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Altered α1-syntrophin expression in myofibers with Duchenne and Fukuyama muscular dystrophies

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Summary. α1-Syntrophin, a scaffolding adapter and modular protein, is a cytoplasmic component of the dystrophin glycoprotein complex. This study investigated immunohistochemically the expression of α1-syntrophin in Duchenne and Fukuyama muscular dystrophies (DMD and FCMD, respectively). Biopsied muscles of five DMD, five FCMD, five normal controls and five disease controls (three myotonic and two facioscapulohumeral dystrophies) were analyzed. Immunoblot analysis showed that anti-α1-syntrophin antibody had a decreased reaction in both DMD and FCMD muscle extracts. Biopsied muscle sections and their serial sections were immunostained with rabbit anti-α1-syntrophin and rabbit anti-muscle-specific ß-spectrin antibodies, respectively. Immunoreactive patterns of sarcolemma were classified into (i) a continuously positive immunostaining pattern, (ii) a partially positive immunostaining pattern, (iii) a negative immunostaining pattern and (iv) a faint but entire surface positive immunostaining pattern. The group mean percentages of α1-syntrophin and ß-spectrin immunonegative myofibers in the DMD group were 39.3% and 10.8%, respectively, while those in the FCMD group were 45.5% and 10.4%, respectively. These values were statistically significant compared with those of disease control and normal control muscles. Thus we found that dystrophin-deficient DMD muscles contained significant numbers of α1-syntrophin-positive fibers and significant numbers of α1-syntrophin-negative fibers were present in dystrophin-positive muscles of severe muscular dystrophy such as FCMD. α-Dystrobrevin immunoreactivity was tested in DMD muscles and appreciable amounts of α-dystrobrevin that binds to syntrophin were found in DMD muscle membranes.

Key words: α1-Syntrophin, Immunohistochemistry, Immunoblot, Skeletal myofiber, Human muscular dystrophies

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Introduction

Duchenne muscular dystrophy (DMD) is the most common form of human muscular dystrophy and is caused by mutations in the megagene encoding a large sarcolemmal protein dystrophin (Hoffman et al., 1987; Koenig et al., 1988). Syntrophins are a family of scaffolding proteins of the dystrophin glycoprotein complex that contains extracellular α-dystroglycan, the transmembrane ß-dystroglycan, α-, ß,-, γ-, δ-sarcoglycan and sarcospan, and cytoplasmic syntrophins and dystrobrevins (Ozawa, 2004). Syntrophins α, ß1, ß2, γ1 and γ2 have been characterized so far (Ahn et al., 1996; Piluso et al., 2000). Each syntrophin isoform shares a similar domain organization and contains two tandem pleckstrin homology domains at the N terminus (Tyers et al., 1988; Chockalingam et al., 1999): a single post-synaptic density-95, Drosophila disc large protein and Zona occludens protein 1 (PDZ) protein-interaction domain (Fanning and Anderson, 1999) and a highly conserved C terminal syntrophin-unique domain (Ahn et al., 1996; Piluso et al., 2000; Ou et al., 2003). Syntrophins bind to two sites in the COOH-terminal region of dystrophin so that two syntrophins can attach to one dystrophin (Newey et al., 2000; Adams et al., 2001). α1-Syntrophin binds at its PDZ domain to various plasma membrane proteins, such as neuronal nitric oxide synthase (nNOS) (Brenman et al., 1996; Adams et al., 2000), SCN4A and SCN5A, Na^+-channel, (Gee et al., 1998; Schultz et al., 1998; Hogan et al., 2001) and kinases (Hasegawa et al., 1999; Lumeng et al., 1999). PDZ domain-disrupted α1-syntrophin transgenic mice lack membrane localization of aquaporin (AQP) 4 (Adams et al., 2001), which is absent at the sarcolemma and perivascular astrocyte endfeet in α1-syntrophin knockout mice (Yokota et al., 2000). PDZ domains of syntrophins ß1, ß2 interact with SCN4A, a skeletal muscle Na^+-channel, and SCN5A, a cardiac muscle and intestinal smooth muscle Na^+-channel, by the intermediary of the C terminal sequence motif (Gee et al., 1998; Schultz et al., 1998; Hogan et al., 2001); however, it is yet unknown if ß1 and ß2 syntrophins interact with the AQP4 molecule. α1-Syntrophin has binding sites for actin cytoskeleton and calmodulin

(Iwata et al., 1998). Syntrophins have been hypothesized to couple SCN5A with the actin cytoskeleton to provide a mechanism for mechanical regulation of voltage-dependent ion channel gating (Ou et al., 2003).

Dystrophin also binds to dystrobrevin, which is a protein having a sequence homology with dystrophin (Sadoulet-Puccio et al., 1996; Peters et al., 1998; Adams et al., 2001) and which can also bind two syntrophins (Newey et al., 2000). Dystrobrevins consist of different isoforms and are encoded by different genes (Blake et al., 1996). Mouse and human α-dystrobrevin (Blake et al., 1996; Sadoulet-Puccio et al., 1996) and ß-dystrobrevin (Peters et al., 1997b; Blake et al., 1998; Puca et al., 1998) have been cloned, and ß-dystrobrevin is expressed in a broader range of tissues than α-dystrobrevin (Enigk and Maimone, 1999). α-Dystrobrevin is the predominant isoform in skeletal muscle and alternative splicing of the dystrobrevin gene in skeletal and cardiac muscle generates α-dystrobrevin 1, 2 and 3 (Blake, 2002). α-Dystrobrevin 1 and 2 have two syntrophins and one dystrophin binding site (Sadoulet-Puccio et al., 1997; Newey et al., 2000). The dystrophin glycoprotein complex can bind up to four syntrophins, which provides a scaffold for multiple functionally interdependent signaling proteins (Adams et al., 2001). Expression of syntrophins and dystrobrevins in human dystrophic muscles has so far been underinvestigated.

Fukuyama type congenital muscular dystrophy (FCMD) is a severe muscular dystrophy widespread in Japan and is characterized by generalized and severe muscle atrophy having an extremely early onset, joint contracture at an early stage and mental retardation (Fukuyama et al., 1981). FCMD is caused by mutation of the fukutin gene (Kobayashi et al., 1998), but the molecular architecture of the skeletal muscle plasma membrane observed by using freeze fracture electron microscopy is similar to that of DMD (Schotland et al., 1981; Wakayama et al., 1984, 1985). The AQP4 expression in the muscle plasma membrane is markedly reduced in DMD and FCMD (Wakayama et al., 2002, 2003). The AQP4 molecule is associated with α1-syntrophin at its PDZ protein-interacting domain (Fanning and Anderson, 1999; Adams et al., 2001). Thus we analyzed α1-syntrophin expression in muscle plasma membranes of both DMD and FCMD muscles and compared this expression in these two muscular dystrophies. We also surveyed α-dystrobrevin expression in DMD myofibers.

