Neurology

Official Journal of the American Academy of Neurology

Merosin-negative congenital muscular dystrophy associated with extensive brain abnormalities

Y. Sunada, MD, PhD; T.S. Edgar, MD; B.P. Lotz, MD; R.S. Rust, MD; and K.P. Campbell, PhD

Article abstract—Congenital muscular dystrophies (CMDs) are autosomal recessive, heterogeneous disorders. The most frequent form in the Caucasian population is classic (occidental) CMD, characterized by exclusive muscle involvement, although abnormal brain white matter signals are occasionally observed on MRI. Recently, deficiency of merosin, the laminin isoform in skeletal muscle, has been identified in classic CMD patients. In skeletal muscle, merosin is a native ligand for dystroglycan linking the extracellular matrix and dystrophin. Thus, merosin deficiency could disrupt the attachment of muscle cell to the extracellular matrix and lead to muscle cell necrosis. Since merosin is also expressed in the nervous system and has biologic activities on neurite outgrowth and Schwann cell migration, deficiency of merosin could affect the development of the nervous system. We report here two patients with merosin-negative CMD presenting extensive brain abnormalities characterized by cortical anomaly, polymicrogyria, and abnormal white matter signals.

NEUROLOGY 1995;45:2084-2089

Congenital muscular dystrophy (CMD) is an autosomal recessive disease of muscle presenting in the first few months of life and is characterized by dystrophic changes on muscle biopsy.[1-3] There are several forms of CMD with heterogeneous etiologies. One form that has become established as a single disease entity is Fukuyama-type CMD, prevalent in Japan; it is typically associated with brain structural anomalies[4,5] and was recently mapped to chromosome 9q31-33.[6] Clinical manifestations of CMD in the Occidental population usually are limited to skeletal muscle without CNS symptoms, even though brain white matter abnormalities are occasionally observed on CT or MRI.[7] Hence, these cases have been termed "classic" or "pure" CMD. Typical presenting signs include hypotonia and arthrogryposis. The predominant histopathologic feature of muscle is extensive connective tissue proliferation.[3] We have identified a particular subset of classic CMD that is characterized by complete absence of merosin (laminin M chain).[8]

Merosin is the predominant homologue of laminin A chain in the basal laminae of striated muscle and Schwann cells.[9-12] Merosin has diverse biologic functions, including mediation of cell attachment and promotion of neurite outgrowth and Schwann cell migration.[13] In skeletal muscle, merosin binds α-dystroglycan, an extracellular 156-kDa dystrophin-associated glycoprotein, and in turn is linked to the subsarcolemmal actin-based cytoskeleton via the dystrophin–glycoprotein complex (DGC).[14] The absence of merosin could disrupt this critical link and cause

From the Howard Hughes Medical Institute and the Department of Physiology and Biophysics (Drs. Sunada and Campbell), University of Iowa College of Medicine, Iowa City, IA; and the Department of Neurology (Drs. Edgar, Lotz, and Rust), University of Wisconsin School of Medicine, Madison, WI.

Supported in part by the Muscular Dystrophy Association. K.P.C. is an investigator of the Howard Hughes Medical Institute.

Received January 4, 1995. Accepted in final form March 10, 1995.

