoglycan complex cause severe symptoms and missense mutations with weak α-sarcoglycan staining are generally associated with mild symptoms (57). Also, α-sarcoglycan null mice demonstrate progressive muscular dystrophy and necrosis, characteristic of LGMD2D (43). In addition, α-sarcoglycan null mice lack sarcolemmal integrity and show increased permeability as observed by the increased Evans blue dye uptake. Furthermore, immunoblot and immunofluorescence analyses demonstrated loss of the entire sarcoglycan complex, sarcospan and a reduced expression of additional members of the DGC, including dystrophin, α- and β-dystroglycan, and nNOS; suggesting that the sarcoglycan complex stabilises the DGC (43). Despite fibre detachment and degeneration of skeletal muscle, α-sarcoglycan null mice surprisingly show a significant increase in muscle mass compared to controls. However, how disruption of the DGC causes an increase in muscle mass in α-sarcoglycan null mice still remains to be resolved.

Overexpression of ε-sarcoglycan ameliorates the phenotype of α-sarcoglycan null mice, as sarcolemmal damage or aberrant muscular contractions have not been detected (58). These findings suggest that there is potential for ε-sarcoglycan to replace α-sarcoglycan in striated muscle (59, 60). This is further supported as α- and ε-sarcoglycan share almost 50% identity at the amino acid level, suggesting that they serve a similar function (Figure 2).

In order to model the most prevalent α-sarcoglycan mutation (R77C) observed in patients, knock-in mice carrying the H77C mutation in the α-sarcoglycan gene (*Sgca*^{H77C}) were generated (61, 62). Surprisingly, these two independent studies determined that *Sgca*$^{H77C/H77C}$ mice demonstrated neither skeletal muscle defects nor an apparent phenotype. Furthermore, expression of human *SGCA*^{R77C} using an adenoviral vector was able to rescue the phenotype and restore expression of the sarcoglycan-sarcospan complex in α-sarcoglycan null mice (61, 62). Despite the presence of SGCA^{R77C} aggregates in the ER, the majority of the mutant protein was correctly transported to the sarcolemma and was fully functional in the skeletal muscle of mice. In contrast, in humans the R77C mutations results in severe LGMD2D with childhood onset (36), suggesting that α-sarcoglycan undergoes species-specific trafficking and protein quality control measures (62).

4.2. β-Sarcoglycan

Expression of β-sarcoglycan is observed predominantly in skeletal and cardiac muscle, but it also present in other tissues such as smooth muscle, the brain, kidney, lung and spinal cord (11, 63). β-sarcoglycan has three asparagine residues that are predicted to be N-linked glycosylation sites (11). Accordingly, glycosylation-defective mutations in β-sarcoglycan results in self-aggregation and disruption of the sarcoglycan complex 47), suggesting that β-sarcoglycan is essential for the correct folding and assembly of the sarcoglycan complex (47, 64). β-sarcoglycan forms the functional core of the sarcoglycan complex together with δ-sarcoglycan (46, 47). The intracellular domain of the β-δ-sarcoglycan core has been suggested to bind to the C-terminal end of dystrophin (64); however, the functional significance of this close association has yet to be clarified.

Mutations in β-sarcoglycan can provoke a broad clinical spectrum of LGMD2E (11, 12). It is interesting to note that even missense mutations can result in a complete loss of all sarcoglycan subunits from the sarcolemma (11), emphasising the importance of β-sarcoglycan within the sarcoglycan complex. These findings are in agreement with the notion that β-sarcoglycan is necessary for the initiation of the assembly of the sarcoglycan complex (47). Furthermore, similar mutations can show large variation in the severity of the disease in different patients. For instance, the Arg91Pro mutation in β-sarcoglycan has been described in patients with severe MD and Arg91Leu is associated with mild MD (65). This clinical variability may be explained by external factors such as the environment or genetic factors; however, no contributing factors have been identified to date.

