PATHOGENETIC ROLE OF THE SARCOGLYCAN-SARCOSPAN COMPLEX IN CARDIOMYOPATHIES

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Abstract. Cardiomyopathy is a multifactorial disease and one of the leading causes of heart failure in the industrial world. The dystrophin-glycoprotein complex has been implicated in the pathogenesis of both hereditary and acquired forms of cardiomyopathy. Clinical features of cardiomyopathy have been reported in patients with mutations in dystrophin and the sarcoglycan genes. We have developed mouse models of cardiomyopathy by ablation of genes encoding components of the sarcoglycan complex. Interestingly, mice deficient for α-sarcoglycan (*Sgca-null*) exhibit a progressive muscular dystrophy but do not develop cardiomyopathy, despite a disruption of the sarcoglycan-sarcospan complex. In marked contrast, mice deficient for β- and δ-sarcoglycan (*Sgcb*- and *Sgcd*-null, respectively) display a more severe muscular dystrophy and develop cardiomyopathy. In addition to perturbation of the sarcoglycan-sarcospan complex in striated muscle, both animal models show an additional disruption of the sarcoglycan-sarcospan complex in vascular smooth muscle. This leads to perturbation of vascular function as shown by multiple microvascular constrictions. Moreover, *Sgcb*- and *Sgcd*-null mice show a disruption of a distinct ε-sarcoglycan containing complex in striated muscle. Taken together, perturbation of vascular function together with disruption of the cardiac muscle sarcoglycan-sarcospan complex represent a novel mechanism in the pathogenesis of cardiomyopathies associated with limb-girdle muscular dystrophy types 2E and F. Characterization of this novel mechanism will ultimately lead to new pharmacological and gene therapeutic approaches targeted not only towards striated muscle but also towards the dysfunction of the vascular system.

Introduction

The dystrophin-glycoprotein complex (DGC) is a multisubunit protein complex composed of integral membrane, peripheral membrane, and cytoplasmic proteins expressed at the sarcolemma of skeletal and cardiac muscle fibers (for review see 1,2). Isolation and cloning of proteins within this complex have provided key insights into the function of the DGC and its role in normal muscle physiology and muscular dystrophy. In striated muscle the DGC is composed of dystrophin; the syntrophins; dystrobrevin; α- and β-dystroglycan; α-, β-, γ-, and δ-sarcoglycan; and sarcospan [1,2].

One characterized function of the DGC is to provide a structural link between the extracellular matrix and the actin cytoskeleton, thereby maintaining the stability of the sarcolemma under contractile forces [3, 4]. This link occurs through dystrophin, which binds to filamentous actin [5, 6] and dystroglycan, with its β-subunit binding to dystrophin and its α-subunit interacting with the extracellular matrix component laminin-2 [7]. Several forms of muscular dystrophy arise from primary mutations in genes encoding components of the DGC and perturbation of the composition of the DGC is thought to play a major role in the pathogenesis of DGC associated forms of muscular dystrophy [1]. Within the DGC, a group of single pass transmembrane glycoproteins forms a tetrameric unit consistent of α-, β-, γ-, and δ-sarcoglycan. Among limb-girdle muscular dystrophies type 2 (LGMD2) four distinct subtypes are caused by mutations in sarcoglycan glycoproteins [8]: LGMD2D (α-sarcoglycan), LGMD2E (β-sarcoglycan), LGMD2C (γ-sarcoglycan), and LGMD2F (δ-sarcoglycan).

Recent evidence is accumulating that hereditary and acquired forms of cardiomyopathy can also be caused by alterations within the dystrophin-glycoprotein complex (DGC) [9-14]. Cardiomyopathies have been associated with mutations within the dystrophin gene, as well as due to mutations of either β- γ- or δ-sarcoglycan. Moreover, mutations in the human δ-sarco-

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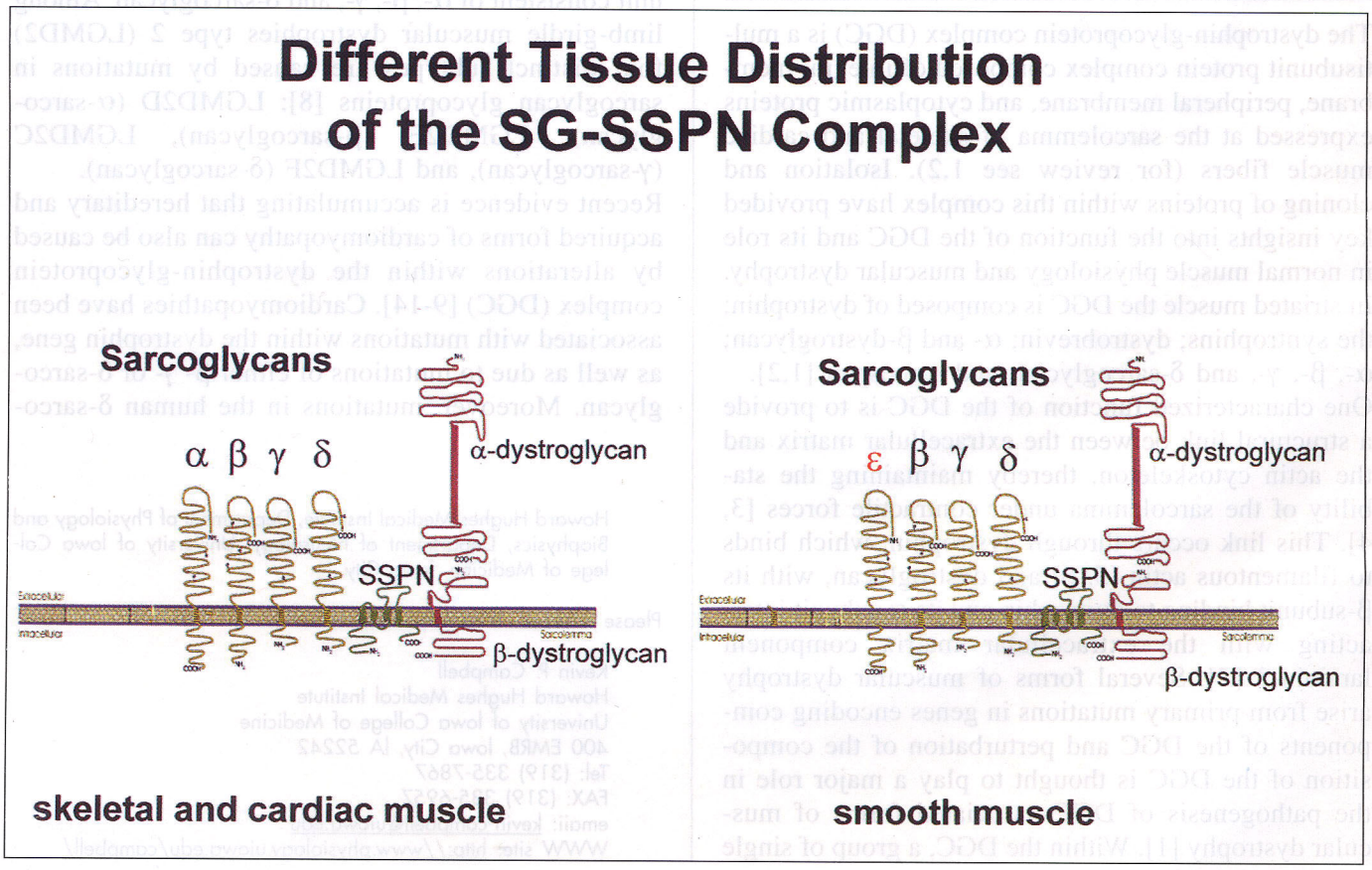
glycan gene have been characterized in patients with familiar and sporadic cases of dilated cardiomyopathy without significant involvement of the skeletal muscle [15].

