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# Partial deficiency of dystrophin-associated proteins in a young girl with sporadic myopathy and normal karyotype

*Kiichiro Matsumura, MD; Ikuya Nonaka, MD; Kiichi Arahata, MD; and Kevin P. Campbell, PhD*

The mechanism by which the absence of dystrophin, a membrane cytoskeletal protein, causes muscle fiber necrosis in Duchenne muscular dystrophy (DMD) has not yet been established. Recently, we demonstrated that dystrophin is associated with a large oligomeric complex of sarcolemmal glycoproteins, including the 156-kd dystroglycan (156 DAG), which provides a linkage to the extracellular matrix component, laminin.[1-3] The absence of dystrophin leads to a drastic reduction of the dystrophin-associated proteins in the sarcolemma of DMD patients.[4,5] Based on the structural organization of the dystrophin-glycoprotein complex as a trans-sarcolemmal link between the subsarcolemmal cytoskeleton and the extracellular matrix,[2,3] we proposed that the disruption of this complex plays a key role in the cascade of events leading to muscle fiber necrosis in DMD.[5] The absence of dystrophin leads to a drastic reduction of the dystrophin-associated proteins in the sarcolemma, causing the disruption of the linkage between the subsarcolemmal cytoskeleton and the extracellular matrix which, in turn, leads to sarcolemmal instability and eventually to muscle fiber necrosis.[5]

A substantial proportion of young girls with a sporadic myopathy of early onset are indeed symptomatic DMD carriers, because immunohistochemistry demonstrates a mosaic of fibers with and without dystrophin.[6,7] According to Minetti et al,[6] the phenotype of these patients is characterized by proximal weakness, calf hypertrophy, and, interestingly, very high serum CK level; the family history is negative for neuromuscular diseases, karyotype is normal, and DNA analysis does not reveal a deletion of the dystrophin gene.[6] Although partial deficiency of dystrophin is presumed to be the cause of the clinical symptoms, the precise mechanism of how it leads to muscle fiber degeneration is unclear. Therefore, based on the aforementioned hypothesis on the mechanism of muscle fiber necrosis in DMD, it was crucial to investigate the status of the dystrophin-associated proteins in the sarcolemma of these patients. Here we report the immunohistochemical analysis of these proteins in the biopsied skeletal muscle from one such patient.

*Case report.* The patient is a 7-year-old girl with no family history of neuromuscular diseases. Weakness of the extremities began around the age of 5 years and progressed gradually. Physical examination at the age of 7 years revealed proximal weakness and calf hypertrophy. Serum CK level was elevated to 40 times of the normal upper limit. Karyotype was normal. Muscle biopsy from the quadriceps femoris muscle showed features consistent with muscular dystrophy. Immunohistochemistry of the same muscle was performed, using specific antibodies against each component of the dystrophin-glycoprotein complex as previously described.[1-5] The results revealed scattered small groups of muscle fibers with no dystrophin staining or with reduced and patchy dystrophin staining along the sarcolemma (figure). Dystrophin-associated proteins including the laminin-binding 156 DAG were drastically diminished in the sarcolemma of these dystrophin-deficient muscle fibers (figure).

*Discussion.* We demonstrated, for the first time, that dystrophin-associated proteins are lost in the sarcolemma of dystrophin-deficient muscle fibers, and thus the linkage between the subsarcolemmal cytoskeleton and the extracellular matrix is disrupted in these muscle fibers. The combination of the characteristic clinical features and the results of dystrophin staining indicate that this patient is indeed a symptomatic DMD carrier very similar to those reported by Minetti et al.[6] Our results indicate that the same sarcolemmal instability as in DMD is responsible for the muscle fiber degeneration and very high serum CK level in these patients. Furthermore, the severity of the loss of the dystrophin-associated proteins may correlate with the severity of the clinical symptoms. Thus, immunochemical analysis of dystrophin-associated proteins, in addition to dystrophin, could be helpful in the evaluation of these patients.