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

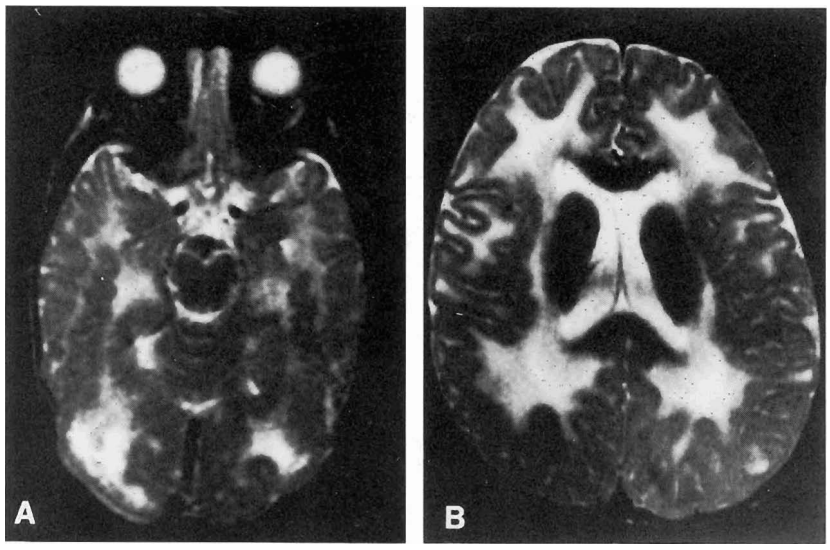

Figure 1. T_2-weighted brain MRI in (A) merosin-negative congenital muscular dystrophy (CMD) patient 1 and (B) merosin-negative CMD patient 2. Both show abnormal high-intensity signals in supratentorial white matter and abnormal gyral formation suggestive of dysmyelination and polymicrogyria.

muscle cell degeneration. Thus, merosin deficiency represents an adequate explanation for certain types of human muscular dystrophy.

We report a clinical presentation and an immunochemical analysis of merosin and dystrophin-associated proteins in two patients with CMD from the United States. Both patients show extensive brain abnormalities with putative dysmyelination and polymicrogyria. This is the first report describing brain cortical anomaly associated with merosin-negative CMD.

Case reports. Patient 1. This 3.2-kg girl was delivered uneventfully to a 31-year-old mother after a term pregnancy. The family history was negative for neuromuscular diseases, and her parents were not consanguineous. Apgar scores were 8/8. Profound hypotonia with marked axioproximal weakness was evident in the neonatal period. There were no cranial nerve abnormalities or hypognathus; palate was normal. Tendon reflexes were diminished. Mild bilateral talipes equinovarus deformities and finger contractures were evident. By 7 months of age, her weight had dropped from the 35th to the 5th percentile. The cranial nerves remained normal. She was severely hypotonic and weak, with a paucity of active movements, proximally more than distally. She was unable to hold her head upright in a supported sitting position. She was visually attentive, and cooed and babbled. She could transfer objects from hand to hand. She had mild facial weakness but no drooling. A pectus excavatum had developed; she had had many respiratory infections. Tendon reflexes were difficult to obtain. The ocular examination was normal, showing no cataracts or retinal abnormalities. Serum CK level was 4,740 IU/ml at 4 weeks of age. EMG findings were consistent with active myopathy. Nerve conduction velocities were normal. Light microscopy of a muscle biopsy specimen showed variation in fiber size with numerous internal nuclei, clusters of degenerating and regenerating fibers, and significant increase in fibrous and fatty connective tissue. Electron microscopy showed disruption of the sarcolemma. Brain MRI showed abnormal cortical gyral formation suggesting polymicrogyria; discrete areas of increased signal on long TR weighting were present deep within the occipital lobe, representing hamartomas, areas of migratory errors, or possibly a double cortex. Myelination was abnormal within the centra semiovale and central white matter tracts of the cerebral lobes, sparing the subcortical U fibers (figure 1A). ECG was normal.