The current manuscript will summarize our previous studies [12, 13, 16, 17, 18] of how the use of gene targeting of several sarcoglycan genes has increased our understanding of the pathogenetic mechanism involved in the development of cardiomyopathy. Moreover, we will outline how the identification of those mechanisms can be used to develop future concepts of therapy in order to prevent and/or mitigate the cardiomyopathic phenotype associated with certain forms of sarcoglycanopathy.

Tissue specific composition of the sarcoglycan-sarcospan complex

By studying the different tissue distribution of the sarcoglycan-sarcospan complex (SG-SSPN), a new avenue of research opened up and revealed new insights into the pathogenesis of sarcoglycanopathies. In skeletal and cardiac muscle the SG-SSPN complex is composed of α, β, γ, δ-sarcoglycan and sarcospan (Figure 1). Recently, ε-sarcoglycan, a transmembrane glycoprotein showing 43% amino acid identity with α-sarcoglycan has been identified [19, 20]. In contrast to ε-sarcoglycan, which has a broad tissue distribution, α-sarcoglycan expression is restricted to skeletal and cardiac muscle. Biochemical fractionation studies demonstrated that ε-sarcoglycan replaces α-sarcoglycan in smooth muscle as an integral component of a unique SG-SSPN complex composed of ε-, β-, δ- sarcoglycan and sarcospan [16, 17]. More recently, it has been demonstrated that γ-sarcoglycan is also expressed in smooth muscle and an integral part of the smooth muscle sarcoglycan-sarcospan complex [18]. The pathogenetic significance of the characterization of an unique sarcoglycan-sarcospan complex in smooth muscle will be shown by analyzing animal models with various targeted deletions of sarcoglycan genes.

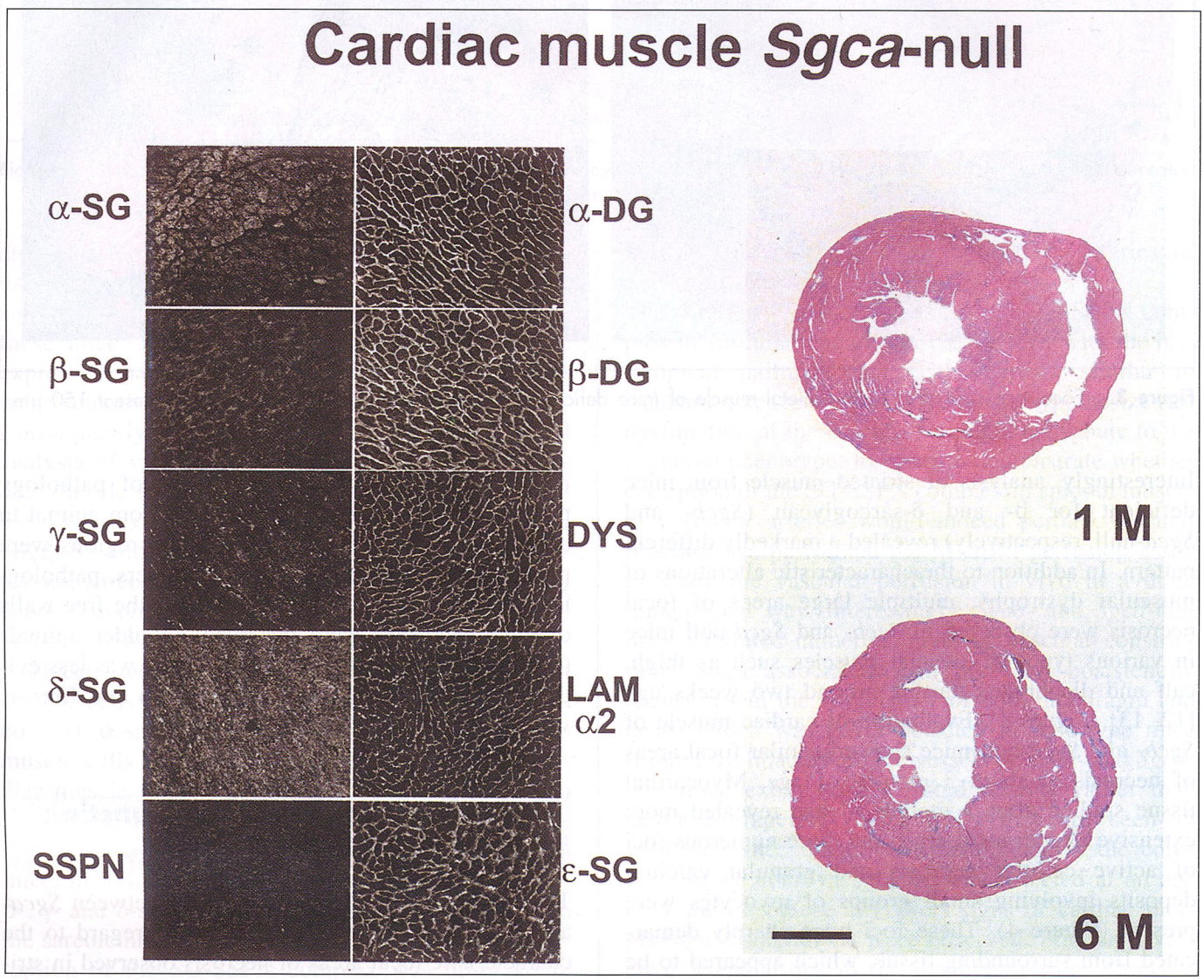
Figure 1. - Integral membrane components of the sarcoglycan-sarcospan complex in striated muscle and smooth muscle. α-sarcoglycan is solely expressed in striated muscle and is replaced by ε-sarcoglycan in smooth muscle.