*From the Howard Hughes Medical Institute and Department of Physiology and Biophysics (Drs. Matsumura and Campbell), University of Iowa College of Medicine, Iowa City, IA; and the National Institute of Neuroscience (Drs. Nonaka and Arahata), National Center of Neurology and Psychiatry, Tokyo, Japan.*

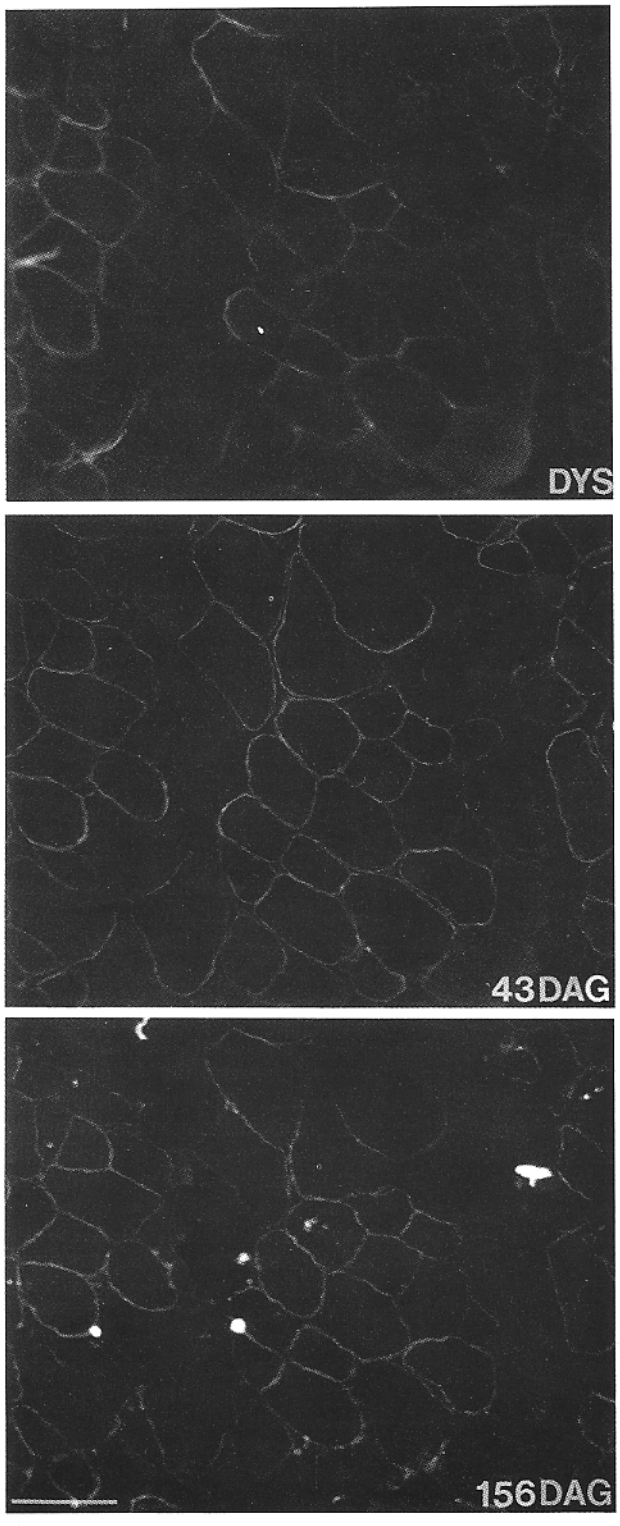


*Figure. Indirect immunofluorescence microscopy of quadriceps femoris muscle from a 7-year-old girl with a sporadic myopathy. Serial transverse cryosections (7 μm) were immunostained with the following antibodies: (1) VIA4$_2$, a monoclonal antibody against dystrophin (DYS); (2) an affinity-purified sheep polyclonal antibody against the 43-kd dystroglycan (43 DAG); and (3) an affinity-purified sheep polyclonal antibody against the 156-kd dystroglycan (156 DAG) fusion protein.[1-5] Bar = 100 μm. 43 DAG and 156 DAG, which binds the extracellular matrix component laminin, were drastically diminished in the sarcolemma of dystrophin-deficient muscle fibers. Other dystrophin-associated proteins (35 DAG, 50 DAG, and 59 DAP) showed the same distribution as the 43 DAG and 156 DAG (not shown).*

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*Address correspondence and reprint requests to Professor Kevin P. Campbell, Howard Hughes Medical Institute, University of Iowa College of Medicine, 400 EMRB, Iowa City, IA 52242.*

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