Materials and methods

Muscle samples

All muscle samples were biopsied from quadriceps femoris muscles under local anesthesia. Muscle samples contained five DMD muscles, five FCMD muscles, three muscles with myotonic dystrophy and two muscles with facioscapulohumeral (FSH) muscular dystrophy. Boys aged 5 to 8 years affected with DMD had proximal muscle weakness, atrophy in various degrees, calf pseudohypertrophy and markedly high serum creatine kinase levels. The diagnosis of DMD was confirmed by examination of leukocyte genomic DNA and negative dystrophin immunostaining of biopsied muscle. Children (3 boys and 2 girls aged 8 months to 2 years) affected with FCMD had muscle weakness and hypotonia from infancy, delayed motor milestone, myopathic faces, mental retardation, high serum creatine kinase values and myopathic changes shown by electromyograms (Fukuyama et al., 1981). Myotonic dystrophy and FSH dystrophy were diagnosed by their characteristic clinical manifestations. Muscle samples of myotonic dystrophy and FSH dystrophy patients (4 males and 1 female aged 23 to 45 years) were the disease control. For normal control specimens, five histochemically normal biopsy specimens of quadriceps femoris muscles were collected from patients (1 male and 4 females aged 10 to 48 years) who were thought to have myopathy, but were free of neuromuscular disorders after histochemical and immunologic examinations. All muscle samples were taken under informed consent. The procedure of muscle biopsy was approved by the ethics committee of Showa University.

Immunoblot analysis

Immunoblot analyses of α1-syntrophin, ß-spectrin and myosin heavy chain in the histochemically normal quadriceps femoris muscles and those of children with DMD and FCMD were done by using previously described methods (Wakayama et al., 2003) with minor modifications. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was done for α1-syntrophin (Wakayama et al., 1997) and myosin heavy chain (Upstate Inc. NY, USA) on a 12.5% homogeneous gel and for ß-spectrin (Wakayama et al., 1995) on a 3-10% gradient gel. The protein was transferred from the gel to a clear blot P membrane sheet (ATTO, Tokyo, Japan) by using horizontal electrophoresis at 108mA for 90 min at room temperature. Immunostainings were done with rabbit anti-α1-syntrophin antibody diluted to 3μg IgG/ml, rabbit anti-ß-spectrin antiserum diluted to 1:200 and mouse monoclonal anti-myosin heavy chain antibody diluted to 1:3000.

Immunohistochemistry

Antibodies against α1-syntrophin and a skeletal muscle specific isoform of ß-spectrin were generated in our laboratory by commercial base (Wakayama et al., 1995, 1997). The muscle biopsy specimens were immediately frozen in isopentane cooled with liquid nitrogen. Frozen 6 μm-thick cross sections of the muscles were placed on cover slips and were incubated with primary rabbit anti-α1-syntrophin antibody diluted to 5 μg IgG/ml. Serial sections of muscles were incubated with primary rabbit anti-ß-spectrin antiserum

diluted to 1:200 and with primary monoclonal Dys 3 antibody (Novocastra Laboratories Ltd. Newcastle upon Tyne, U.K.) diluted to 1:30. Other serial muscle section was further histochemically stained with myosin ATPase at pH 4.6. Muscle sections from the five DMD boys were immunostained with mouse monoclonal anti-α-dystrobrevin antibody (BD Bioscience CA, USA) diluted to 1:50. Muscle sections from the five FCMD children were immunostained with mouse monoclonal anti-neonatal myosin antibody (Vector Laboratory Inc. CA, USA) diluted to 1:50. Indirect immunofluorescent staining was done as previously described (Wakayama et al., 1995).

Analysis of immunohistochemically stained muscles

Muscle specimens with immunofluorescent staining by anti-ß-spectrin and anti-α1-syntrophin antibodies were examined, and photographs were taken and printed at a final magnification of x 250. The immunostaining patterns of the surface membrane of myofibers were classified from these printed photographs into the following four patterns: group 1, continuously positive immunostaining pattern with more than 90% myofiber surface immunolabeling and normal immunoreactivity; group 2, partially positive immunostaining pattern with 10 to 90% myofiber surface immunolabeling and normal immunoreactivity; group 3, negative immunostaining pattern with less than 10% myofiber surface immunolabeling; group 4, faint but entire surface positive immunostaining pattern. The immunostaining patterns in the muscle samples stained both with anti-ß-spectrin and anti-α1-syntrophin antibodies were analyzed in each muscle by using about 200 myofibers in each case. The group mean percentage of each immunostaining pattern was calculated for DMD, FCMD, disease control and normal control groups. The difference in the group mean percentage in each immunostaining pattern in each muscle group was statistically compared by using the two-tailed t test. DMD muscle sections immunostained with anti-α-dystrobrevin antibody and FCMD muscle sections immunostained with anti-neonatal myosin antibody were evaluated qualitatively.

Results

Immunoblot analysis

Immunoblot analysis showed that anti-ß-spectrin antibody reacted with 270kDa protein extracts of normal muscles. The reactions of anti-ß-spectrin antibody both in DMD and FCMD muscle extracts slightly decreased compared with those of extracts in normal control muscles (Fig. 1A). Anti-α1-syntrophin antibody reacted with 59kDa protein extracts of normal muscles, but the reactions of anti-α1-syntrophin antibody decreased both in DMD and FCMD muscle extracts (Fig. 1B). The reactions for anti-myosin heavy chain antibody showed a fairly broad band and its center was about 220kDa in normal, DMD and FCMD muscles. The reactions for anti-myosin heavy chain antibody in DMD and FCMD