Histopathological features of mice deficient for α-, β- and δ-sarcoglycan

A shared feature of the four types of sarcoglycanopathies is that mutations in a single sarcoglycan gene result in abnormal expression of all sarcoglycans at the sarcolemma [1] (Figure 2). Consequently, targeted deletion of α-sarcoglycan in mice leads to loss of the SG-SSPN in skeletal and cardiac muscle and perturbation of the dystroglycan complex [21]. Histological analyses of skeletal muscle sections from mice deficient for α-sarcoglycan (*Sgca*-null) reveals the characteristic alterations observed in muscular dystrophy with cell necrosis, centrally nucleated muscle fibers, calcification and endomysial fibrosis. However, although the SG-SSPN complex is not expressed at the sarcolemma of cardiac muscle, no pathological abnormalities of the heart can be observed in these mice up to 2 years of age [12] (Figure 3). These findings are in accordance with the clinical data observed in patients with primary mutations in the α-sarcoglycan gene, which also do not or only mildly display cardiomyopathic phenotypes [14].

Figure 2. - Loss of the sarcoglycan-sarcospan complex in cardiac muscle of mice deficient for α-sarcoglycan. However, no pathological abnormalities can be observed in the heart. α-SG = α-Sarcoglycan; β-SG = β-Sarcoglycan; γ-SG = γ-Sarcoglycan; δ-SG = δ-Sarcoglycan; ε-SG = ε-Sarcoglycan; α-DG = α-Dystroglycan; β-DG = β-Dystroglycan; DYS = Dystrophyn; LAM = Lamine. Scale bar represents 1.5 mm.