Patient 2. This 3.6-kg boy, born after a 41-week pregnancy, was the second child of a 25-year-old mother. The family history was negative for neuromuscular diseases, and his parents were not consanguineous. Apgar scores were 8/8. Cranial nerve function and ocular examination were normal. He had generalized weakness at birth, emphasized in axial and proximal muscles. A mild contracture of the left biceps and early talipes equinovarus deformities were present. Tendon reflexes were absent. There was no hypognathus and no palatal abnormality. By 22 months, his weight had dropped below the 5th percentile. Spontaneous movements of the limbs, trunk, and face were markedly diminished, and examination showed severe hypotonia and weakness, which were emphasized axioproximally and included facial weakness with drooling. He was visually alert, reached for interesting objects, and could pass them from hand to hand. He was able to use single words appropriately, but did so with mechanical impairment due to weakness. Pectus excavatum had developed. He was barely able to hold his head upright with significant bobbing, but he could not sit unsupported. Serum CK level was 4,004 IU/ml at 6 weeks of age. EMG findings suggested an active myopathy. Nerve conduction velocities were normal for age. Light microscopy of a muscle biopsy specimen showed dystrophic pathology similar to that of patient 1. Brain MRI showed abnormal gyral contours involving the posterior temporal, parietal, and occipital lobes and suggesting polymicrogyria. Both T_1- and T_2-weighted pulse sequences showed diffuse abnormality of supratentorial white matter compatible with dysmyelination (figure 1B). ECG was normal.

Methods. *Muscle biopsy specimens.* Biopsies of quadriceps muscle from the above two merosin-negative CMD patients, four merosin-positive CMD patients, and five normal control subjects were studied. The diagnosis of CMD was based on (1) neonatal onset of muscle weak-