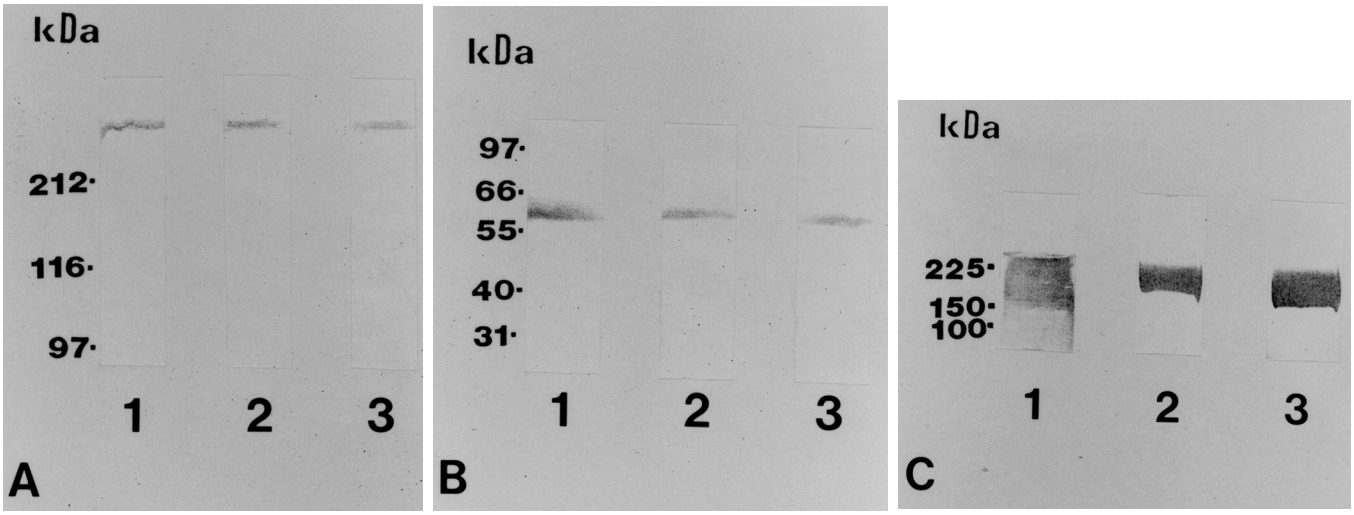


Fig. 1. Immunoblots of ß-spectrin **(A)**, α1-syntrophin **(B)** and myosin heavy chain **(C)** expressions in normal human quadriceps femoris muscles (lane 1 of A, B and C, respectively), in muscles of boys with Duchenne muscular dystrophy (DMD) (lane 2 of A, B and C, respectively) and in muscles of children with Fukuyama-type congenital muscular dystrophy (FCMD) (lane 3 of A, B and C, respectively). Electrophoresis and blotting were done as described in the Materials and Methods. In normal human muscle extracts, bands of ß-spectrin and α1-syntrophin were at 270kDa and 59kDa, respectively (A and B, respectively); the band of myosin heavy chain was rather broad and its center was about 220kDa **(C)**. The reaction for ß-spectrin in DMD and FCMD muscle extracts showed a slight decrease in comparison with normal control muscle extracts **(A)**; the reaction for α1-syntrophin both in DMD and FCMD muscle extracts moderately decreased compared with normal muscle extracts **(B)**. Reactions for myosin heavy chain in extracts of normal, DMD and FCMD muscles had almost the same intensity **(C)**. Numbers to the left of each figure are the molecular masses of standards.

muscle extracts were almost as intense as the reaction of normal muscle extract (Fig. 1C).

Immunohistochemistry and its analysis

The immunoreaction of normal skeletal myofibers stained with anti-ß-spectrin and anti-ß1-syntrophin antibodies was at the myofiber surface (Fig. 2A, B). All myofibers including both slow twitch type 1 fibers and fast twitch type 2A and 2B fibers (Fig. 2C), were similarly immunostained.

In DMD muscles, scattered myofibers were present with variously surface-immunostained myofibers stained with anti-ß-spectrin antibody. These myofibers included continuous surface immunolabeling myofibers, discontinuous surface immunolabeling myofibers and negative surface immunolabeling myofibers (Fig. 3A1, 2); but dystrophin immunostaining of these myofibers showed a negative immunoreaction (Fig. 3B1, 2). Immunostaining with anti-α1-syntrophin antibody in DMD myofibers showed similar immunostaining patterns to those with anti-ß-spectrin antibody, and the number of immunopositive myofibers with anti-α1-syntrophin antibody was large, irrespective of the absence of dystrophin (Fig. 3B1, B2, C1, C2). However the percentage of immunopositive surface labeling