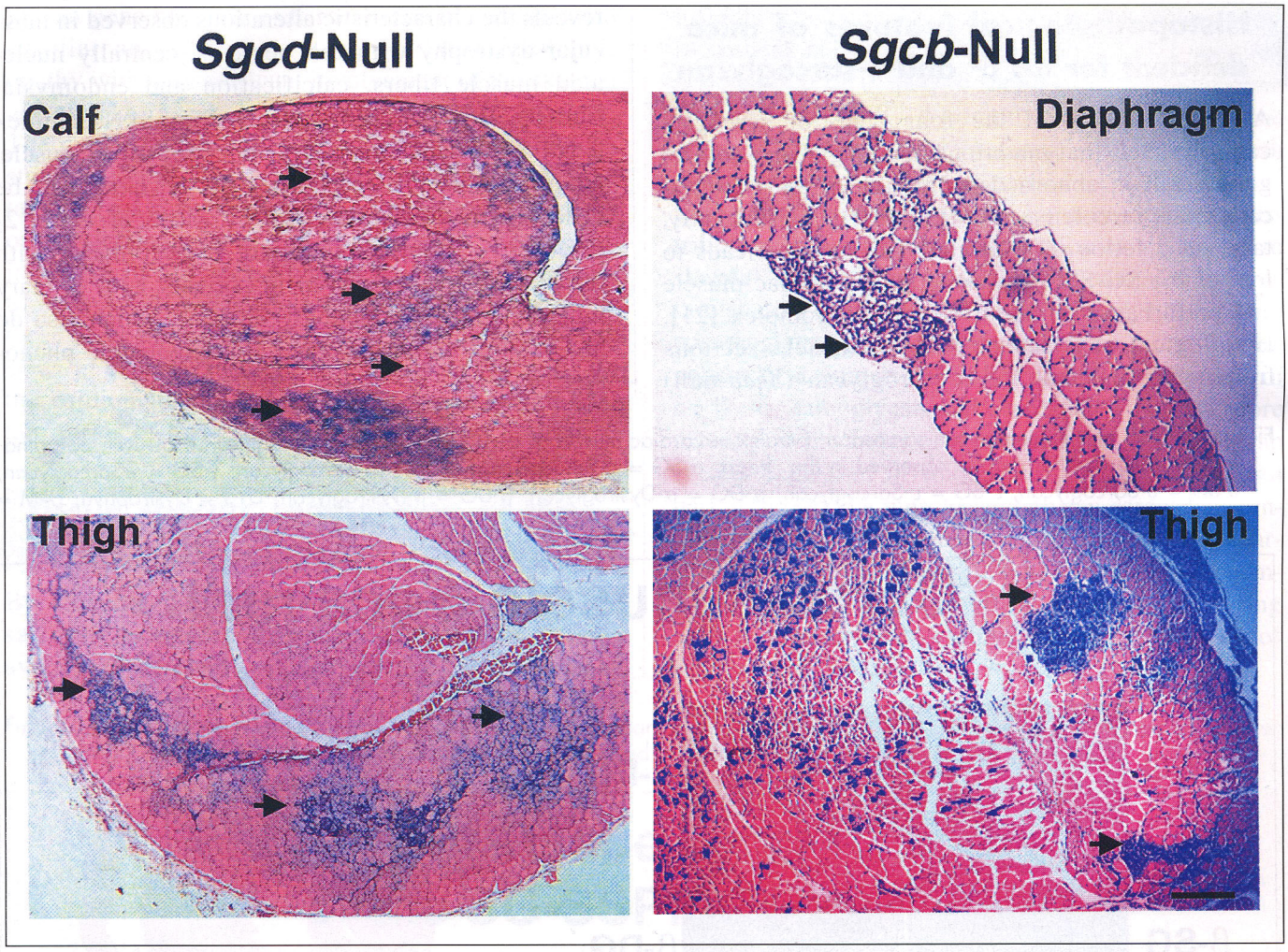


Figure 3. - Focal areas of necrosis in skeletal muscle of mice deficient for β- or δ-sarcoglycan. Scale bars represent 150 μm.

Interestingly, analysis of striated muscle from mice deficient for β- and δ-sarcoglycan (*Sgcb*- and *Sgcd*-null, respectively) revealed a markedly different pattern. In addition to the characteristic alterations of muscular dystrophy, multiple large areas of focal necrosis were observed in *Sgcb*- and *Sgcd*-null mice in various types of skeletal muscles such as thigh, calf and diaphragm starting around two weeks age [12, 13] (Figure 3). Evaluation of cardiac muscle of *Sgcb*- and *Sgcd*-null mice showed similar focal areas of necrosis at about 3 months of age. Myocardial tissue studied after 3 months of age revealed more extensive alterations. Larger and more numerous foci of active cellular necrosis and granular calcium deposits involving small groups of myocytes were present (Figure 4). These foci were sharply demarcated from surrounding tissue, which appeared to be normal. The localization and extent of pathology predilection sites varied considerably from animal to animal. In some hearts subendocardial regions were predominantly affected, whereas in others, pathological changes in the outer two-thirds of the free walls of both ventricles were observed. In older animals (5-6 months) active myocardial necrosis was less evident, but various stages of calcification and fibrosis were observed [12, 13].

Perturbation of vascular function in *Sgcb*- and *Sgcd*-null mice

The marked phenotypical differences between *Sgca*- and *Sgcb*/*Sgcd*-null mice especially in regard to the characteristic focal areas of necrosis observed in stri-

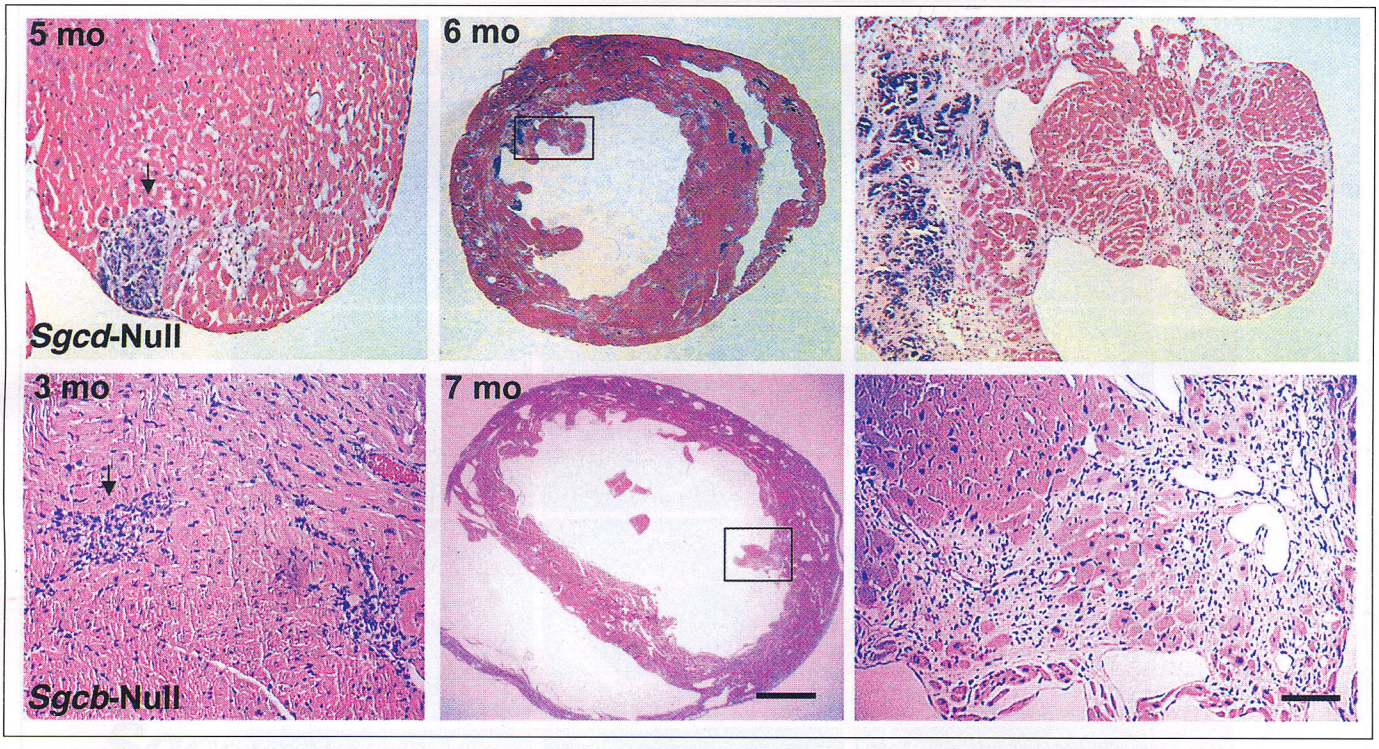


Figure 4. - Development of severe cardiomyopathy in mice deficient for β- or δ-sarcoglycan starting with focal, well demarcated areas of necrosis. Scale bars represent 1.5 mm and 150 μm, respectively.