β-sarcoglycan null mice display progressive muscular dystrophy as a result of degeneration of muscle fibres (66). β-sarcoglycan deficient mice also demonstrate muscular hypertroph, reflecting the clinical features of patients suffering from LGMD2E (67). In addition to skeletal muscle, β-sarcoglycan knockout mice develop cardiomyopathy with large areas of necrosis and fibrosis in the heart musculature (68). Northern blots demonstrated that α-, γ-, and δ-sarcoglycan and sarcospan transcripts were present in the skeletal muscle of β-sarcoglycan deficient mice (66). However, immunohistochemical analyses of homozygous mutant mice revealed a complete loss of all muscle sarcoglycan subunits and sarcospan at the sarcolemma. These findings suggest that defects in the assembly of the sarcoglycan complex may lead to the proteolytic degradation of the sarcoglycan subunit, resulting in loss of immunoreactivity for the entire complex (69).

Interestingly, treatment of β- and γ-sarcoglycan null mice with Verapamil, a calcium channel blocker with vasodilator properties, can mitigate cardiac abnormalities (70). Specifically, Verapamil treatment prevented cardiomyopathy caused by ischemic damage and significantly reduced serum levels of cardiac troponin I to normal levels. The authors suggested that the vasodilator properties of Verapamil restored cardiac muscle morphology, highlighting its potential as a therapeutic strategy for patients with cardiomyopathy caused by mutations in the sarcoglycans. These results further support the notion of calcium overload as the molecular basis responsible for the progression of disease in sarcoglycanopathies. However, it should also be noted that long-term Verapamil treatment did not restore or improve cardiac morphology in dystrophin-deficient *mdx* mice (70), predicting a different mechanism in the development of cardiomyopathy in Duchenne MD and LGMD.

4.3. γ-Sarcoglycan

Mutations in γ-sarcoglycan are known to cause LGMD2C, which clinically resembles features of Duchenne MD and is characterised by limb-girdle weakness and calf hypertrophy (13). Congruent with the disease, γ-sarcoglycan is expressed exclusively in skeletal and cardiac muscle (13).

Immunoblotting experiments in cultured rat skeletal muscle cells showed that muscle contractions induced tyrosine phosphorylation of γ- and α-sarcoglycan (7). Phosphorylation of tyrosine residues of γ- and α-sarcoglycan is mediated by focal adhesion kinase (FAK) or a FAK-stimulated tyrosine kinase (7). It has been demonstrated that tyrosine phosphorylation of these two sarcoglycan subunits regulate bidirectional signalling with integrins to facilitate cell adhesion to the extracellular matrix (7). Furthermore, γ-sarcoglycan deficient mice show an upregulation of integrins in skeletal muscle (71), suggesting that upregulation of integrins acts as a compensatory mechanism for the loss of the sarcoglycan complex. The integrin-associated complex and DGC have overlapping roles as they both function as an anchor for the cytoskeleton to the extracellular matrix in skeletal muscle. These findings support the notion that the sarcoglycan complex is involved in linking the cytoskeleton to the extracellular matrix (71).

Several patients have been identified carrying a missense mutation (C283Y) in the conserved extracellular cysteine residues of γ-sarcoglycan (72). Homozygous patients displayed a severe muscular dystrophy phenotype and were diagnosed with LGMD2C. Surprisingly, even asymptomatic heterozygous carriers had elevated levels of creatine kinase, a marker of muscle damage and inflammation; indicating the functional significance of the conserved cysteine residues in the extracellular domain. Additionally, two patients homozygous for the Δ525T mutation in γ-sarcoglycan have been identified (73). This frameshift mutation resulted in the production of truncated γ-sarcoglycan and removes

part of the extracellular domain along with conserved cysteine residues. Interestingly, despite premature truncation, all sarcoglycan subunits and sarcospan showed appropriate expression and localisation and these patients developed severe muscular dystrophy and cardiomyopathy. These findings suggest that the extracellular domain of γ-sarcoglycan is critical for the function of the entire sarcoglycan complex and plays a key role in the survival of muscle cells, independent from the rest of the DGC (73).