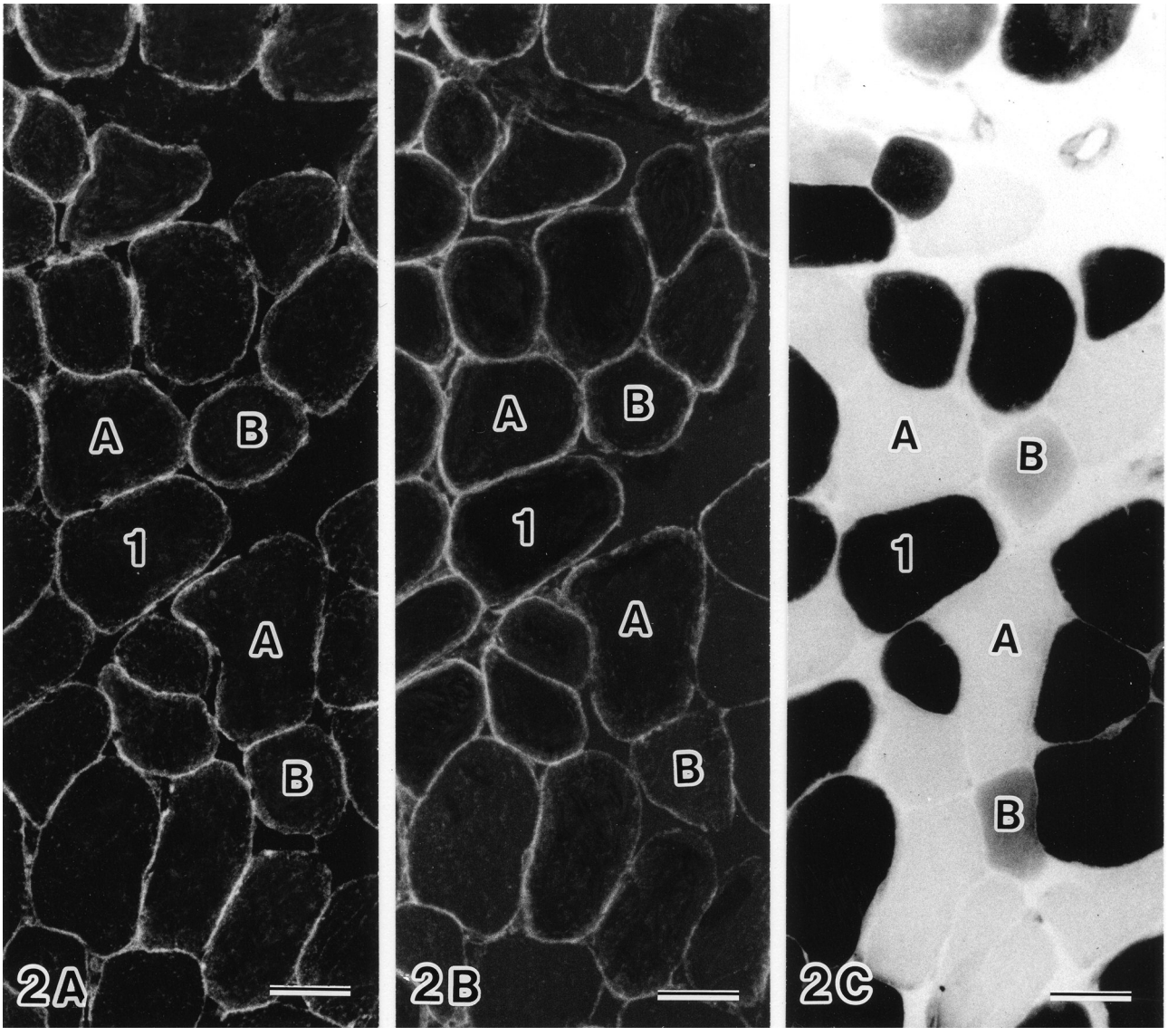


Fig. 2. Immunohistochemical staining of serial normal muscle sections with anti-ß-spectrin **(A)**, anti-α1-syntrophin **(B)** antibodies and myosin ATPase (pH 4.6) **(C)**. The cell surface of individual myofibers in normal muscle shows a thin layer of immunofluorescense with these antibodies. The muscle section with myosin ATPase staining **(C)** contains type 1 fiber (1), type 2A fiber **(A)** and type 2B fiber **(B)**. The immunostaining pattern has no fiber type selectivity. Scale bars: 50 µm. x 250

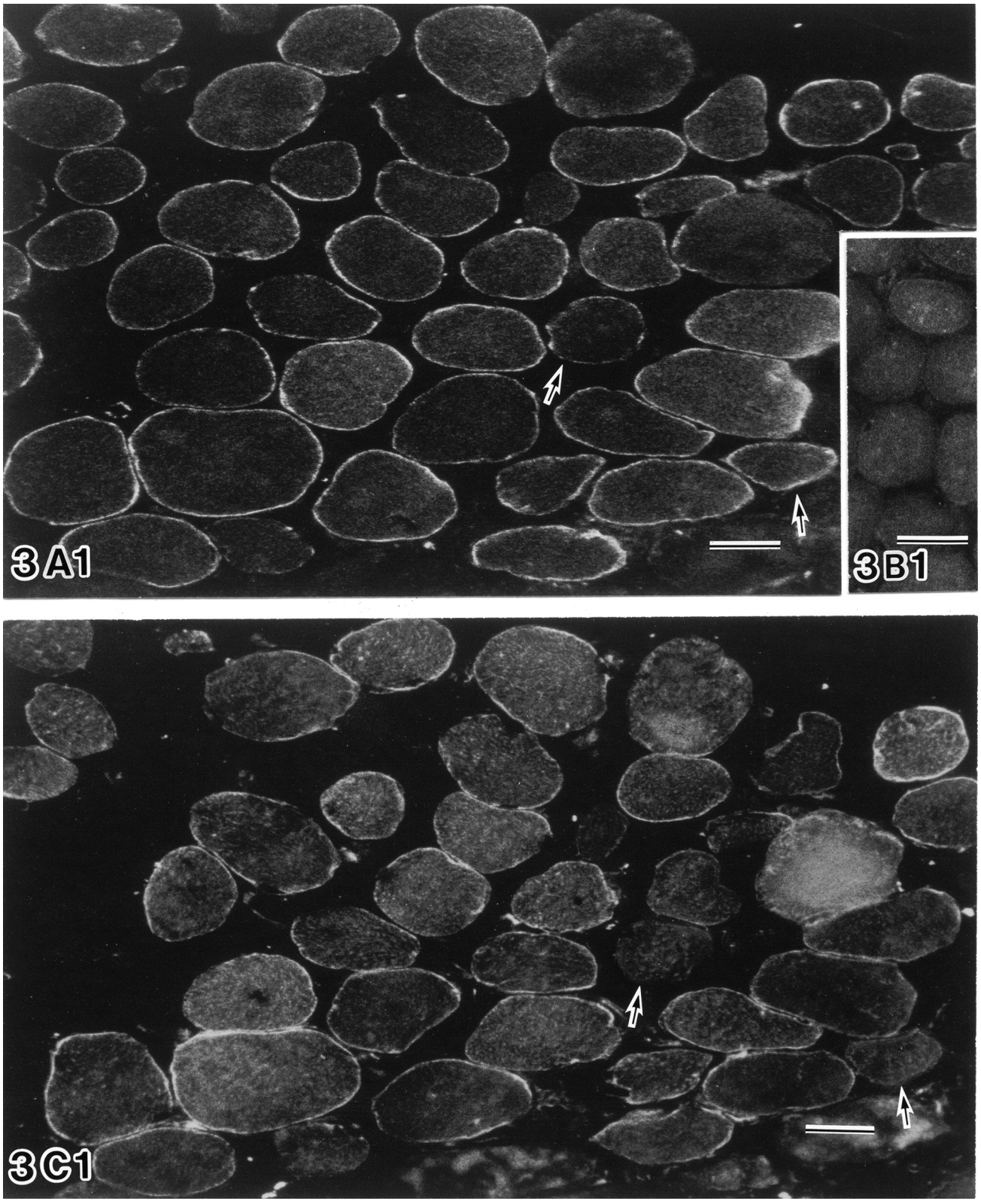


Fig. 3A1-C1. Immunohistochemical staining of serial sections of Duchenne muscular dystrophy (DMD) muscle with anti-ß-spectrin **(A1)**, anti-dystrophin **(B1)** and anti-α1-syntrophin **(C1)** antibodies. Immunoreactions for both ß-spectrin **(A1)** and α1-syntrophin **(C1)** at the surface of DMD myofibers show a positive immunoreaction at many DMD myofibers in various degrees and negative immunoreaction in scattered remaining myofibers; the immunoreactivity for dystrophin was negative **(B1)**. Generally, the number of myofibers with negative immunoreactivity for α1-syntrophin was more than for ß-spectrin. Positively immunostained myofibers with anti-ß-spectrin antibody (**A1**, arrows) showed negative immunoreaction for anti-α1-syntrophin antibody in the serial muscle section (**C1**, arrows). Scale bars: 50 µm. x 250