ated muscle of *Sgcb*- and *Sgcd*-null mice, prompted us to investigate whether abnormalities in vascular smooth muscle might contribute to the phenotype in these mice. For this purpose, we first studied the expression pattern of the SG-SSPN complex in vascular smooth muscle of our animal models.

Consequently, we performed immunohistochemical analysis of various components of the DGC in cardiac muscle fibers and smooth muscle cells of coronary arteries from wild type, *Sgca*- and *Sgcd*-null mice. In wild type mice, α-, β-, γ-, δ-, and ε-sarcoglycan, sarcospan and β-dystroglycan were homogeneously expressed at the cardiac muscle fiber membranes. β-, δ- and ε-sarcoglycan, sarcospan and β-dystroglycan were also strongly expressed in the smooth muscle cells of the coronary arteries (Figure 5). In contrast, α-sarcoglycan was not expressed in smooth muscle cells of coronary arteries (Figure 5). In cardiac muscle fibers of *Sgca*-null mice, α-sarcoglycan was absent from the sarcolemma, whereas δ-sarcoglycan was absent from the sarcolemma of *Sgcd*-null mice. In addition, there was a concomitant loss of α-, β-, γ- and δ-sarcoglycan. Sarcospan was absent from the sarcolemma of both animal models. A similar disruption of the SG-SSPN complex was observed in vascular smooth muscle of pulmonary arteries of *Sgcb*-null mice (data not shown).

The combination of disruption of the SG-SSPN complex in vascular smooth muscle together with the histological hallmark of focal necrosis similar to ischemic-like lesions prompted us the hypothesize that dysfunction of the vasculature might contribute to the observed phenotype. In order to demonstrate whether disruption of the SG-SSPN complex in smooth muscle of coronary arteries would indeed perturb vascular function, we performed the Microfil perfusion assay to study the vascular perfusion in vivo in a three dimensional way. Interestingly, *Sgcb*- and *Sgcd*-null mice exhibited numerous areas of vascular constrictions often associated with pre- and poststenotic aneurysms in the vasculature of both diaphragm and heart, which was never detected in wild-type mice (Figure 6). In addition, the vessels of *Sgcb*- and *Sgcd*-null mice exhibited a serrated contour rather than smoothly tapered vessel walls that are seen in wild-type mice. Functional disturbance of the coronary artery microvasculature was detected at an age of 4 weeks, before any overt signs of cardiac morphological alterations were observed. Similarly, vascular irregularities in the diaphragm were observed

