

γ -Sarcoglycan Deficiency Leads to Muscle Membrane Defects and Apoptosis Independent of Dystrophin

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Abstract. γ -Sarcoglycan is a transmembrane, dystrophin-associated protein expressed in skeletal and cardiac muscle. The murine γ -sarcoglycan gene was disrupted using homologous recombination. Mice lacking γ -sarcoglycan showed pronounced dystrophic muscle changes in early life. By 20 wk of age, these mice developed cardiomyopathy and died prematurely. The loss of γ -sarcoglycan produced secondary reduction of β - and δ -sarcoglycan with partial retention of α - and ϵ -sarcoglycan, suggesting that β -, γ -, and δ -sarcoglycan function as a unit. Importantly, mice lacking γ -sarcoglycan showed normal dystrophin content and localization, demonstrating that myofiber degeneration occurred independently of dystrophin alteration. Furthermore, β -dystroglycan and laminin were left intact, implying that the dystrophin–dystroglycan–laminin

mechanical link was unaffected by sarcoglycan deficiency. Apoptotic myonuclei were abundant in skeletal muscle lacking γ -sarcoglycan, suggesting that programmed cell death contributes to myofiber degeneration. Vital staining with Evans blue dye revealed that muscle lacking γ -sarcoglycan developed membrane disruptions like those seen in dystrophin-deficient muscle. Our data demonstrate that sarcoglycan loss was sufficient, and that dystrophin loss was not necessary to cause membrane defects and apoptosis. As a common molecular feature in a variety of muscular dystrophies, sarcoglycan loss is a likely mediator of pathology.

Key words: muscular dystrophy • sarcolemma • dystrophin • extracellular matrix • apoptosis

PROGRESS in human genetics has identified a number of proteins at the muscle membrane that are associated with muscular dystrophy. Dystrophin, the protein product of the Duchenne muscular dystrophy (DMD)¹ gene, is an elongated cytoskeletal protein that binds actin and a complex of dystrophin-associated proteins, forming the dystrophin-glycoprotein complex (DGC; Burghes et al., 1987; Campbell and Kahl, 1989; Ervasti and Campbell, 1991; Koenig et al., 1987; Monaco et al., 1986; Rybakova et al., 1996; Yoshida and Ozawa, 1990). More recently, a subcomplex of the DGC, sarcoglycan, was associated with mutations that result in human auto-

somal recessive muscular dystrophy (Bönnemann et al., 1996a; Campbell, 1995; Duggan et al., 1997; Straub and Campbell, 1997). There are at least five subunits of sarcoglycan: α , β , γ , δ , and ϵ (Ettinger et al., 1997; Lim et al., 1995; McNally et al., 1998; Nigro et al., 1996; Noguchi et al., 1995; Roberds et al., 1993). Three of these sarcoglycans— β , γ , and δ —share homology as type II transmembrane proteins with cysteine-rich EGF-like repeats in their extracellular domains. Mutations in any single sarcoglycan gene result in varying degrees of secondary reduction of the other sarcoglycan units, suggesting interaction between the sarcoglycan subunits. α -sarcoglycan is a type I transmembrane protein expressed exclusively in skeletal and cardiac muscle. A widely expressed fifth sarcoglycan, ϵ -sarcoglycan, was recently identified with significant homology to α -sarcoglycan (Ettinger et al., 1997; McNally et al., 1998). ϵ -sarcoglycan is present in skeletal and cardiac muscle, but is also expressed in most tissues studied, demonstrating that sarcoglycan-like proteins are present in nonmuscle tissues.

In the presence of β -D-octyl-glycoside, the sarcoglycan subunits can be separated from the remainder of the DGC, but the exact interaction between sarcoglycan and

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1. *Abbreviations used in this paper:* DMD, Duchenne muscular dystrophy; LGMD, limb girdle muscular dystrophy; CK, creatine kinase; EBD, Evans blue dye; DGC, dystrophin-glycoprotein complex; ECM, extracellular matrix.

the remainder of the DGC, particularly dystrophin, is ill-defined (Ozawa et al., 1995; Yoshida et al., 1994). Studies of human muscle biopsies have provided limited information regarding the nature of the sarcoglycan–dystrophin interaction. Quantitative assessment of dystrophin content showed a secondary decrease of dystrophin content in patients with mutations in β -, γ -, and δ -sarcoglycan; the reduction in dystrophin content ranged from 40 to 90% of normal (Vainzof et al., 1996). This finding suggests that a secondary decrease in dystrophin may be responsible for the pathology of muscular dystrophy in patients with primary sarcoglycan mutations.

In addition to the sarcoglycans, the DGC includes dystroglycan, a highly glycosylated protein whose α subunit directly binds the extracellular matrix (ECM) protein laminin (Ibraghimov-Beskrovnaya et al., 1992). Dystroglycan is widely expressed, and mice homozygously lacking dystroglycan have an early embryonic lethality (day 6.5 after conception) arising from the role of dystroglycan in nonmuscle tissue, particularly the extraembryonic structure Reichert's membrane (Williamson et al., 1997). Dystroglycan binds laminin- α 2, an extracellular matrix protein expressed in peripheral nerve, skeletal, and cardiac muscle (Ervasti and Campbell, 1993; Yamada et al., 1994). In humans, laminin- α 2 (merosin) mutations have been associated with a severe phenotype, congenital muscular dystrophy (Helbling-Leclerc et al., 1995; Nissinen et al., 1996). A murine model for laminin- α 2 deficiency, the *dy* mouse, demonstrates a severe phenotype resulting in marked skeletal muscle degeneration and early death (Sunada et al., 1994; Xu et al., 1994). In contrast, the murine model for dystrophin deficiency, the *mdx* mouse (Sicinski et al., 1989), has a milder phenotype than its human counterpart. *mdx* mice have a normal lifespan, but demonstrate many of the histologic features seen in human muscular dystrophy, including fiber size variation and centrally placed nuclei. Human DMD patients exhibit replacement of myofibers with fatty and fibrous infiltration within the first decade, yet *mdx* mice show progressive fibrosis relatively later in life. Although the *mdx* mouse has a milder phenotype than human DMD patients, it has proved useful for studies identifying the nature of the membrane defect in muscular dystrophy. Vital staining studies of *mdx* and *dy* mice suggested different mechanisms of membrane degeneration since dystrophin deficiency produces membrane defects, while laminin deficiency did not (Matsuda et al., 1995; Straub et al., 1997). This observation and its cytoskeletal localization has led to the hypothesis that dystrophin participates in a mechanical link that stabilizes muscle membrane. Contraction-induced force exerted on a membrane defective in dystrophin is thought to lead directly to membrane disruptions and myofiber degeneration.

To investigate the role of sarcoglycan in muscular dystrophy, we targeted the murine γ -sarcoglycan gene. In early life, mice lacking γ -sarcoglycan developed muscular dystrophy that preferentially affected the proximal musculature. Mice lacking γ -sarcoglycan developed cardiomyopathy that affected both the right and left ventricle. Vital staining of mice lacking γ -sarcoglycan revealed membrane disruptions demonstrating that sarcoglycan deficiency has a similar mechanism to dystrophin-deficiency and is unlike

laminin deficiency. Membrane disruptions in γ -sarcoglycan-deficient muscle were primarily observed in proximal skeletal muscles similar to humans with limb-girdle muscular dystrophy (LGMD). Moreover, apoptotic myonuclei were abundant in γ -sarcoglycan-deficient muscle. Importantly, dystrophin, dystroglycan, and laminin were intact and normally present at the sarcolemma in γ -sarcoglycan-