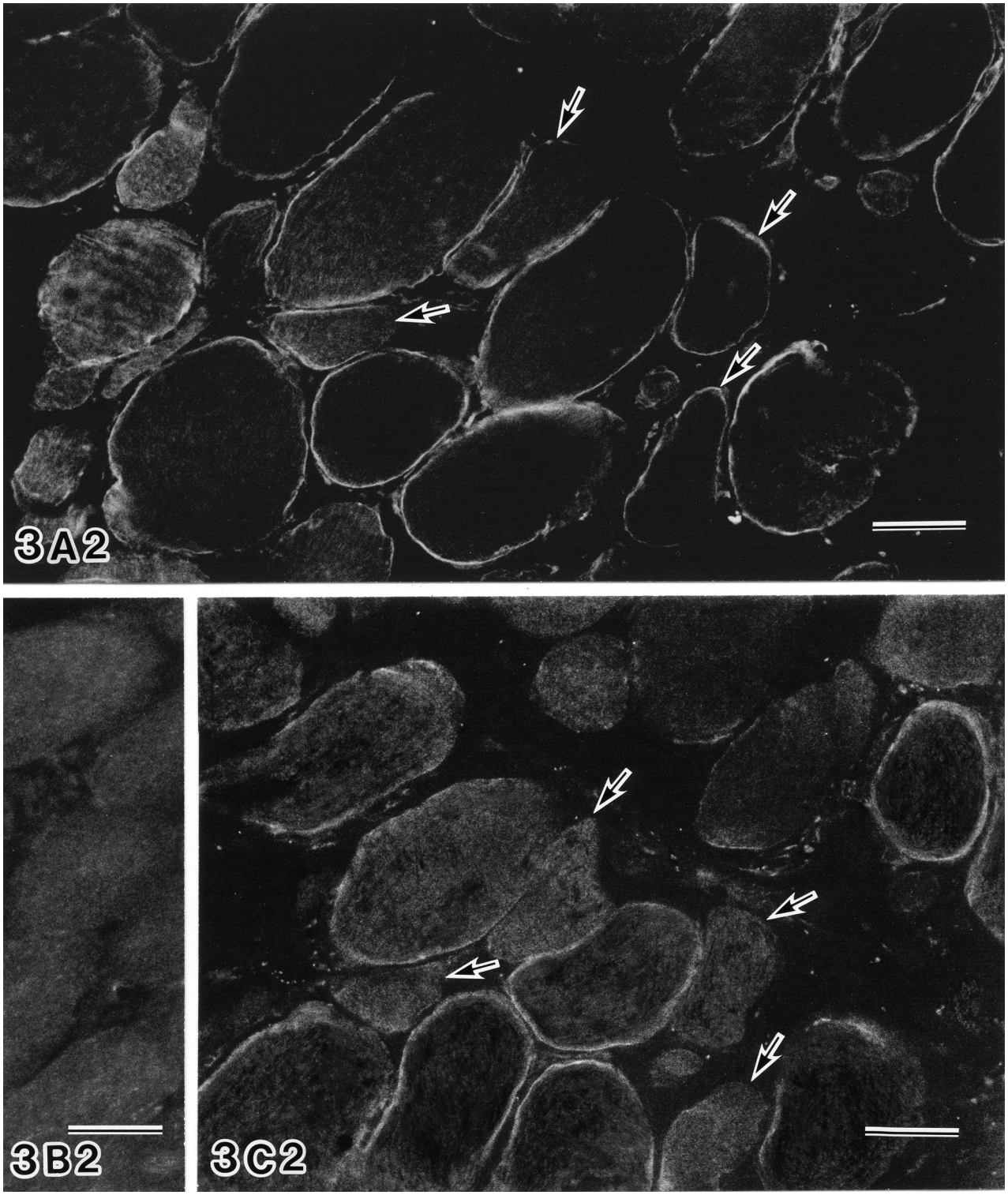


Fig. 3A2-C2. Immunohistochemical staining of serial muscle sections with anti-ß-spectrin **(A2)**, anti-dystrophin **(B2)** and anti-α1-syntrophin **(C2)** antibodies from another boy with Duchenne muscular dystrophy (DMD). Anti-α1-syntrophin immunopositive myofibers **(C2)** are less numerous than those seen in Fig. 3C1, although the immunostaining patterns at the myofiber surface for both ß-spectrin **(A2)** and dystrophin **(B2)** in this case reveal similar patterns to those observed in Fig. 3A1 and Fig. 3B1, respectively. Again the number of myofibers with negative immunoreactivity for α1-syntrophin was more than for ß-spectrin. Positively immunostained myofibers with anti-ß-spectrin antibody (**A2**, arrows) disclosed negative immunoreaction for anti-α1-syntrophin antibody in the serial DMD muscle section (**C2**, arrows). Scale bars: 50 µm. x 330