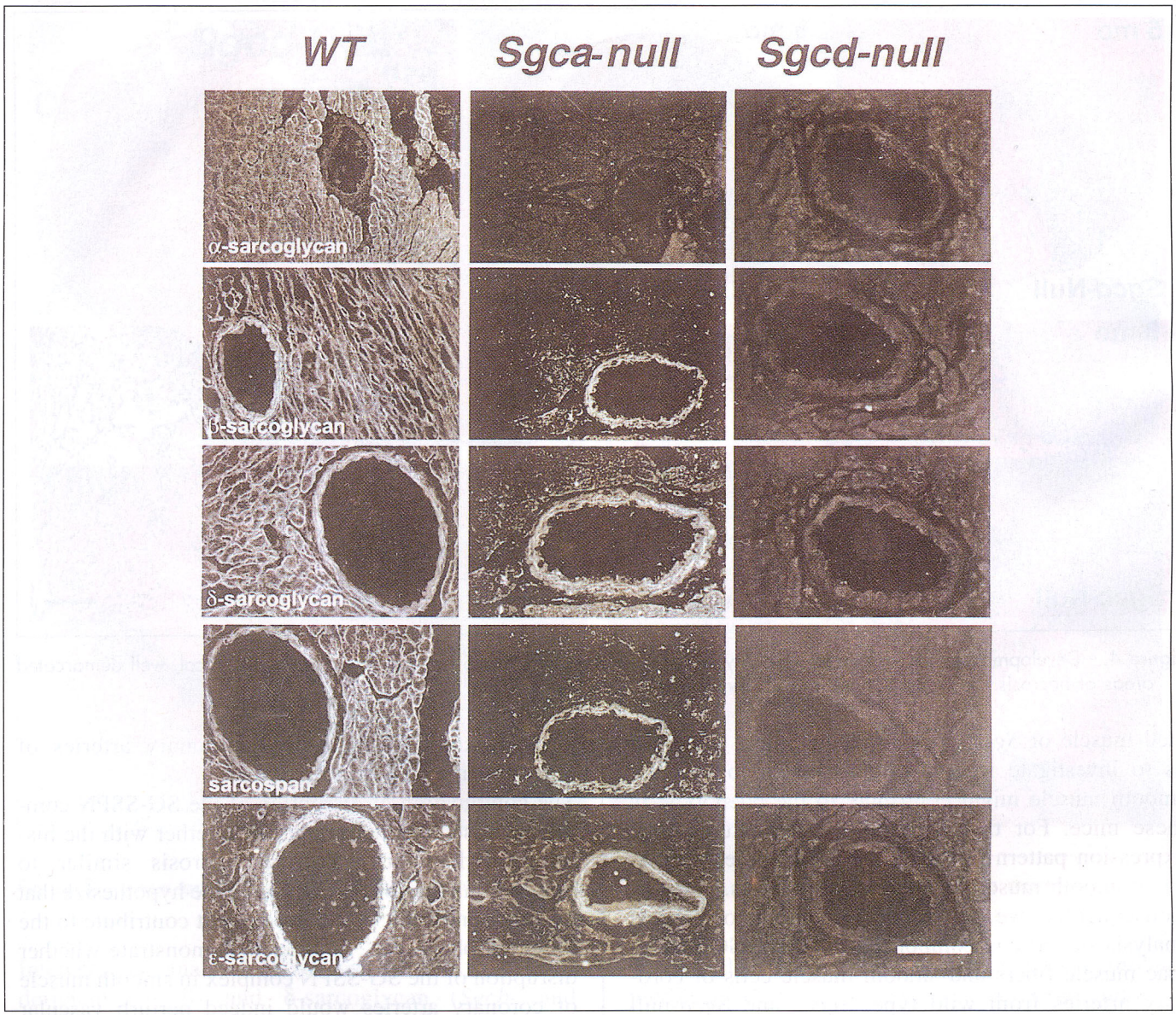


Figure 5. - Disruption of the sarcoglycan sarcospan-complex in vascular smooth muscle of coronary arteries of *Sgcd*-null. Note the preserved expression of the sarcoglycan-sarcospan complex in vascular smooth muscle of mice deficient for α-sarcoglycan. Scale bar represents 20 μm.

in 4-wk-old *Sgcb*- and *Sgcd*-null mice, at a time when acute necrosis starts to occur in the skeletal muscle. These observations indicate that the disturbance of the vasculature precedes the onset of ischemic-like lesions in *Sgcb*- and *Sgcd*-null mice. To further ascertain that the vascular phenotype is independent of the muscular dystrophy phenotype, we also analyzed Microfil®-perfused diaphragms from *Sgca*-null mice. *Sgca*-null mice develop a progressive muscular dystrophy and fibrotic areas are detected in diaphragms of *Sgca*-null mice [21]. However, since α-sarcoglycan is not expressed in vascular smooth muscle, *Sgca*-null mice should not display any vascular perturbations, at any age (Figure 6). Indeed, no constrictions were detected in diaphragms and coronary arteries of one-year-old *Sgca*-null mice. Together these data demonstrate that the muscle degeneration does not initiate the vascular phenotype.

Because extensive histological studies failed to reveal any morphological or structural abnormalities in vascular smooth muscle, we hypothesized that the observed abnormalities of vascular perfusion could