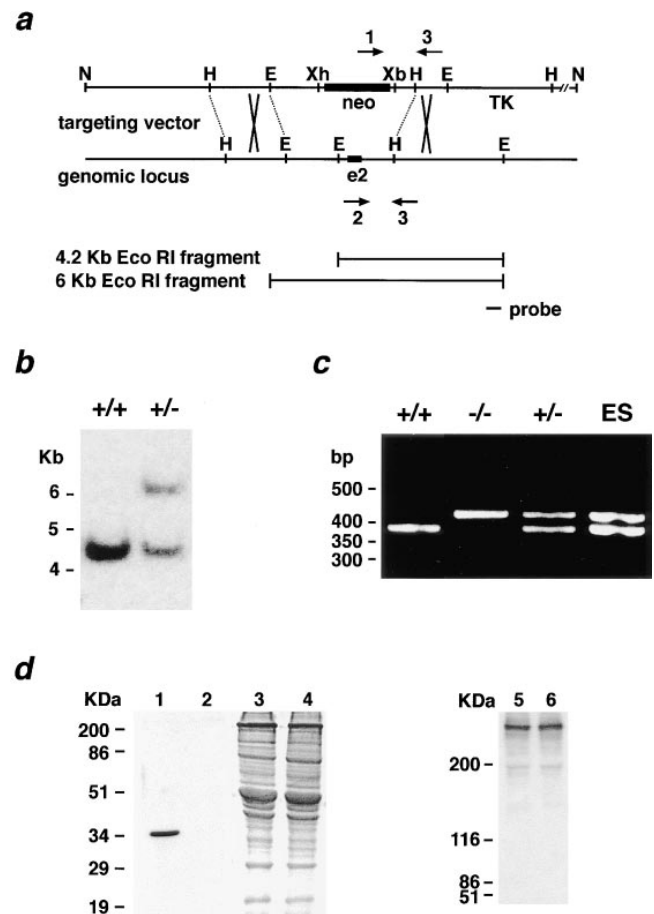


Figure 1. Homologous recombination in the murine γ -sarcoglycan locus. (a) The strategy for replacing exon 2 of murine γ -sarcoglycan with neomycin phosphotransferase (neo) is shown. The probe used for Southern blotting and the primers (arrows) used for genotyping progeny from *gsg*^{+/-} crosses are indicated. E, EcoRI; H, HindIII; N, NotI; Xb, XbaI; Xh, XhoI. (b) Southern blot of EcoRI-digested genomic DNA from ES cells. Homologous recombination in the γ -sarcoglycan locus produces the loss of a single EcoRI site. (c) PCR genotyping of progeny from *gsg*^{+/-} interbreeding. The PCR product generated by the mutant allele is 410 bp, and that generated by the wild-type allele is 376 bp. (d) Immunoblotting of total protein from wild-type (lanes 1 and 5) and mutant *gsg*^{-/-} (lanes 2 and 6) muscle with a monoclonal antibody directed at γ -sarcoglycan amino acids encoded by exon 6 (NCL-35DAG, lanes 1 and 2), or with a polyclonal anti-dystrophin antibody (AB6-10, lanes 5 and 6). No γ -sarcoglycan is detectable in *gsg*^{-/-} muscle. Dystrophin is normal in *gsg*^{-/-} muscle. Polyclonal antisera raised to the complete γ -sarcoglycan protein also shows the absence of γ -sarcoglycan protein in *gsg*^{-/-} mice (data not shown). A Coomassie-stained gel, *gsg*^{+/-} (lane 3) and *gsg*^{-/-} (lane 4), demonstrates equivalent loading. γ -sarcoglycan and dystrophin were resolved on 12.5% and 5% SDS-PAGE, respectively.

deficient muscle. Therefore, γ -sarcoglycan deficiency was sufficient to cause muscle membrane instability and the dystrophic process independently of dystrophin, placing sarcoglycan genetically downstream of dystrophin.

Materials and Methods

Gene Targeting

Genomic phage encoding exon 2 of γ -sarcoglycan was isolated from a murine 129SVJ library (Stratagene, La Jolla, CA) and characterized by restriction mapping, nucleotide sequencing, long-range PCR, and Southern hybridization. 5 kb of the first intron and 1.7 kb of the second intron were cloned into pPNT (Tybulewicz et al., 1991). Targeted RW4 ES cells were selected as described previously (Scott et al., 1994). Recombinants were screened by PCR and confirmed by Southern blotting of EcoRI-digested genomic DNA using a probe shown in Fig. 1 *a*. Recombinant ES cell clones were injected into C57BL/6 blastocysts and implanted into pseudopregnant foster mothers. Chimeric males were identified by coat color, and were mated to C57BL/6 females. Germline transmission was identified by coat color, and was confirmed by PCR and Southern blotting. PCR genotyping of progeny was performed on genomic DNA isolated from tail clippings using the primers shown in Fig. 1 *a*. Animals were housed and treated according to standards set by the University of Chicago Institutional Animal Care and Use Committee.

Immunoblotting

Immunoblots to detect γ -sarcoglycan protein were performed as described previously (Nicholson et al., 1989), using either affinity-purified polyclonal (McNally et al., 1996) or monoclonal (NCL-35DAG; Novocastra Laboratories, Newcastle upon Tyne, UK) anti- γ -sarcoglycan.

General Characterization, Vital Staining, and Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick End-labeling (TUNEL) Assay

Animals were observed for gait abnormalities weekly through 24 wk of age. Serum creatine kinase (CK) levels were measured on blood drawn from tail veins at 4 ($n = 24$) and 8 ($n = 24$) wk of age. Blood was collected in a Microstainer Serum Separator tube (Becton Dickinson, Franklin Lakes, NJ) and analyzed using a Vitros DT60II discrete chemistry analyzer (Eastman Kodak, Rochester, NY). Vital staining was performed as described previously using filter-sterilized Evans blue dye (Sigma-Aldrich, St. Louis, MO) resuspended in PBS at 1 mg/0.1 ml/10 g body weight (Matsuda et al., 1995; Straub et al., 1997). Intraperitoneal injections were performed on mice at 2 ($n = 5$), 4 ($n = 4$) and 8 ($n = 4$) wk of age; mice were killed 12 h after injection. TUNEL was performed on fixed, paraffin-embedded muscle using the ApopTagTM-fluorescein kit (Oncor Inc., Gaithersburg, MD). Double-labeling with polyclonal anti-dystrophin antibodies was performed after TUNEL-labeling, as described below.

Histology and Immunocytochemistry

Muscle from 4-, 8-, and 20-wk-old animals was fixed for 24–48 h in 10% neutral buffered formalin and embedded in paraffin. 10- μ M sections were cut and stained with Masson trichrome. Immunostaining was performed as described (McNally et al., 1996). The following primary antibodies were used: polyclonal anti-dystrophin AB6-10 (Lidov et al., 1990), polyclonal anti- γ -sarcoglycan (McNally et al., 1996), polyclonal anti- β -sarcoglycan (Bönnemann et al., 1996b), monoclonal anti- α and γ -sarcoglycans (NCL-50DAG and NCL-35DAG, respectively), monoclonal anti- β -dystroglycan (NCL- β -DG), monoclonal anti-utrophin (NCL-DRP2), and monoclonal anti-merosin (NCL-merosin; Novocastra Laboratories). Polyclonal anti- δ -sarcoglycan was raised to a glutathione-S-transferase fusion protein containing amino acids 85–291 of the human protein (Genbank number X95191), as previously described (McNally et al., 1996). Polyclonal anti- ϵ -sarcoglycan was raised similarly using amino acid residues 31–413 of the human ϵ -sarcoglycan sequence (Genbank no. AF036364). Cy3-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) were used. Integrin- β 1 antibody (MAB1997) was from Chemicon International, Inc. (Temecula, CA). Counterstaining with 4',6-diamidino-2-phenylindole (DAPI) was performed at 20 μ g/ml for 1 min.

Sections were photographed on a Zeiss Axiophot epifluorescence microscope.

Electron Microscopy

Mice were perfusion-fixed with 2% paraformaldehyde and 2% glutaraldehyde. Skeletal muscle was processed for EM by postfixation with 1% osmium tetroxide in water for 1 h, washed in water, and dehydrated with ethanol and propylene oxide. Dehydrated tissues were embedded in plastic, sectioned at 60 nm, and stained with 5% uranyl acetate and 1% lead. Sections were viewed and photographed on a Hitachi 600 EM.