γ-sarcoglycan knockout mice (*gsg*$^{-/-}$) develop a dystrophic phenotype, reminiscent of the pathology seen in LGMD2C patients (44). *gsg*$^{-/-}$ mice show increased Evans blue dye uptake, indicative of sarcolemmal damage and membrane permeability defects. TUNEL-positive nuclei were abundant in γ-sarcoglycan deficient skeletal muscle, demonstrating that apoptosis induced myofibre degeneration. In addition, *gsg*$^{-/-}$ mice displayed cardiomyopathy and a significant increase in the thickness of the left and right ventricular walls, resulting in an increased mortality. Despite the severe muscle defect, dystrophin, laminin, and β-dystroglycan localisation was unaffected, indicating the mechanical link of the cytoskeleton to the extracellular matrix provided by the DGC was maintained despite loss of γ-sarcoglycan. Interestingly, disruption of the γ-sarcoglycan gene caused a secondary reduction of α-, β- and δ-sarcoglycan protein, but did not have an effect on mRNA levels. These findings suggest that loss of γ-sarcoglycan results in a deficiency in the transport and/or assembly of the sarcoglycan complex (44).

Intriguingly, overexpression of γ-sarcoglycan in mice induces severe muscular dystrophy (74). In skeletal muscle fibres, overexpression of γ-sarcoglycan resulted in an upregulation of α- and β-sarcoglycan, but did not affect δ-sarcoglycan or dystrophin levels. Furthermore, cytoplasmic γ-sarcoglycan-positive aggregates were identified, which interfered with normal γ-sarcoglycan localisation to the sarcolemma. From this result, it can be inferred that the assembly, localisation and function of the sarcoglycan complex is dependent on the stoichiometric ratios of the sarcoglycan subunits (74). These findings should be heavily considered for future studies aiming to develop a gene therapy for patients suffering from LGMD.

4.4. δ-Sarcoglycan

Cross-linking experiments using cultured mouse myotubes showed that the extracellular domain of δ-sarcoglycan binds to α- as well as β-dystroglycan, consequently forming a complex that strengthens the association between other components of the DGC (48). In contrast, immunoprecipitation experiments demonstrated that γ-sarcoglycan associates directly with only β-dystroglycan during formation of the sarcoglycan complex (46). Another study showed that all four sarcoglycans bind to β-dystroglycan using pull-down binding assays with recombinant proteins (75). As all these findings are inconsistent, further experiments are necessary in order to verify the molecular structure and interactions of the sarcoglycan and dystroglycan complex during assembly and transport.

In addition to binding to the DGC, both γ- and δ-sarcoglycan have been shown to bind and interact with filamin 2 (FLN2) (76). Filamin proteins have been shown to play an essential role in actin polymerisation and signalling cascades involved in regulating cell migration, growth, differentiation, and survival (reviewed in (77)). Additionally, filamins have been shown to bind to β1-integrin (78) and caveolin-1 (79), thereby regulating intracellular signalling to control stress fibre formation and actin polymerisation. Because of the interaction with FLN2, the sarcoglycan complex may be involved in cell signalling. If any additional undiscovered ligands that bind to the sarcoglycan subunits exist, identification of these will be critical to further our understanding of the signalling role of the sarcoglycans in maintaining skeletal muscle integrity.

The BIO14.6. hamster develops muscular dystrophy and cardiomyopathy at one to two months of age as a result of a deletion in the δ-sarcoglycan gene (80, 81). Interestingly, multiple sub-strains with the same deletion have different heart phenotypes and display either a dilated or hypertrophic cardiomyopathy (75). The different heart phenotypes observed arise from variation in the genetic background and potenially unidentified modifier genes. In the BIO14.6. hamster, loss of δ-sarcoglycan consequently results in a deficiency in α-, β- and γ-sarcoglycan, as well as a reduction in α-dystroglycan (45). In addition, sarcospan is absent from the sarcolemma. Sarcospan and α-dystroglycan localisation was only restored by injection of recombinant δ-sarcoglycan protein (30, 33). These findings suggest that the sarcoglycan complex, and δ-sarcoglycan in particular, plays a critical role in the localisation of sarcospan (30, 33).