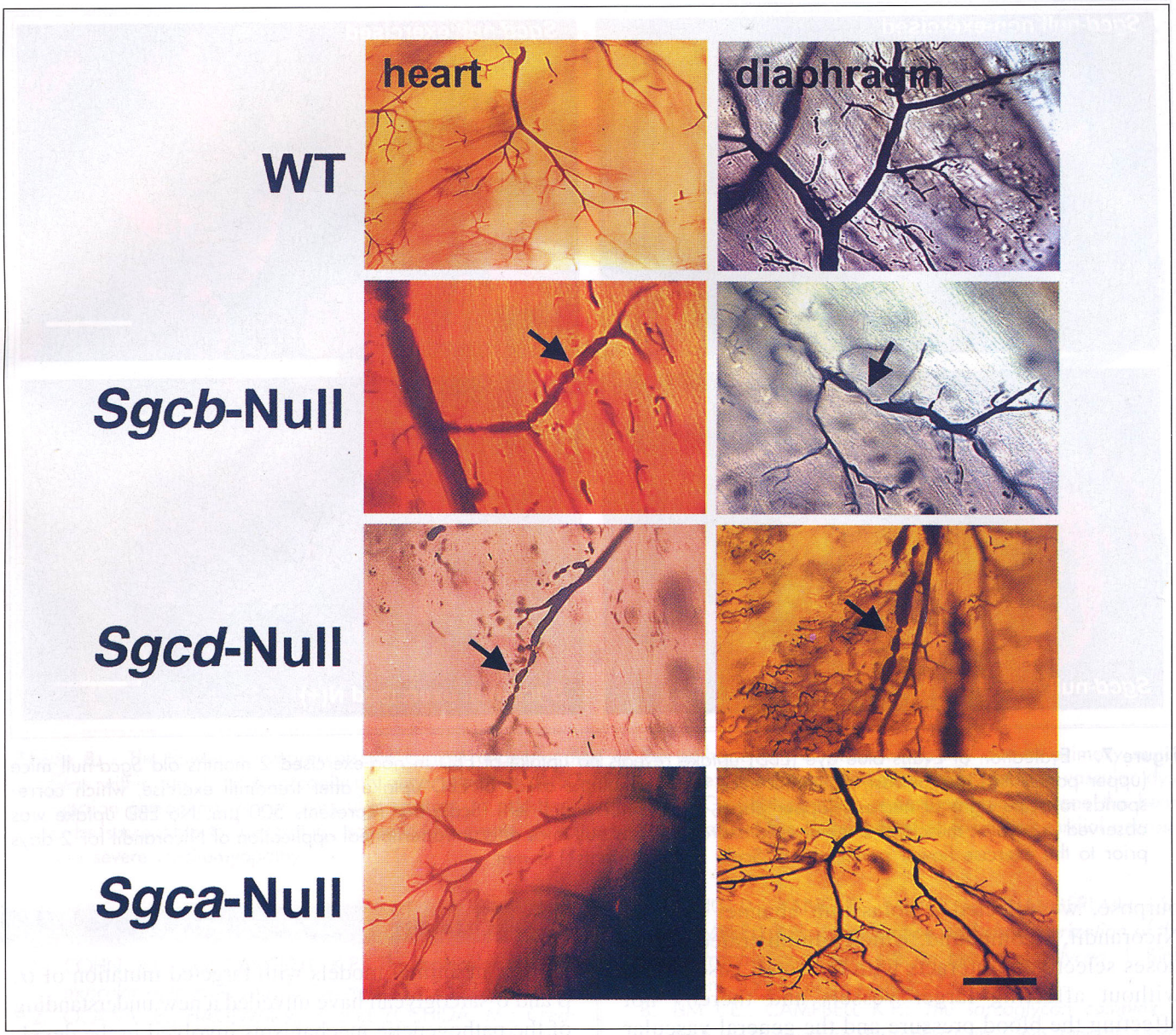


Figure 6. - Vessels of 4-week-old heart and diaphragm from wild-type mice showed smoothly tapered vessel walls. In contrast, vessels of the heart and diaphragm from *Sgcb*- and *Sgcd*-null mice showed numerous constrictions associated with pre- and poststenotic aneurysms. Note that vessels of the diaphragm from one year old *Sgca*-null mice did not show any constrictions. Arrows denote constrictions. Bar, 40 µm.

possibly represent a dynamic hyper-reactivity of the vasculature. In order to test this hypothesis, wild type and *Sgcd*-null mice underwent acute extensive treadmill exercise. The exercised mice were studied at the age of 2-3 months a time where *Sgcd*-null mice do not show any overt signs of cardiac muscle necrosis, but do have microvessel abnormalities as revealed by our perfusion studies. Interestingly, acute treadmill exercise lead to the development of multiple ischemic-like focal myocardial necrosis in *Sgcd*-null mice [12] (Figure 7).

To finally prove that the observed vascular perfusion abnormalities were responsible for the acutely induced myocardial lesions, we wanted to test the hypothesis whether administration of a vascular smooth muscle relaxant would alleviate the vascular constrictions and thereby prevent the exercise-induced onset of myocardial necrosis. For this

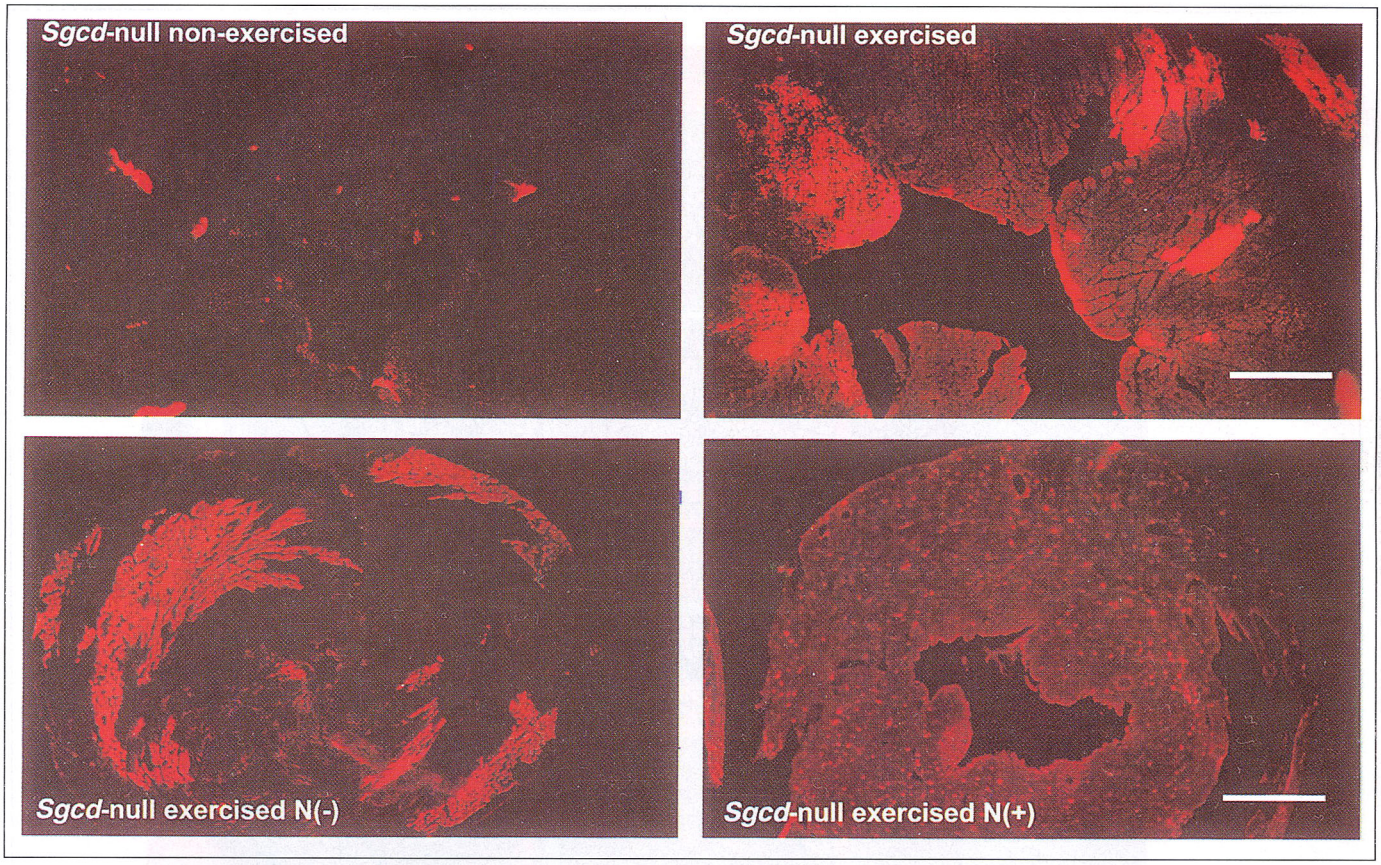


Figure 7. - Evaluation of Evans blue dye (EBD) uptake reveals no uptake of EBD in non-exercised 2 months old *Sgcd*-null mice (upper panel, left). In contrast, *Sgcd*-null mice display multiple areas of EBD uptake after treadmill exercise, which corresponds to acute signs of necrosis (upper panel, right lower panel, left). Scale bar represents 500 μm. No EBD uptake was observed in cardiac muscle of 2 months old exercised *Sgcd* null mice after intraperitoneal application of Nicorandil for 2 days prior to the exercise (lower panel, right). Scale bar represents 1.5 mm.