Results

Targeted Disruption of γ -Sarcoglycan and Generation of Null Mice

Given the large size of the γ -sarcoglycan gene, exon 2 was chosen for targeting. Exon 2 specifies the initiator methionine, the cytoplasmic tail, and the transmembrane domain. Homologous recombination replaces exon 2 with the phosphoglycerate kinase promoter/neomycin phosphotransferase cDNA (Fig. 1 *a*). A representative Southern blot of a targeted ES cell clone is shown in Fig. 1 *b*. Heterozygous $gsg^{+/-}$ mice were viable, fertile, and ap-

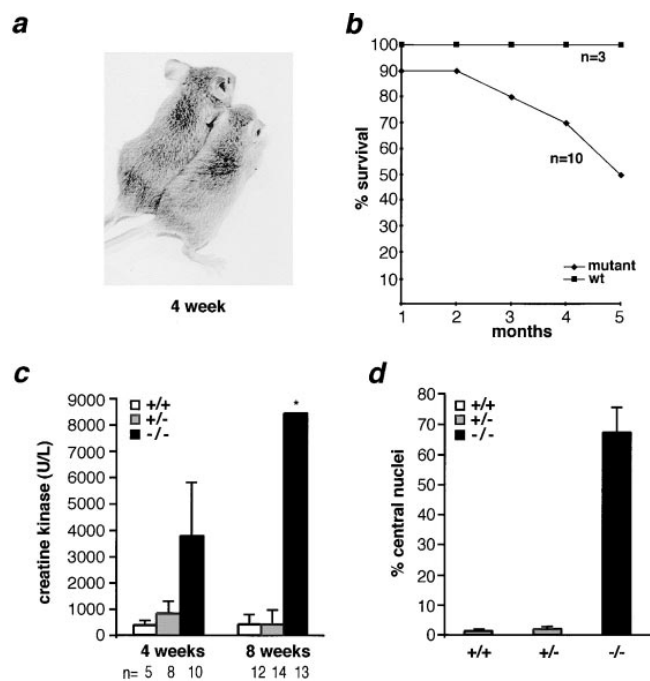


Figure 2. Characterization of mice lacking γ -sarcoglycan. (*a*) Left, wild-type $gsg^{+/+}$ mouse; right, $gsg^{-/-}$ mouse displaying stunted growth and abnormal stance. (*b*) Survival curve for $gsg^{-/-}$ vs. wild-type $gsg^{+/+}$ littermates. By 4–5 mo of age, nearly 50% of $gsg^{-/-}$ animals die. (*c*) Serum creatine kinase (CK) levels of wild-type $gsg^{+/+}$ (white), heterozygous $gsg^{+/-}$ (gray), and mutant $gsg^{-/-}$ (black) animals at 4 and 8 wk of age. At 4 wk, the mean serum CK was elevated $\sim 10\times$ that seen in wild-type mice, and at 8 wk the mean CK was elevated more than $40\times$ that seen in wild-type mice. The standard deviation (*) of serum CK values in 8-wk-old mice was $\pm 22,830$; 4/13 mice in this group had serum CK values of 17,750, 28,400, 33,400, and 80,500 U/liter (normal range 68–1070 U/liter). (*d*) The percentage of myofibers with centrally placed nuclei is greatly increased in $gsg^{-/-}$ mice.

peared healthy. $gsg^{+/-}$ mice were bred to obtain $gsg^{-/-}$ mice (Fig. 1 c). Immunoblot analysis showed no detectable γ -sarcoglycan protein in homozygous mutant $gsg^{-/-}$ muscle (Fig. 1 d), indicating that this mutation created a null allele. Notably, immunoblotting for dystrophin revealed that normal amounts of dystrophin remain in γ -sarcoglycan-deficient muscle (Fig. 1 d).

γ -Sarcoglycan-deficient Mice Develop Muscular Dystrophy

Most mutant $gsg^{-/-}$ mice were of normal size and appearance, but displayed a variably abnormal gait that was characterized by a stiff walking posture and a widened hind-leg spacing (Fig. 2 a). Less than 10% (4 of 50) of mutant $gsg^{-/-}$ mice had stunted growth or died as early as 3 wk of age. By 5 mo of age, 50% of $gsg^{-/-}$ animals died (Fig. 2 b). The average weight of mutant, heterozygous, and wild-type mice did not differ significantly at 8 wk of age. However, by 20 wk of age mutant mice weighed less than wild-type (28.3 ± 1.9 vs. 34.1 ± 0.3 g). Mutant mice were less active than wild-type littermates, exhibited less climbing and burrowing activity, and were slow to initiate walking or running from a sitting position. Elevation of serum CK, indicating disruption of muscle membrane integrity, was detected at 2 wk of age ($1.7\times$ wild-type controls). By 4 wk of age, serum CK levels were elevated in $gsg^{-/-}$ mice to an average of ten times that seen in normal mice (Fig. 2 c). This elevation continued in 8-wk-old $gsg^{-/-}$ mice and ranged from 3–240 \times the serum CK levels of normal controls (Fig. 2 c).

Diaphragm muscle from $gsg^{-/-}$ mice appeared hypertrophied and opaque when compared with $gsg^{+/+}$ and $gsg^{+/-}$ littermates. The thickness of $gsg^{-/-}$ diaphragm muscle was greater than that of wild-type controls without an increase in myofiber size or content (Fig. 3 a). Such pseudohypertrophy is a hallmark of progressive proximal muscular dystrophy, and commonly affects the gastrocnemius muscles of patients with LGMD and DMD (Dubowitz, 1995). Histologic analyses of muscle from $gsg^{-/-}$ mice revealed severe dystrophic changes, including wide variation in fiber size, inflammatory infiltrate, and abnormal calcification with fatty and fibrous replacement. Dystrophic changes were evident throughout the entire diaphragm muscle of $gsg^{-/-}$ mice (Fig. 3 a). Quadriceps and gastrocnemius muscle also showed pronounced dystrophic changes (Fig. 3, b and c). In these muscles, unlike the diaphragm, regions of marked degeneration were juxtaposed with segments of intact myofibers. Most of these intact myofibers, however, had centrally placed nuclei consistent with regeneration having occurred. The number of centrally placed nuclei in 8-wk-old $gsg^{-/-}$ quadriceps muscle was greatly increased (45 \times) above wild-type littermate controls (Figs. 2 d and 3 d).

Cardiomyopathy in $gsg^{-/-}$ Mice

The hearts of 20-wk-old $gsg^{-/-}$ mice were examined. Both the right and left ventricular walls appeared thickened, resulting in near obliteration of the ventricular cavities. $gsg^{-/-}$ hearts weighed less than wild-type hearts (167 ± 17 vs. 190 ± 0 mg, but total body mass was decreased in $gsg^{-/-}$ mice). The heart-to-body mass index was increased (5.91 ± 0.30 mg/g for $gsg^{-/-}$ vs. 5.57 ± 0.05 mg/g for wild-type animals).

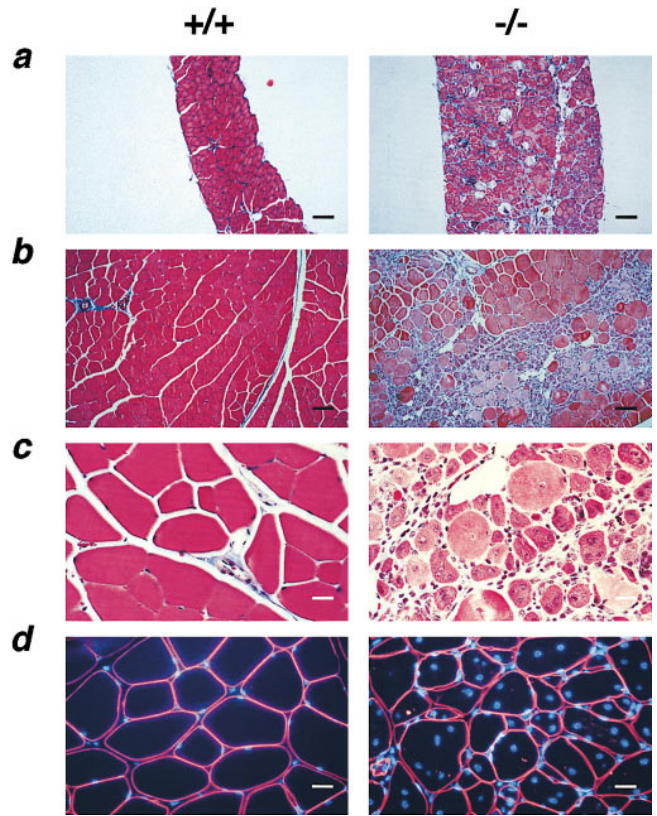


Figure 3. Histologic analysis of skeletal muscle lacking γ -sarcoglycan. *a–c* show Masson Trichrome staining of muscle. (*a*) Low-power view of diaphragm muscle from normal ($gsg^{+/+}$) and mutant ($gsg^{-/-}$) mice. Of note, the magnification is equal in the two photos. Diaphragm tissue from $gsg^{-/-}$ mice was hypertrophied. Marked replacement by connective and adipose tissue is seen throughout the diaphragm of $gsg^{-/-}$ muscle. Areas of calcification are visible. (*b*) Low-power magnification of $gsg^{-/-}$ quadriceps muscle (*right*) shows regions of inflammatory infiltrate adjacent to normal appearing muscle. (*a* and *b*) Bar, 100 μ m. (*c*) High-power view of quadriceps muscle showing an area of infiltrate and regeneration. (*d*) Immunostaining of muscle plasma membrane with an anti-dystrophin antibody counterstained with DAPI to reveal centrally placed nuclei seen in $gsg^{-/-}$ muscle. (*c* and *d*) Bar, 25 μ m.