Similar to the δ-sarcoglycan deficient hamster, δ-sarcoglycan loss-of-function mice display signs of muscular dystrophy and cardiomyopathy, reminiscent of LGMD2F (42). Severe dystrophic features are present in the musculature of knockout mice, including increased central nucleation, regions of regeneration, necrosis, and fatty infiltration. Furthermore, δ-sarcoglycan deficiency results in coronary artery vascular irregularities, as mice show multiple vascular constrictions and deformities of the coronary vascular bed. Consistent with the BIO14.6. hamster, loss of δ-sarcoglycan in mice results in a secondary loss of the sarcoglycan complex in skeletal and cardiac muscle. Treadmill exercise triggered an earlier onset of myocardial necrosis and lesions in δ-sarcoglycan null mice. Interestingly, this early onset of myocardial necrosis was prevented in δ-sarcoglycan

deficient mice by intraperitoneal injection of Nicorandil, a vascular smooth muscle relaxant (42). These results signify that deficiencies of the sarcoglycan complex in smooth muscle disrupt vascular function, contributing to severe cardiomyopathy and muscular dystrophy (42).

Sarcoglycan orthologues have also been identified and characterised in zebrafish (82), a well-established model organism for muscle development in vertebrates (83). Knockdown of δ-sarcoglycan using antisense oligonucleotides causes skeletal and cardiac muscle defects (84, 85). Furthermore, knockdown of δ-sarcoglycan disrupts the left-right asymmetry of the heart, delays cardiac development (84), disorganises myofibrils and induces a down-regulation of β- and γ-sarcoglycan (85). Therefore, morpholino-based knockdown of δ-sarcoglycan in zebrafish features aspects of the human condition and can be used to study the function of the sarcoglycan complex and the molecular mechanism of disease; thus, contributing to novel insight to the progression of muscular dystrophies.

5. CONCLUSION

Research has shown that the sarcoglycan complex is not just part of the DGC, but can be considered as a complex on its own. The sarcoglycan complex has an essential role in stabilising the DGC and strengthening the interaction between dystrophin and dystroglycan, as well as the fundamental signalling role in maintaining skeletal muscle integrity. Generally, loss of one of the sarcoglycan subunits results in a complete loss or reduction of all other sarcoglycan components. The utilisation of different animal models for sarcoglycanopathies, such as mouse and zebrafish, have contributed to a better understanding of both the functional role of the sarcoglycan complex, and how deficiencies provoke these maladies. This in turn has advanced our knowledge on the progression of muscular dystrophies and the critical role of the sarcoglycan complex in skeletal muscle function and integrity. Currently, it is hypothesised that deficiencies in the assembly and localisation of the sarcoglycan complex may damage the sarcolemma or induce defects in calcium channels, leading to an increase in cytoplasmic calcium and ultimately causing cell death. To date there is no resolutive cure for sarcoglycanopathies and more research must to be conducted in order to determine the molecular mechanism of disease. Ultimately, this can have direct implications for the development of a cure or treatment for this group of debilitating disorders.

6. ACKNOWLEDGEMENTS

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Abbreviations: *50-DAG*: 50 kDa dystrophin-associated glycoprotein; *DAG*: dystroglycan; *DMD*: dystrophin gene; DTN: dystrobrevin; ECM: extracellular matrix; *EGF*: epidermal growth factor; *FLN2*: filamin 2; *FAK*: focal adhesion kinase; *gsg*: γ-sarcoglycan; LGMD: Limb girdle muscular dystrophy; LGMD2C: Limb girdle muscular dystrophy type 2C; LGMD2D: Limb girdle muscular dystrophy type 2D; LGMD2E: Limb girdle muscular dystrophy type 2E; LGMD2F: Limb girdle muscular dystrophy type 2F; MD: Muscular dystrophy; *nNOS*: neuronal nitric-oxide synthase; SCARMD: severe congenital autosomal recessive muscular dystrophy; *SGC*: sarcoglycan; *SNT*: syntrophin; *SSPN*: sarcospan.

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