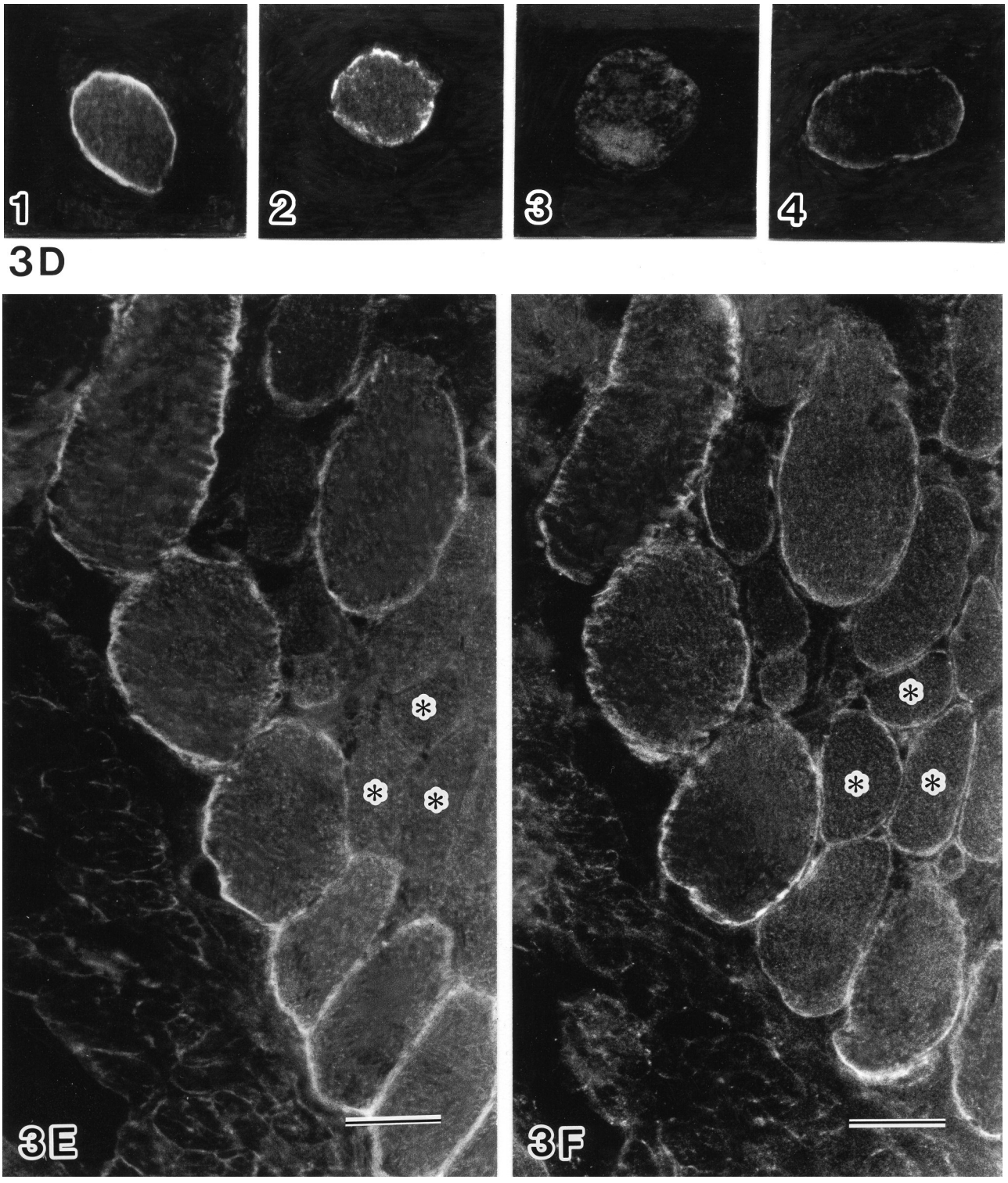


Fig. 3D1-F. Representative myofibers immunostained with anti-ß-spectrin and anti-α1-syntrophin antibodies. **Fig. 3D1, 3D2, 3D3, 3D4** are examples of myofibers in group 1, 2, 3, 4, respectively. **3E**, **F**. Immunohistochemical staining of serial DMD muscle sections with anti-α1-syntrophin **(E)** and anti-α-dystrobrevin **(F)** antibodies. Appreciable amounts of α1-syntrophin **(E)** and α-dystrobrevin **(F)** are in DMD muscle membranes. α1-Syntrophin-negative myofibers (**E**, asterisks) are α-dystrobrevin immunopositive myofibers (**F**, asterisks). Scale bars: 50 µm. x 330

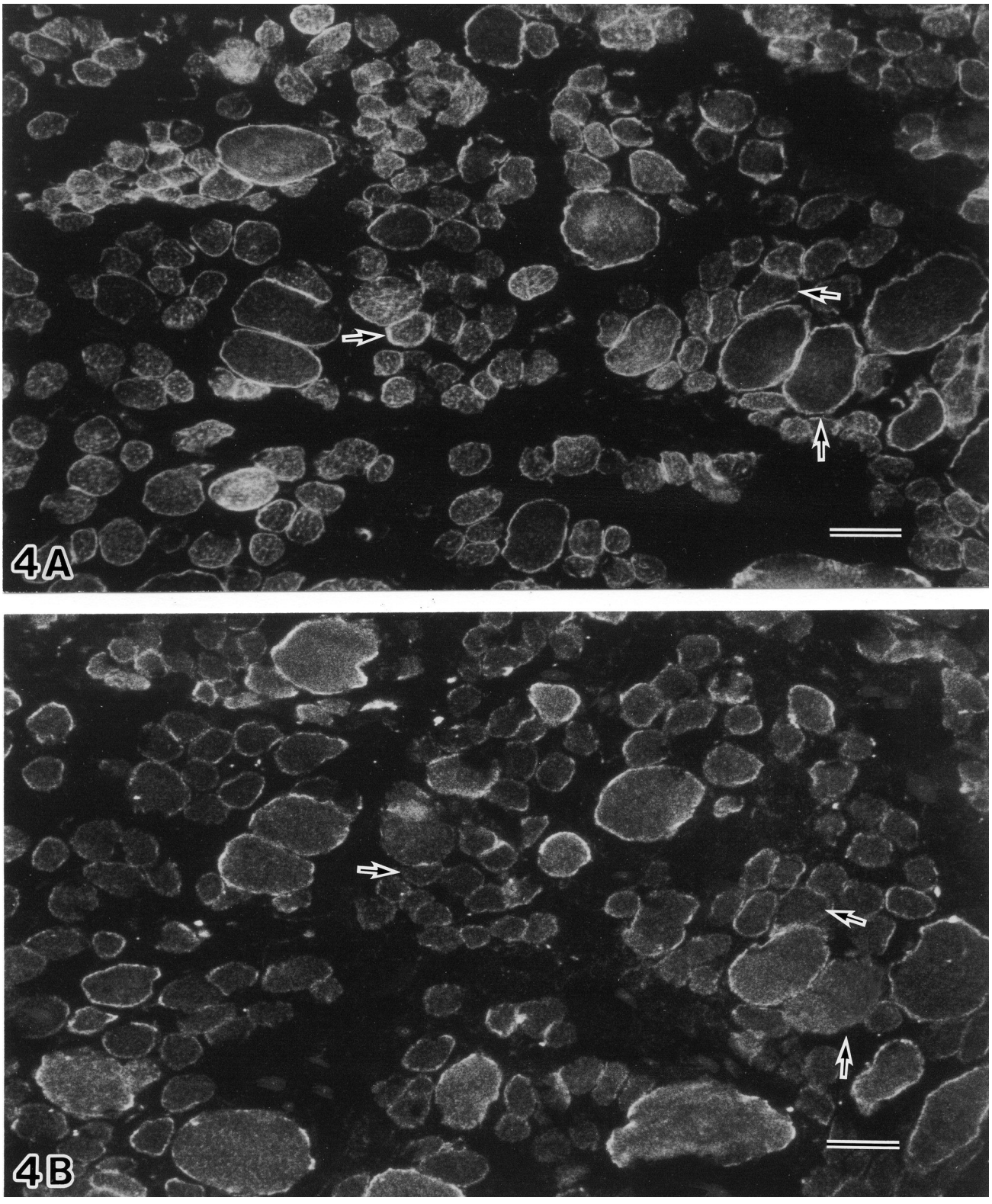
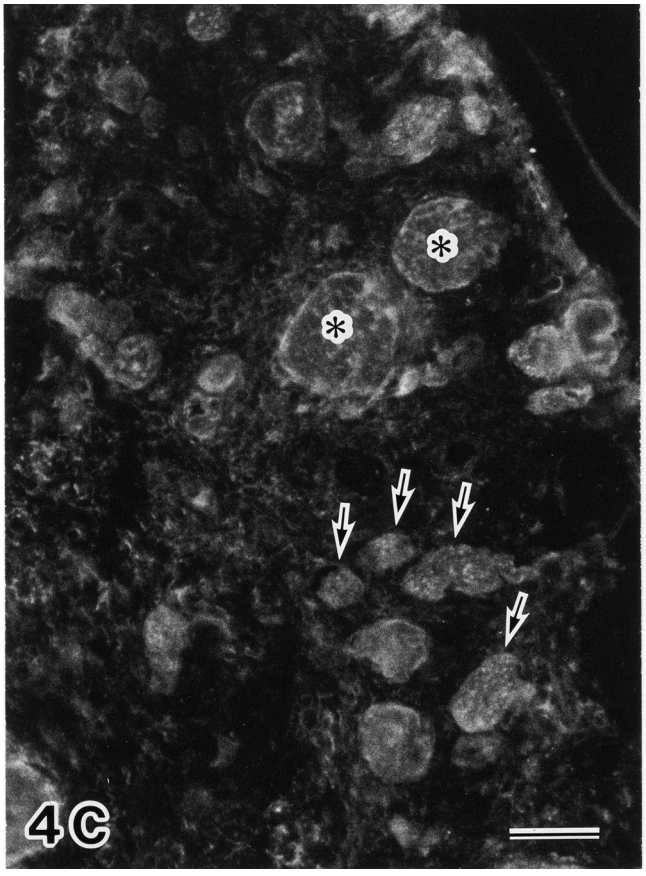