Mice examined at earlier time points, including 4 and 8 wk of age, did not show the same degree of cardiomyopathy (data not shown). Fig. 4 a shows representative hearts from 20-wk-old $gsg^{-/-}$ animals that were killed for the study. Mutant hearts showed marked thickening of the ventricular walls; the heart in Fig. 4 a also showed significant fibrosis. Fibrosis was evident throughout the right and left ventricle, but a prominent fibrotic area was present in the posterior segment of the left ventricle (Fig. 4 c). Perivascular regions of fibrosis were also seen (Fig. 4 d). Fibrosis was not seen in $gsg^{-/-}$ hearts at 4 and 8 wk of age (data not shown). Of a cohort of ten $gsg^{-/-}$ mice, five died before 5 mo of age. Postmortem analysis of these $gsg^{-/-}$ mice revealed areas of ventricular fibrosis that were visible on gross examination (data not shown).

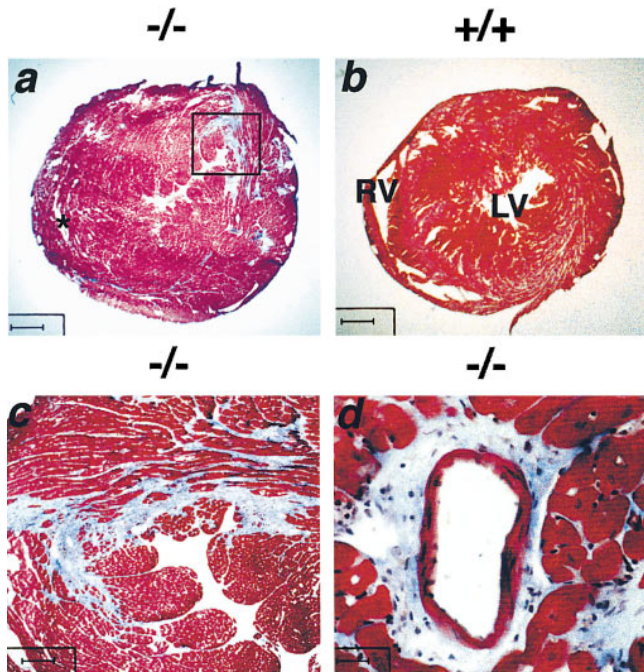


Figure 4. Cardiomyopathy in *gsg*^{-/-} mice. (a and b) Masson trichrome-stained hearts taken from 20-wk-old littermates. Bar, 625 μ m. LV, left ventricle; RV, right ventricle. (a) A representative *gsg*^{-/-} heart with significant increases in right and left ventricular wall thickness. (c) The region within the box of a. The *gsg*^{-/-} heart shown in a shows marked fibrosis, seen as blue staining. Wall thickness of the right ventricle is markedly increased (a); the right ventricular cavity is marked with an asterisk. (c) Bar, 100 μ m. (d) A higher magnification view of perivascular fibrosis is seen. (d) Bar, 25 μ m.

Sarcoglycan Loss Causes Muscular Dystrophy Without Alteration in Dystrophin

Immunostaining of γ -sarcoglycan-deficient and dystrophin-deficient skeletal muscle is shown in Fig. 5 a. *mdx* muscle showed a marked secondary deficiency of β -, γ -, and δ -sarcoglycan in addition to the primary defect of dystrophin loss. In *gsg*^{-/-} muscle, dystrophin staining is intact, but β - and δ -sarcoglycan show severe secondary reduction at the sarcolemma. In *mdx* muscle, revertant fibers are commonly seen that reexpress dystrophin. Dystrophin-positive revertant fibers in *mdx* also reexpress sarcoglycan as shown for δ -sarcoglycan (Fig. 5 a, bottom middle). No such revertant phenomenon is seen in *gsg*^{-/-} muscle. Partial retention of α -sarcoglycan at the sarcolemma was seen (Fig. 5 b, top). Immunoblotting for α -sarcoglycan shows that 10–30% of α -sarcoglycan is expressed in *gsg*^{-/-} muscle (Fig. 5 c). Normal levels of ϵ -sarcoglycan are present in *gsg*^{-/-} muscle, yet ϵ -sarcoglycan is completely absent in *mdx* muscle. β -dystroglycan and laminin- α 2 (merosin) staining was intact at the sarcolemma of *gsg*^{-/-} muscle. In muscle lacking dystrophin, the dystrophin-related protein utrophin is upregulated to compensate for the lack of dystrophin protein (Helliwell et al., 1992; Karpati et al., 1993; Love et al., 1989). Utrophin staining in *gsg*^{-/-} muscle is

unchanged from wild-type controls, consistent with a normal dystrophin content in γ -sarcoglycan-deficient animals. Integrin- β 1 was also unchanged in *gsg*^{-/-} muscle.

Membrane Defects in Muscular Dystrophy

Evans blue dye (EBD) is a small molecular mass tracer that tightly complexes with serum albumin. Normal muscle fibers are impermeable to the EBD-albumin complex, while *mdx* muscle fibers are positively stained, reflecting a loss of membrane integrity (Matsuda et al., 1995; Straub et al., 1997). EBD was injected into mutant and normal mice to determine the extent sarcolemmal disruption as a result of sarcoglycan deficiency. No EBD staining was seen in *gsg*^{+/+} and *gsg*^{+/-} animals at any age (data not shown). In contrast, 2-wk-old *gsg*^{-/-} mice showed EBD staining of the diaphragm, shoulder girdle, and pectoralis major muscles on gross examination (data not shown). By 4 wk of age, *gsg*^{-/-} animals displayed significant staining in nearly all muscle groups examined. Bands of EBD-positive tissue gave the *gsg*^{-/-} diaphragm a striking, radially streaked appearance not seen in wild-type littermate controls (Fig. 6 a). The adductor muscles of the leg were commonly EBD-positive, as were the pectoralis major, latissimus dorsi, rectus abdominus, external oblique, and triceps brachii muscles. Mutant muscles lacking macroscopic evidence of EBD uptake showed microscopic evidence of EBD-positive fiber clusters demonstrating the extensive nature of the dystrophic process in *gsg*^{-/-} mice (Fig. 6 b).

Myofiber Loss is Mediated by Apoptosis

To investigate the mechanism of myofiber degeneration in γ -sarcoglycan deficiency, TUNEL was performed on quadriceps muscle from 8-wk-old *gsg*^{+/+}, *gsg*^{+/-}, and *gsg*^{-/-} mice. No TUNEL-positive myonuclei were detected in *gsg*^{+/+} and *gsg*^{+/-} skeletal muscle (Fig. 6 c, left). In contrast, TUNEL-positive nuclei were commonly found in *gsg*^{-/-} muscle (Fig. 6 c, right), indicating that apoptosis contributed to muscle degeneration in γ -sarcoglycan-deficient muscular dystrophy. TUNEL-positive nuclei were most commonly seen in degenerating areas of *gsg*^{-/-} muscle, but were seen also in intact or partially degenerated muscle fibers (Fig. 6 d). The presence of TUNEL-positive myonuclei in intact, dystrophin-positive muscle fibers suggests that sarcoglycan deficiency leads to apoptosis as an early event in the dystrophic process.