purpose, we selected a potassium channel agonist, Nicorandil, a compound, which administrated at low doses selectively interacts with the small vasculature without affecting larger vessels and thereby not affecting the blood pressure and the general vascular systemic resistance. Remarkably, *Sgcd*-null mice injected with Nicorandil for 2 days prior to exercise did not develop any exercise induced myocardial necrosis nor did they show any kind of vascular dysfunction [12] (Figure 7).

Conclusion

Studies in animal models with targeted mutation of α, β and δ-sarcoglycan have unveiled a new understanding of the pathogenetic mechanisms involved in the development of cardiomyopathy associated with limb-girdle muscular dystrophy types 2E and F (Figure 8). Disruption of the SG-SSPN complex in vascular smooth muscle leads to perturbation of vascular function as demonstrated by multiple vascular constrictions.

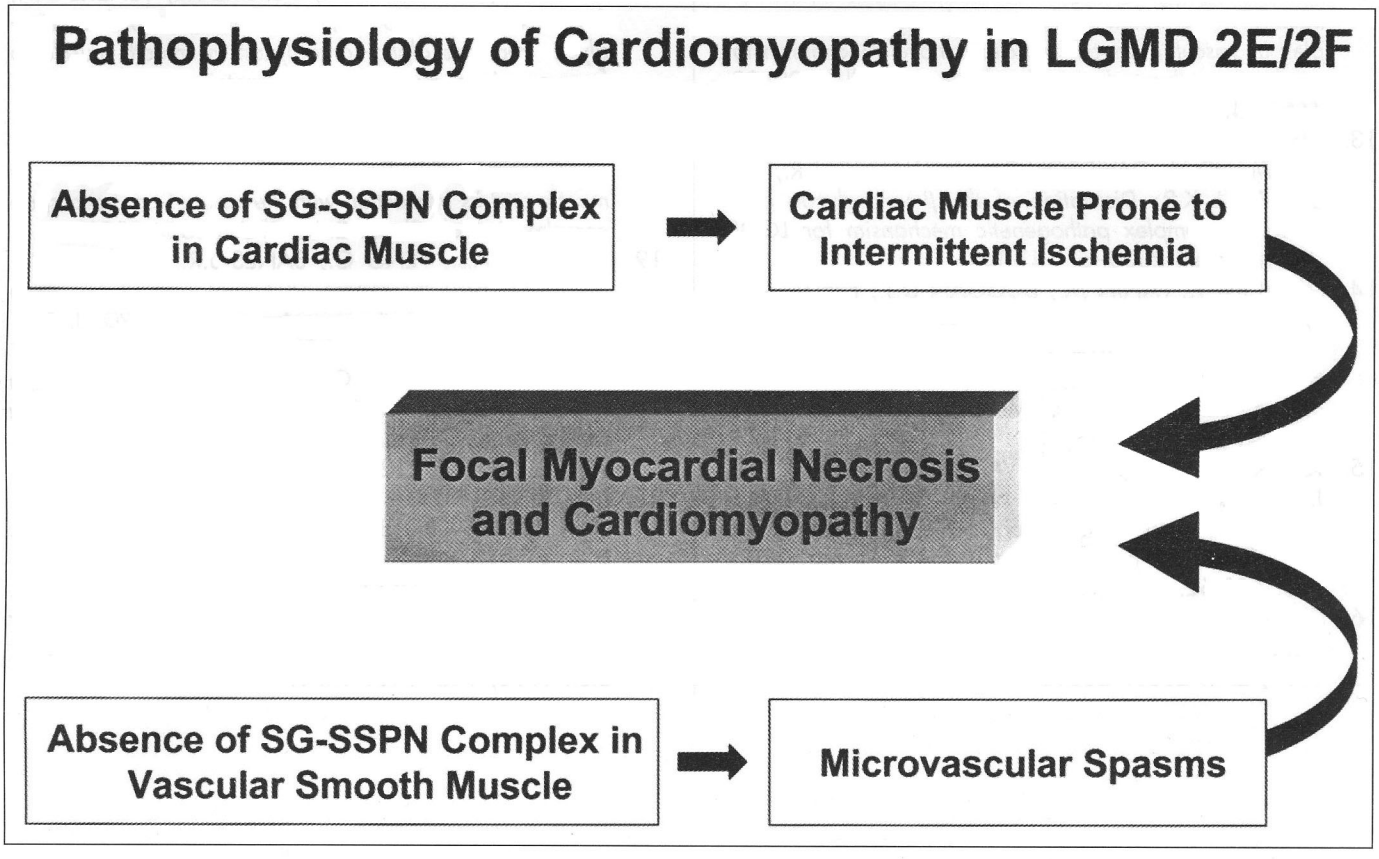


Figure 8. - The flow chart represents the current understanding of the pathogenesis of cardiomyopathy due to primary mutations within the β- or δ-sarcoglycan gene. Absence of SG-SSPN complex in vascular smooth muscle leads to vascular dysfunction presenting as microvascular spasms. In addition, loss of the SG-SSPN complex in cardiac muscle prone the heart to be susceptible to intermittent ischemic-like events, which eventually leads to the development of focal myocardial necrosis and severe cardiomyopathy.

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