Fig. 4. Immunohistochemical staining of serial sections of Fukuyama-type congenital muscular dystrophy (FCMD) muscle with anti-ß-spectrin **(A)** and anti-α1-syntrophin **(B)** antibodies. Immunoreactivities for both anti-ß-spectrin and anti-α1-syntrophin antibodies in FCMD myofibers show positive immunoreaction in various degrees in many FCMD myofibers and negative immunostaining in the remaining myofibers. The number of FCMD myofibers with negative immunostaining by anti-α1-syntrophin antibody was more than by anti-ß-spectrin antibody. Positively immunostained myofiber with anti-ß-spectrin antibody (**A**, arrows) shows negative immunoreaction for anti-α1-syntrophin antibody in the serial muscle section (**B**, arrows). Immunohistochemical staining of serial FCMD muscle sections with anti-α1-syntrophin **(C)** and anti-neonatal myosin **(D)** antibodies. Partially α1-syntrophin-expressed FCMD myofibers at their surface membranes (**C**, asterisks) show negative immunostaining with anti-neonatal myosin antibody (**D**, asterisks). α1-Syntrophin immunonegative FCMD myofibers at their surface membranes (**C**, arrows) show positive immunostaining with anti-neonatal myosin antibody (**D**, arrows). Scale bars: 50 µm. x 250

myofibers with anti-α1-syntrophin antibody was less than these myofibers stained with anti-ß-spectrin antibody. From the classification of immunostaining patterns into group 1 to group 4 (Fig. 3D1-3D4), the group mean percentages of groups 1, 2, 3 and 4 DMD myofibers stained with anti-α1-syntrophin antibody were 15.4%, 39.6%, 39.3% and 5.7%, respectively, and those of groups 1, 2, 3 and 4 DMD myofibers stained with anti-ß-spectrin antibody were 50.2%, 39.0%, 10.8% and 0%, respectively (Table 1). DMD myofibers immunostained with anti-dystrobrevin antibody showed numerous dystrobrevin positive myofibers. Appreciable amounts of α-dystrobrevin, a molecule of syntrophin-dystrobrevin subcomplex, were found in the DMD muscle membranes. Dystrobrevin-immunopositive DMD myofibers appeared to be more numerous than α1-syntrophin-immunopositive DMD myofibers in all five DMD muscles used to survey the two antibody-stained serial muscle sections (Fig. 3E, F).

FCMD muscles immunostained with anti-ß-spectrin and anti-α1-syntrophin antibodies showed immunostaining patterns similar to those of DMD muscles (Fig. 4A, B). The number of immunonegative myofibers stained with anti-α1-syntrophin antibody was large (Fig. 4B) irrespective of the presence of dystrophin. The percentage of immunonegative myofibers stained with anti-α1-syntrophin antibody was more than these myofibers stained with anti-ß-spectrin antibody. From the classification into group 1 to group 4, the group mean percentages of groups 1, 2, 3 and 4 FCMD myofibers stained with anti-α1-syntrophin antibody were 15.3%, 31.9%, 45.5% and 7.3%, respectively, and those of groups 1, 2, 3 and 4 FCMD myofibers stained with anti-ß-spectrin antibody were 52.7%, 36.4%, 10.4% and 0.5%, respectively (Table 1). Immunonegative FCMD myofibers stained with α1-syntrophin at the myofiber surface were consistent with neonatal myosin immunopositive FCMD myofibers in serial muscle sections (Fig. 4C, D).

The disease control muscle samples immunostained with anti-ß-spectrin and anti-α1-syntrophin antibodies contained fewer myofibers with group 2 and 3 staining patterns (Table 1). The group mean percentages of α1-syntrophin- and ß-spectrin-immunonegative myofibers were 39.3% and 10.8%, respectively, in the DMD group, and were 45.5% and 10.4%, respectively, in the FCMD group. The group mean percentages of α1-syntrophin-immunonegative myofibers in DMD and FCMD groups were statistically significant compared with those of ß-spectrin-immunonegative myofibers (Table 2).



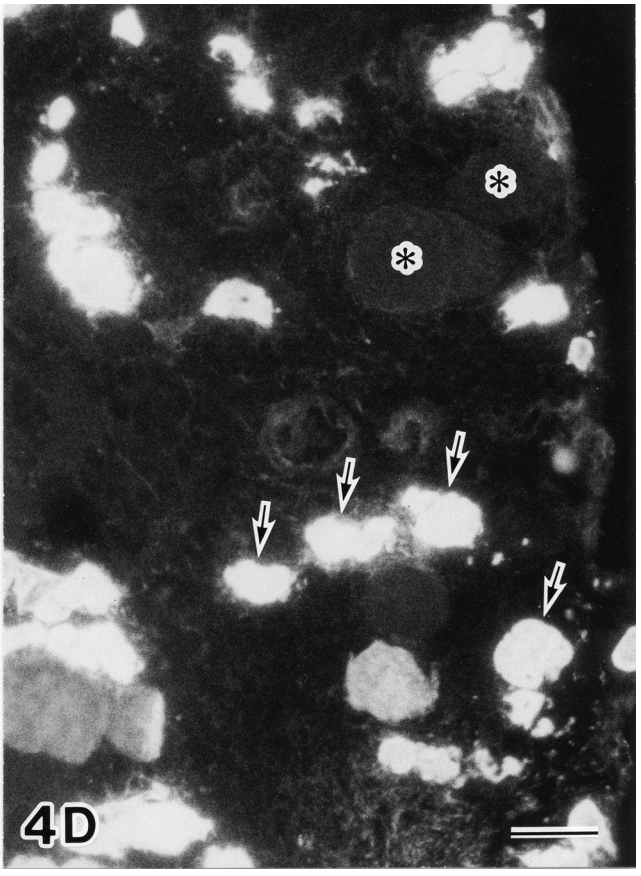


Table 1. Immunoreaction for ß-spectrin and α1-syntrophin in dystrophic muscles.