Normal Sarcomeres, Neuromuscular Junctions, and Basement Membrane in γ -Sarcoglycan Deficiency

The sarcoglycans are found throughout the sarcolemma, and are not excluded at the neuromuscular junctions. The sarcomeres, membrane, and neuromuscular junctions of *gsg*^{-/-} mice were examined by EM and compared with wild-type littermates. No ultrastructural differences were seen in the sarcomeres, membrane, or neuromuscular junctions of *gsg*^{-/-} mice (Fig. 7), suggesting that membrane disruptions visualized by EBD staining are below the limit of resolution of EM. Gross ultrastructural abnormalities are not a feature of sarcoglycan deficiency.

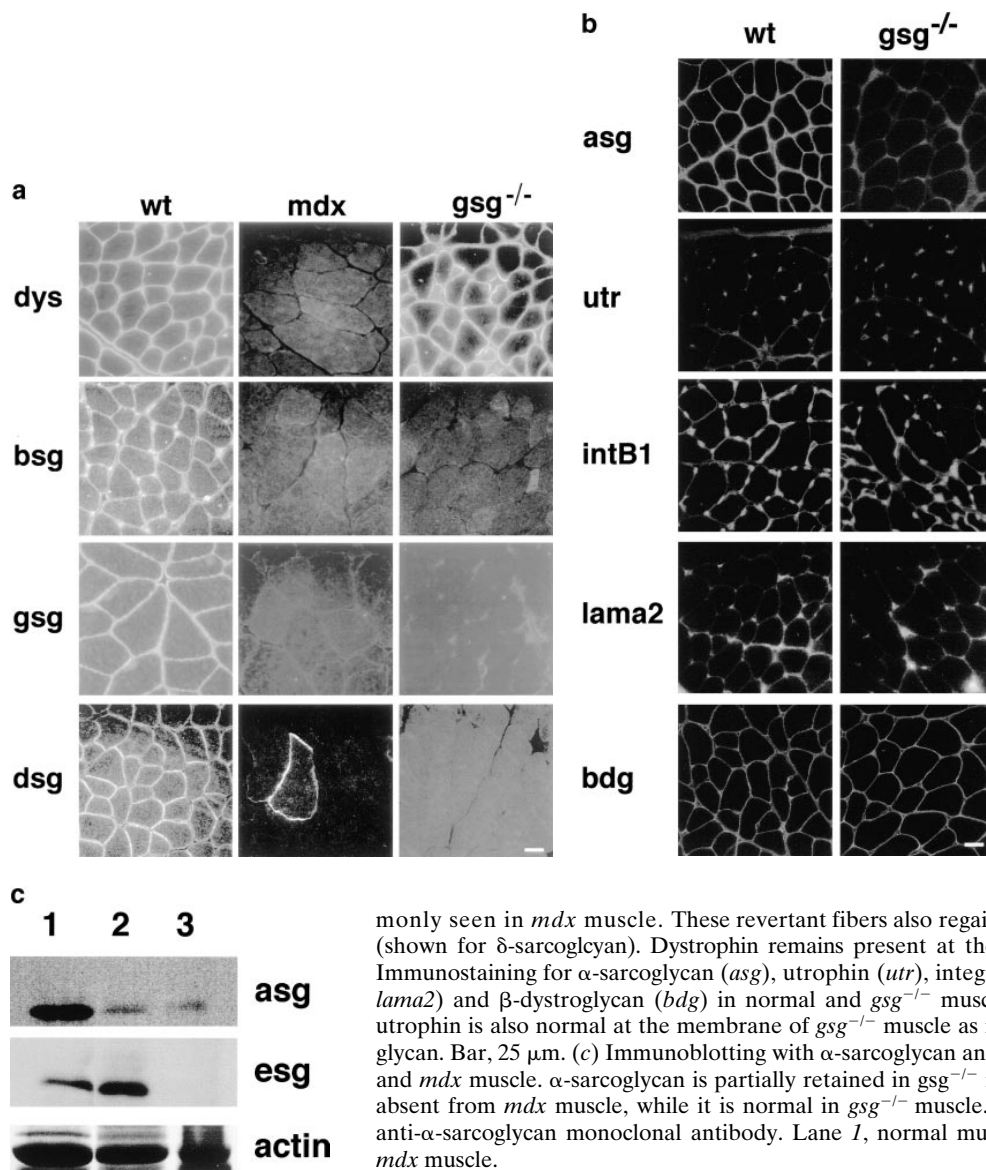


Figure 5. Immunostaining for components of the dystrophin-glycoprotein complex (DGC) in *mdx* and *gsg*^{-/-} muscle. (a) Immunostaining for dystrophin (*dys*), β -sarcoglycan (*bsg*), γ -sarcoglycan (*gsg*), and δ -sarcoglycan (*dsg*). β -, γ , and δ -sarcoglycan are absent in both dystrophin-deficient muscle (*mdx*) and γ -sarcoglycan deficient muscle (*gsg*^{-/-}). Revertant fibers that regain the ability to express dystrophin are com-

monly seen in *mdx* muscle. These revertant fibers also regain the ability to express sarcoglycan (shown for δ -sarcoglycan). Dystrophin remains present at the membrane in *gsg*^{-/-} muscle. (b) Immunostaining for α -sarcoglycan (*asg*), utrophin (*utr*), integrin- β 1 (*intB1*), laminin- α 2 (*merosin*, *lama2*) and β -dystroglycan (*bdg*) in normal and *gsg*^{-/-} muscle. The dystrophin-related protein utrophin is also normal at the membrane of *gsg*^{-/-} muscle as is laminin- α 2 (*merosin*) and dystroglycan. Bar, 25 μ m. (c) Immunoblotting with α -sarcoglycan and ϵ -sarcoglycan antibodies in *gsg*^{-/-} and *mdx* muscle. α -sarcoglycan is partially retained in *gsg*^{-/-} muscle. ϵ -sarcoglycan is completely absent from *mdx* muscle, while it is normal in *gsg*^{-/-} muscle. (Lanes 1-3) Immunoblotting with anti- α -sarcoglycan monoclonal antibody. Lane 1, normal muscle; lane 2, *gsg*^{-/-} muscle; lane 3, *mdx* muscle.

Discussion

γ -Sarcoglycan is Critical for Cardiac and Skeletal Muscle

We have developed mice lacking γ -sarcoglycan through homologous recombination in embryonic stem cells. Unlike *mdx* mice that display a more mild phenotype than DMD patients, mice lacking γ -sarcoglycan develop a phenotype that closely parallels the human disease exhibiting the clinical and histopathologic features of LGMD, including pseudohypertrophy, elevated serum CK, degeneration, and regeneration of skeletal muscle. Furthermore, *gsg*^{-/-} mice develop marked cardiomyopathy that is likely to play a role in their early death. The degree of left ventricular wall thickening is consistent with an intrinsic cardiomyopathy that is likely hypertrophic in nature. The presence of

marked fibrosis in the posterior segment of the left ventricle likely arises from similar pathogenetic mechanisms that affect skeletal muscle in sarcoglycan deficiency. It is possible that wall thickening seen in the right ventricle of *gsg*^{-/-} hearts arises in part as a secondary consequence of restrictive lung disease and elevated pulmonary vascular resistance. The marked dystrophic process that affects the respiratory muscles, particularly the diaphragm, may lead to a restrictive lung disease and hence, a secondary cardiomyopathy.

The phenotype in mice lacking γ -sarcoglycan is similar to that seen in the Syrian hamster, BIO 14.6, that displays both cardiomyopathy and muscular dystrophy (Homburger et al., 1962). The BIO 14.6 hamster is a spontaneously arising mutant that has a large, not fully defined deletion that encompasses part of an alternative first exon of the

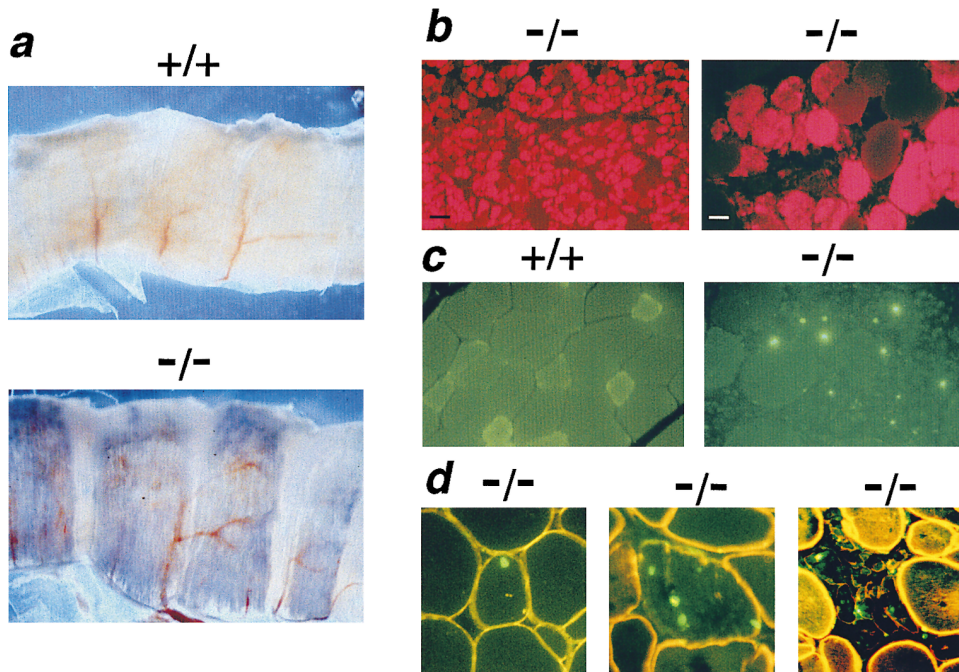


Figure 6. Vital staining with Evans blue dye (EBD) reveals disruption of membrane integrity in sarcoglycan-deficient muscle. Mice were injected with EBD and killed after 12 h. (a) Low-power magnification (5 \times) of diaphragm muscle from EBD-injected wild-type and mutant mice. Diaphragm muscle from $gsg^{-/-}$ mice exhibits segmental EBD uptake (seen as blue areas) while no EBD uptake is seen in wild-type diaphragm. (b) Regions of EBD uptake are seen as red cytoplasmic staining on fluorescence microscopy. No evidence of EBD microscopic staining is seen in $gsg^{+/+}$ muscle (data not shown). (Left) A region of $gsg^{-/-}$ triceps muscle with marked EBD staining (black bar, 100 μ m). (Right) A higher power view of $gsg^{-/-}$ gastrocnemius muscle showing EBD staining (white bar, 25 μ m). (c) Apop-

tosis in γ -sarcoglycan-deficient muscle. TUNEL-labeling was performed on quadriceps muscle from a $gsg^{-/-}$ mouse. TUNEL-positive nuclei appear green. No TUNEL-positive nuclei were found in normal $gsg^{+/+}$ myofibers (left). In contrast, myofibers with TUNEL-positive nuclei were seen commonly in $gsg^{-/-}$ muscle (right). (d) Counterstaining with an anti-dystrophin antibody (yellow-orange) demonstrates that TUNEL-positive nuclei are found within $gsg^{-/-}$ myofibers. Myofibers undergoing degeneration and demonstrating decreased membrane staining for dystrophin also show evidence of apoptosis.

hamster δ -sarcoglycan gene (Nigro et al., 1997; Sakamoto et al., 1997). Recently, membrane defects as observed by EBD staining were described in the BIO 14.6 hamster (Holt et al., 1998). The similar phenotype in $gsg^{-/-}$ mice and the BIO 14.6 hamster argues that both γ - and δ -sarcoglycan have critical and nonredundant roles for cardiac and skeletal muscle. The cardiomyopathic phenotype of $gsg^{-/-}$ mice is strikingly different from that seen in mdx mice since mdx mice display little to no cardiomyopathy. This result further suggests that the sarcoglycans, particularly γ - and δ -sarcoglycan, are critical for cardiac muscle function.

$gsg^{-/-}$ mice display a more severe skeletal muscle phenotype than mdx mice since $gsg^{-/-}$ mice show a greater serum CK elevation, early and more extensive fibrosis, and a higher percentage of centrally placed nuclei (McArdle et al., 1994; Deconinck et al., 1997). mdx mice show a period of intense muscle necrosis at 3–8 wk of age followed by a relative plateau period. $gsg^{-/-}$ mice show marked necrosis at 3–8 wk of age. Areas of necrosis were commonly seen in $gsg^{-/-}$ muscle from older animals, but regions of necrosis appeared less often than in 8-wk animals. Serum CK in $gsg^{-/-}$ animals peaks at 8 wk of age, but remain grossly elevated at 16 wk of age at levels twice that seen in mdx (data not shown).

$gsg^{-/-}$ mice die at a similar age as the recently described double knock-out mice that lack both dystrophin and utrophin (Deconinck et al., 1997; Grady et al., 1997). However, $gsg^{-/-}$ mice do not show gross skeletal abnormalities such as kyphoscoliosis described in the double knockout mice.

Interactions Within the DGC

The absence of γ -sarcoglycan produces a nearly complete secondary reduction of both β - and δ -sarcoglycan. β - and δ -sarcoglycan mRNA was normally produced in $gsg^{-/-}$ myofibers (data not shown). Thus, γ -sarcoglycan is necessary for the proper assembly and membrane localization of β - and δ -sarcoglycan proteins. Curiously, α -sarcoglycan appears to be partially retained in $gsg^{-/-}$ muscle, suggesting that other components can stabilize α -sarcoglycan at the membrane. ϵ -sarcoglycan is completely absent in mdx muscle, while it is produced at normal levels in γ -sarcoglycan deficiency. Since ϵ -sarcoglycan expression mirrors dystrophin expression in mdx and $gsg^{-/-}$ muscle, ϵ -sarcoglycan may be more closely associated with dystrophin.

Most importantly, the normal placement of dystrophin, β -dystroglycan, and laminin- α 2 (merosin) in γ -sarcoglycan-deficient muscle indicates that this cytoskeleton-membrane-ECM interaction is intact and unaffected by γ -sarcoglycan deficiency. The normal ultrastructural appearance of $gsg^{-/-}$ sarcomeres, basement membrane, and neuromuscular junctions may favor alternative cellular defects such as aberrant cell signaling as a mediator of muscular dystrophy. Sarcoglycan has the primary structure suggestive of a cell surface receptor. The cytoplasmic domain of γ -sarcoglycan has five tyrosine residues, and recent studies imply bidirectional signaling with integrins may involve the sarcoglycan subunits (Yoshida et al., 1998). Identifying the extracellular and intracellular ligands that normally bind the subunits of sarcoglycan will be an im-

+/+

-/-

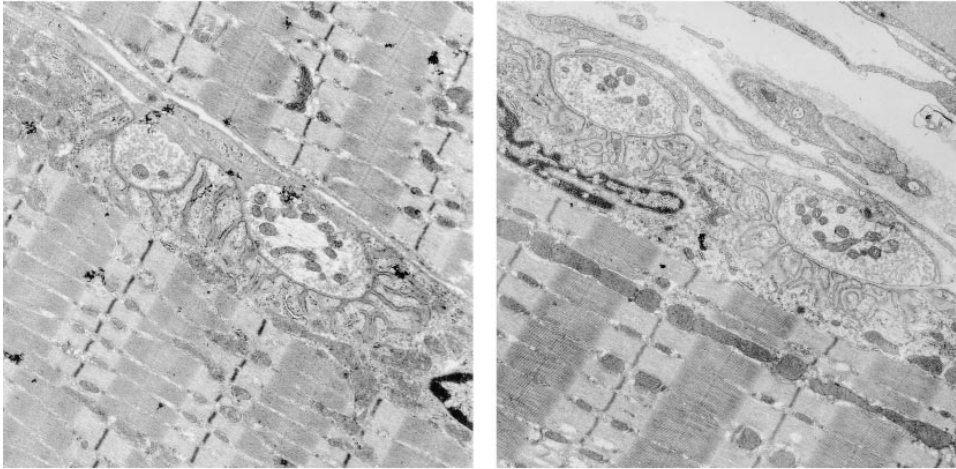


Figure 7. Ultrastructural analysis of $gsg^{-/-}$ and normal muscle. (5,000 \times) Neuromuscular junction, sarcomeres, and membrane from $gsg^{-/-}$ and wild-type muscle ($gsg^{+/+}$) shows that neuromuscular junctions form normally with normal post synaptic folds. The sarcolemma is intact throughout its length, and the basement membrane is continuous and apparently normal at this level of ultrastructure. Sarcomere formation is normal in $gsg^{-/-}$ muscle. Bar, 200 nm.

portant step in defining any potential cytoskeleton-sarcoglycan-ECM complex or signaling cascade.