CASES	(1)	(2)	(3)	(4)
Anti-ß-spectrin immunoreactiviy*				
5 DMD	50.22±8.24	39.01±6.33	10.78±2.51	0
5 FCMD	52.73±8.51	36.43±5.40	10.38±3.08	0.47±0.30
5 DC	84.93±3.15	13.34±2.38	1.73±1.65	0
5 NC	96.40±0.76	3.60±0.76	0	0
Anti-α1-syntrophin immunoreactivity*				
5 DMD	15.41±7.29	39.60±1.56	39.31±7.71	5.67±1.60
5 FCMD	15.31±6.37	31.89±4.21	45.51±10.25	7.30±1.22
5 DC	78.73±1.80	17.38±1.32	3.89±0.98	0
5 NC	95.03±1.43	4.97±1.43	0	0

(1)=group 1: Continuously positive immunostaining pattern with more than 90% myofiber surface immunolabeling and normal immunoreactivity. (2)=group 2: Partially positive immunostaining pattern with 10 to 90% myofiber surface immunolabeling and normal immunoreactivity. (3)=group 3: Negative immunostaining pattern with less than 10% myofiber surface immunolabeling. (4)=group 4: Faint but entire surface positive immunostaining pattern. *: Group mean percentage of myofibers ± standard error of the mean. DMD: Duchenne muscular dystrophy. FCMD: Fukuyama type congenital muscular dystrophy. DC: Disease control (myotonic dystrophy + facioscapulohumeral dystrophy). NC: Normal control.

Table 2. Immunonegative fiber ratio (group 3).

	ANTI-ß-SPECTRIN ANTIBODY IMMUNOREACTIVITY*	ANTI-α1-SYNTROPHIN ANTIBODY IMMUNOREACTIVITY*	P
5 DMD	10.78±2.51%	39.31±7.71%	P<0.01
5 FCMD	10.38±3.08%	45.51±10.25%	P<0.02
5 Disease control (3 Myotonic dystrophy + 2 facioscapulohumeral dystrophy)	1.73±1.65%	3.89±0.98%	P>0.1
5 Normal control	0 %	0 %	

*Group mean percentage of myofibers ± standard error of the mean. P values were calculated by two tailed t test. DMD: Duchenne muscular dystrophy. FCMD: Fukuyama type congenital muscular dystrophy

Discussion

Syntrophins are important molecules because they function as scaffolding adapter molecules that anchor various functionally interdependent signaling molecules at the muscle plasma membrane. Syntrophins are multigene family proteins that include several isoforms named α-, ß1-, ß2-, γ1- and γ2-syntrophin (Jones et al., 2003). Among them γ1-syntrophin is mainly expressed in the brain (Piluso et al., 2000) and other syntrophins are in skeletal muscle (Jones et al., 2003). α1-Syntrophin is localized in the human skeletal myofiber (Wakayama et al., 1997). Immunostaining with anti-α-syntrophin antibody shows surface staining of all normal human myofibers; immunostaining with anti-ß1-syntrophin antibody shows type 2 human myofiber staining with greater intensity than type 1 myofiber and immunostaining with anti-ß2-syntrophin antibody shows type 1 human myofiber staining with greater intensity than type 2 myofiber (Jones et al., 2003). In mouse skeletal muscle, localization of ß2-syntrophin is mainly confined to the neuromuscular junction (Peters et al., 1994, 1997a). In this study, we analyzed quantitatively the expression of α1-syntrophin in muscles with muscular dystrophies, such as DMD and FCMD. α1-Syntrophin is a member of the dystrophin glycoprotein complex. Dystrophin is absent in DMD, and its associated protein α1-syntrophin is assumed to be absent in DMD muscles. However, surprisingly, myofibers expressing α1-syntrophin, including partially expressed myofibers, were 55% of all DMD myofibers in this study, which was consistent with our previous results of AQP4 present at the myofiber surface in many DMD myofibers (Wakayama et al., 2002). We do not know what is the binding partner molecule of α1-syntrophin in DMD muscles, but utrophin or dystrobrevin is a potential candidate for this molecule (Kramarcy et al., 1994; Yang et al., 1995). Utrophin expression in DMD muscles has been found by several studies and so this finding is well established (Mizuno et al., 1994; Sewry et al., 1994; Di Blasi et al., 1996). Dystrobrevin is a dystrophin-associated protein that may be a muscle cell-signaling protein (Grady et al., 1999; Blake, 2002). Appreciable amounts of the large isoform of dystrobrevin, α-dystrobrevin-I, are in the DMD muscle membrane (Blake, 2002), but different results have been described (Metzinger et al., 1997; Puca et al., 1998). Our qualitative finding of the dystrobrevin expression in DMD myofibers supports the findings by Blake (2002). Dystrobrevin has two syntrophin-binding sites (Newey et al., 2000), and syntrophin expressed in DMD myofibers in our study may be binding with dystrobrevin expressed in DMD myofibers in this study. We do not know other binding partners of α-dystrobrevin in DMD myofibers, but newly identified dystrobrevin-binding partners include dysbindin (Benson et al., 2001), syncoilin (Newey et al., 2001) and desmuslin (Mizuno et al., 2001). Syncoilin and desmuslin are both intermediate filament proteins (Mizuno et al., 2001; Newey et al., 2001). Syncoilin is concentrated at the neuromuscular junction of normal skeletal muscle (Newey et al., 2001).

In DMD muscles, morphometric analysis showed that motor nerve terminals are normal, but at nearly 50 percent of the end plates, focal degeneration of the junctional folds is seen and some postsynaptic regions are simpler than normal (Jerusalem et al., 1974). Desmuslin is at the Z-line and binds to desmin (Mizuno et al., 2001). Desmuslin expression in DMD myofibers has not been reported so far, but desmuslin expression in DMD muscles is plausible. The appreciable amounts of α-dystrobrevin expressed in DMD muscles in this study may bind desmuslin despite the absence of dystrophin.

FCMD muscles have dystrophin molecules, but dystrophin-associated protein α1-syntrophin was absent in about 45% FCMD myofibers in this study. This finding was also consistent with our previous finding of the expression of the α1-syntrophin-binding molecule AQP4 being markedly reduced in FCMD muscles (Wakayama et al., 2003). The mechanism of the downregulated expression of AQP4 in FCMD muscle is unknown, but the immaturity of the FCMD myofibers is one cause. Immunostaining of FCMD myofibers with anti-neonatal myosin antibody shows that α1-syntrophin negative FCMD myofibers are immature, because this antibody immunostained the human fatal myosin (Ecob-Prince et al., 1989). Present immunohistochemical studies of α1-syntrophin generally agreed with the results of our immunoblot analysis. Further studies of other isoforms of syntrophin, such as ß1-, ß2- and γ2-syntrophins and a detailed study of dystrobrevins will throw light on the precise pathogenesis of human muscular dystrophies.

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