Sarcoglycan Deficiency Mediates Pathology in DMD

In DMD and *mdx*, primary dystrophin mutations lead to a secondary reduction or mislocalization of a number of dystrophin-associated proteins, including the sarcoglycan subunits (Ohlendieck and Campbell, 1992; Ohlendieck et al., 1993). In mice lacking γ -sarcoglycan, the normal content and location of dystrophin suggests that sarcoglycan deficiency is sufficient to cause muscular dystrophy. It is clear that in $gsg^{-/-}$ muscle, the presence of dystrophin is insufficient to stabilize sarcoglycan, and cannot prevent the dystrophic process in both cardiac and skeletal muscle. Given the macromolecular nature of the DGC, the absence of sarcoglycan may lead to abnormal interactions within the DGC and the presence of muscular dystrophy. Whether restoration of the sarcoglycan complex, specifically β -, γ -, and δ -sarcoglycan will be sufficient to prevent the dystrophic phenotype is unknown. Previous data suggested that restoration of the DGC is not sufficient to prevent the dystrophic phenotype (Cox et al., 1994; Greenberg et al., 1994). However, DGC analysis in those studies was before the discovery of β -, γ -, and δ -sarcoglycan. Since sarcoglycan deficiency is the common feature in both DMD and LGMD, it is a likely mediator of the dystrophic process in both these disorders. Moreover, inhibiting apoptosis or developing new strategies to stabilize the sarcoglycan subunits, particularly β -, γ -, and δ -sarcoglycan, are reasonable therapeutic approaches to the treatment of Duchenne and the Duchenne-like muscular dystrophies.

We thank Celeste Simon, Renée Hackenmiller, and Jeff Leiden for advice and comments, and Lisa Gottschalk for her help preparing figures.

A.A. Hack is supported by the Medical Scientist Training Program and the Training Program in Development Biology. E.M. McNally is supported by the National Institutes of Health, Muscular Dystrophy Association, Heart Research Foundation, and a Research Resources award from the Howard Hughes Medical Institute to the University of Chicago Biological Sciences Division.

Received for publication 20 May 1998 and in revised form 31 July 1998.

References

- Bönnemann, C.G., E.M. McNally, and L.M. Kunkel. 1996. Beyond dystrophin: current progress in the muscular dystrophies. *Curr. Opin. Pediatrics*. 8:569-582.
- Bönnemann, C.G., M.R. Passos-Bueno, E.M. McNally, M. Vainzof, E. de Sa Moreira, S.K. Marie, R.C. Pavanello, S. Noguchi, E. Ozawa, M. Zatz, and L.M. Kunkel. 1996. Genomic screening for beta-sarcoglycan gene mutations: missense mutations may cause severe limb-girdle muscular dystrophy type 2E (LGMD 2E). *Am. J. Hum. Genet.* 59:1040-1047.
- Burghes, A.H., C. Logan, X. Hu, B. Belfall, R.G. Worton, and P.N. Ray. 1987. A cDNA clone from the Duchenne/Becker muscular dystrophy gene. *Nature*. 328:434-437.
- Campbell, K.P. 1995. Three muscular dystrophies: loss of cytoskeleton-extracellular matrix linkage. *Cell*. 80:675-679.
- Campbell, K.P., and S.D. Kahl. 1989. Association of dystrophin and an integral membrane glycoprotein. *Nature*. 338:259-262.
- Cox, G.A., Y. Sunada, K.P. Campbell, and J.S. Chamberlain. 1994. Dp71 can restore the dystrophin-associated glycoprotein complex in muscle but fails to prevent dystrophy. *Nat. Genet.* 8:333-339.
- Deconinck, A.E., J.A. Rafael, J.A. Skinner, S.C. Brown, A.C. Potter, L. Metzinger, D.J. Watt, J.G. Dickson, J.M. Tinsley, and K.E. Davies. 1997. Utrophin-dystrophin-deficient mice as a model for Duchenne muscular dystrophy. *Cell*. 90:717-727.
- Dubowitz, V. 1995. *Muscle Disorders in Childhood*. First edition. W.B. Saunders Co., Ltd., London. 19-50.
- Duggan, D.J., J.R. Gorospe, M. Fanin, E.P. Hoffman, and C. Angelini. 1997. Mutations in the sarcoglycan genes in patients with myopathy. *N. Engl. J. Med.* 336:618-624.
- Ervasti, J.M., and K.P. Campbell. 1991. Membrane organization of the dystrophin-glycoprotein complex. *Cell*. 66:1121-1131.
- Ervasti, J.M., and K.P. Campbell. 1993. A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. *J. Cell Biol.* 122:809-823.
- Ettinger, A.J., G. Feng, and J.R. Sanes. 1997. Epsilon-sarcoglycan, a broadly expressed homologue of the gene mutated in limb-girdle muscular dystrophy 2D. *J. Biol. Chem.* 272:32534-32538.
- Grady, R.M., H. Teng, M.C. Nichol, J.C. Cunningham, R.S. Wilkinson, and J.R. Sanes. 1997. Skeletal and cardiac myopathies in mice lacking utrophin and dystrophin: a model for Duchenne muscular dystrophy. *Cell*. 90:729-738.
- Greenberg, D.S., Y. Sunada, K.P. Campbell, D. Yaffe, and U. Nudel. 1994. Exogenous Dp71 restores the levels of dystrophin associated proteins but does not alleviate muscle damage in *mdx* mice. *Nat. Genet.* 8:340-344.
- Helbling-Leclerc, A., X. Zhang, H. Topaloglu, C. Cruaud, F. Tesson, J. Weissenbach, F.M. Tomé, K. Schwartz, M. Fardeau, K. Tryggvason, and P. Guicheney. 1995. Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. *Nat. Genet.* 11:216-218.
- Helliwell, T.R., N.T. Man, G.E. Morris, and K.E. Davies. 1992. The dystrophin-related protein, utrophin, is expressed on the sarcolemma of regenerating human skeletal muscle fibres in dystrophies and inflammatory myopathies. *Neuromusc. Dis.* 2:177-184.
- Holt, K.H., L.E. Lim, V. Straub, D.P. Venzke, F. Duclos, R.D. Anderson, B.L.

- Davidson, and K.P. Campbell. 1998. Functional rescue of the sarcoglycan complex in the BIO 14.6 hamster using δ -sarcoglycan gene transfer. *Mol. Cell.* 1:841–848.
- Homburger, F., J.R. Baker, C.W. Nixon, and R. Whitney. 1962. Primary, generalized polymyopathy and cardiac necrosis in an inbred line of Syrian hamsters. *Med. Exp.* 6:339–345.
- Ibraghimov-Beskrovnaya, O., J.M. Ervasti, C.J. Leveille, C.A. Slaughter, S.W. Sernett, and K.P. Campbell. 1992. Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. *Nature.* 355:696–702.
- Karpati, G., S. Carpenter, G.E. Morris, K.E. Davies, C. Guerin, and P. Holland. 1993. Localization and quantitation of the chromosome 6-encoded dystrophin-related protein in normal and pathological human muscle. *J. Neuro-path. Exp. Neurol.* 52:119–128.
- Koenig, M., E.P. Hoffman, C.J. Bertelson, A.P. Monaco, C. Feener, and L.M. Kunkel. 1987. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. *Cell.* 50:509–517.
- Lidov, H.G., T.J. Byers, S.C. Watkins, and L.M. Kunkel. 1990. Localization of dystrophin to postsynaptic regions of central nervous system cortical neurons. *Nature.* 348:725–728.
- Lim, L.E., F. Duclos, O. Broux, N. Bourg, Y. Sunada, V. Allamand, J. Meyer, I. Richard, C. Moomaw, C. Slaughter, et al. 1995. Beta-sarcoglycan: characterization and role in limb-girdle muscular dystrophy linked to 4q12. *Nat. Genet.* 11:257–265.
- Love, D.R., D.F. Hill, G. Dickson, N.K. Spurr, B.C. Byth, R.F. Marsden, F.S. Walsh, Y.H. Edwards, and K.E. Davies. 1989. An autosomal transcript in skeletal muscle with homology to dystrophin. *Nature.* 339:55–58.
- Matsuda, R., A. Nishikawa, and H. Tanaka. 1995. Visualization of dystrophic muscle fibers in mdx mouse by vital staining with Evans blue: evidence of apoptosis in dystrophin-deficient muscle. *J. Biochem.* 118:959–964.
- McArdle, A., R.H.T. Edwards, and M.J. Jackson. 1994. Time course of changes in plasma membrane permeability in the dystrophin-deficient mdx mouse. *Muscle Nerve.* 17:1378–1384.
- McNally, E.M., D. Duggan, J.R. Gorospe, C.G. Bönnemann, M. Fanin, E. Pegoraro, H.G. Lidov, S. Noguchi, E. Ozawa, R.S. Finkel, et al. 1996. Mutations that disrupt the carboxyl-terminus of gamma-sarcoglycan cause muscular dystrophy. *Hum. Mol. Genet.* 5:1841–1847.
- McNally, E.M., C.T. Ly, and L.M. Kunkel. 1998. Human epsilon sarcoglycan is highly related to alpha-sarcoglycan (adhalin), the limb girdle muscular dystrophy type 2D gene. *FEBS Lett.* 422:27–32.
- Monaco, A.P., R.L. Neve, F.C. Colletti, C.J. Bertelson, D.M. Kurnit, and L.M. Kunkel. 1986. Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene. *Nature.* 323:646–650.
- Nicholson, L.V., K. Davison, G. Falkous, C. Harwood, E. O'Donnell, C.R. Slater, and J.B. Harris. 1989. Dystrophin in skeletal muscle. I. Western blot analysis using a monoclonal antibody. *J. Neurol. Sci.* 94:125–136.
- Nigro, V., G. Piluso, A. Belsito, L. Politano, A.A. Puca, S. Papparella, E. Rossi, G. Viglietto, M.G. Esposito, C. Abbondanza, et al. 1996. Identification of a novel sarcoglycan gene at 5q33 encoding a sarcolemmal 35 kDa glycoprotein. *Hum. Mol. Genet.* 5:1179–1186.
- Nigro, V., Y. Okazaki, A. Belsito, G. Piluso, Y. Matsuda, L. Politano, G. Nigro, C. Ventura, C. Abbondanza, A.M. Molinari, et al. 1997. Identification of the Syrian hamster cardiomyopathy gene. *Hum. Mol. Genet.* 6:601–607.
- Nissinen, M., A. Helbling-Leclerc, X. Zhang, T. Evangelista, H. Topaloglu, C. Cruaud, J. Weissenbach, M. Fardeau, F.M. Tomé, et al. 1996. Substitution of a conserved cysteine-996 in a cysteine-rich motif of the laminin alpha2-chain in congenital muscular dystrophy with partial deficiency of the protein. *Am. J. Hum. Genet.* 58:1177–1184.
- Noguchi, S., E.M. McNally, K. Ben Othmane, Y. Hagiwara, Y. Mizuno, M. Yoshida, H. Yamamoto, C.G. Bönnemann, E. Gussoni, et al. 1995. Mutations in the dystrophin-associated protein gamma-sarcoglycan in chromo-some 13 muscular dystrophy. *Science.* 270:819–822.
- Ohlendieck, K., and K.P. Campbell. 1992. Dystrophin-associated proteins are greatly reduced in skeletal muscle from mdx mice. *J. Cell Biol.* 115:1685–1694.
- Ohlendieck, K., K. Matsumura, V.V. Ionasescu, J.A. Towbin, E.P. Bosch, S.L. Weinstein, S.W. Sernett, and K.P. Campbell. 1993. Duchenne muscular dystrophy: deficiency of dystrophin-associated proteins in the sarcolemma. *Neurology.* 43:795–800.
- Ozawa, E., M. Yoshida, A. Suzuki, Y. Mizuno, Y. Hagiwara, and S. Noguchi. 1995. Dystrophin-associated proteins in muscular dystrophy. *Hum. Mol. Genet.* 4:1711–1716.
- Roberds, S.L., R.D. Anderson, O. Ibraghimov-Beskrovnaya, and K.P. Campbell. 1993. Primary structure and muscle-specific expression of the 50-kDa dystrophin-associated glycoprotein (adhalin). *J. Biol. Chem.* 268:23739–23742.
- Rybakova, I.N., K.J. Amann, and J.M. Ervasti. 1996. A new model for the interaction of dystrophin with F-actin. *J. Cell Biol.* 135:661–672.
- Sakamoto, A., K. Ono, M. Abe, G. Jasmin, T. Eki, Y. Murakami, T. Masaki, T. Toyooka, and F. Hanaoka. 1997. Both hypertrophic and dilated cardiomyopathies are caused by mutation of the same gene, delta-sarcoglycan, in hamster: an animal model of disrupted dystrophin-associated glycoprotein complex. *Proc. Natl. Acad. Sci. USA.* 94:13873–13878.
- Scott, E., M.C. Simon, J. Anastasi, and H. Singh. 1994. Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. *Science.* 265:1573–1577.
- Scinski, P., Y. Geng, C. Ryder, E. Barnard, M. Darlison, and P. Barnard. 1989. The molecular basis of muscular dystrophy in the mdx mouse: a point mutation. *Science.* 244:1578–1580.
- Straub, V., and K.P. Campbell. 1997. Muscular dystrophies and the dystrophin-glycoprotein complex. *Curr. Opin. Neurol.* 10:168–175.
- Straub, V., J.A. Rafael, J.S. Chamberlain, and K.P. Campbell. 1997. Animal models for muscular dystrophy show different patterns of sarcolemmal disruption. *J. Cell Biol.* 139:375–385.
- Sunada, Y., S.M. Bernier, C.A. Kozak, Y. Yamada, and K.P. Campbell. 1994. Deficiency of merosin in dystrophic dy mice and genetic linkage of laminin M chain gene to dy locus. *J. Biol. Chem.* 269:13729–13732.
- Tybulewicz, V., C. Crawford, P. Jackson, R. Bronson, and R. Mulligan. 1991. Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-abl proto-oncogene. *Cell.* 65:1153–1163.
- Vainzof, M., M.R. Passos-Bueno, M. Canovas, E.S. Moreira, R.C. Pavanello, S.K. Marie, L.V. Anderson, C.G. Bonnemann, E.M. McNally, V. Nigro, et al. 1996. The sarcoglycan complex in the six autosomal recessive limb-girdle muscular dystrophies. *Hum. Mol. Genet.* 5:1963–1969.
- Williamson, R.A., M.D. Henry, K.J. Daniels, R.F. Hrstka, J.C. Lee, Y. Sunada, O. Ibraghimov-Beskrovnaya, and K.P. Campbell. 1997. Dystroglycan is essential for early embryonic development: disruption of Reichert's membrane in Dag1-null mice. *Hum. Mol. Genet.* 6:831–841.
- Xu, H., P. Christmas, X.R. Wu, U.M. Wewer, and E. Engvall. 1994. Defective muscle basement membrane and lack of M-laminin in the dystrophic dy/dy mouse. *Proc. Natl. Acad. Sci. USA.* 91:5572–5576.
- Yamada, H., T. Shimizu, T. Tanaka, K.P. Campbell, and K. Matsumura. 1994. Dystroglycan is a binding protein of laminin and merosin in peripheral nerve. *FEBS Lett.* 352:49–53.
- Yoshida, M., and E. Ozawa. 1990. Glycoprotein complex anchoring dystrophin to sarcolemma. *J. Biochem.* 108:748–752.
- Yoshida, M., A. Suzuki, H. Yamamoto, S. Noguchi, Y. Mizuno, and E. Ozawa. 1994. Dissociation of the complex of dystrophin and its associated proteins into several unique groups by n-octyl beta-D-glucoside. *Eur. J. Biochem.* 222:1055–1061.
- Yoshida, T., Y. Pan, H. Hanada, Y. Iwata, and M. Shigekawa. 1998. Bidirectional Signaling between Sarcoglycans and the Integrin Adhesion System in Cultured L6 Myocytes. *J. Biol. Chem.* 273:1583–1590.