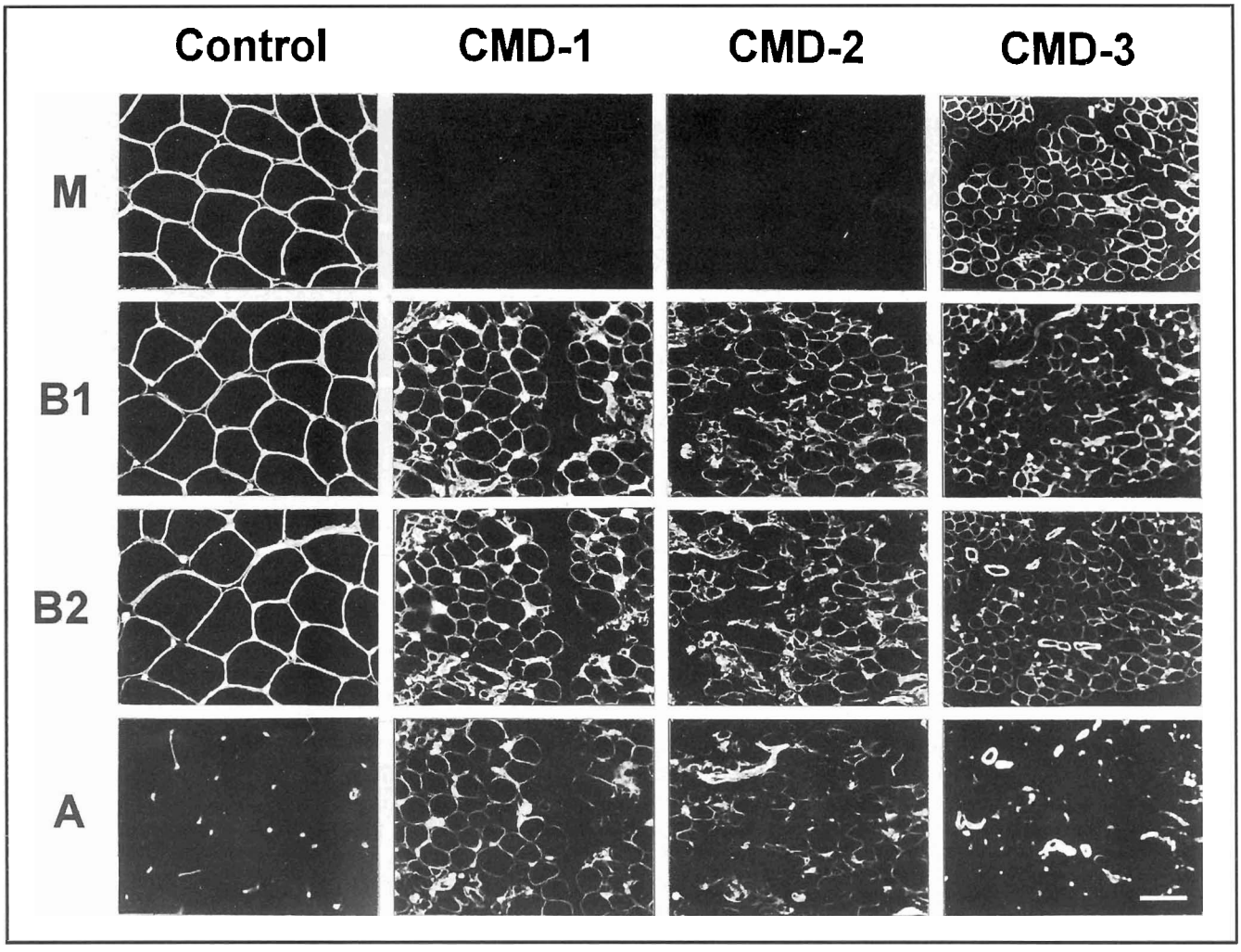

Figure 2. Immunohistochemical analysis of laminin subunits on skeletal cryosections. Merosin (M) was undetectable in classic CMD patients 1 and 2; however, classic CMD patient 3 shows normal merosin expression. Laminin B1 (B1) and B2 (B2) chains were normally expressed in CMD patients 1 and 2. Laminin A chain (A) was apparently overexpressed in CMD patients 1 and 2. Bar indicates 50 μm.

ness with hypotonia, (2) dystrophic changes of biopsied skeletal muscle, and (3) elevated serum CK value. The biopsy specimens were snap-frozen in isopentane cooled in liquid nitrogen and stored at −80 °C.

Antibodies. Monoclonal antibodies against merosin (laminin M chain),[10,11] laminin A chain,[11] laminin B1 chain,[15] and laminin B2 chain[15] were purchased from GIBCO–BRL. A rabbit polyclonal antibody against a recombinant merosin fragment was a generous gift from Dr. Y. Yamada at the National Institutes of Health, Bethesda, MD. Polyclonal antibodies against dystrophin, α-dystroglycan, 59 DAP, β-dystroglycan, and 35 DAG were affinity-purified from sheep antiserum against purified DGC as previously described.[16] A monoclonal antibody against adhalin (50 DAG) was previously characterized.[17]

Immunohistochemistry. Indirect immunofluorescence microscopy of 7-μm-thick cryosections from skeletal muscle biopsy specimens was performed as previously described.[18] The sections were examined under a Zeiss Axioplan fluorescence microscope. Photographs were taken under identical conditions with the same exposure time. For comparison, cryosections from a normal control and a merosin-positive CMD patient were processed identically.

Immunoblotting. Biopsied skeletal muscle cryosections, 20 μm thick, were homogenized in 20 volumes of buffer A (50 mM TRIS-HCl [pH 7.4], 150 mM NaCl, 1 mM PMSF, 0.75 mM benzamidine, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin A). Tissue residues were collected by centrifugation for 15 minutes at 13,000× *g*, 4 °C. This wash cycle was then repeated. The pellet was resuspended in five volumes of buffer A containing 10 mM EDTA and was extracted for 2 hours at 4 °C with gentle shaking. After centrifugation, the protein concentration of each sample was determined by Lowry's method. Samples containing 200 μg protein were separated on 3 to 12% gradient SDS-PAGE[19] in the presence of 1% 2-mercaptoethanol and transferred to nitrocellulose.[20] The nitrocellulose transfer was stained with a rabbit polyclonal antibody against a recombinant mouse M chain fragment.

Results. *Analysis of laminin subunits.* In normal human skeletal muscle, immunofluorescence using a monoclonal antibody against merosin (M chain) showed uniform labeling of the basal lamina surrounding each muscle fiber (figure 2). In contrast, immunolabeling of merosin was undetectable in CMD patients 1 and 2; however, other CMD pa-

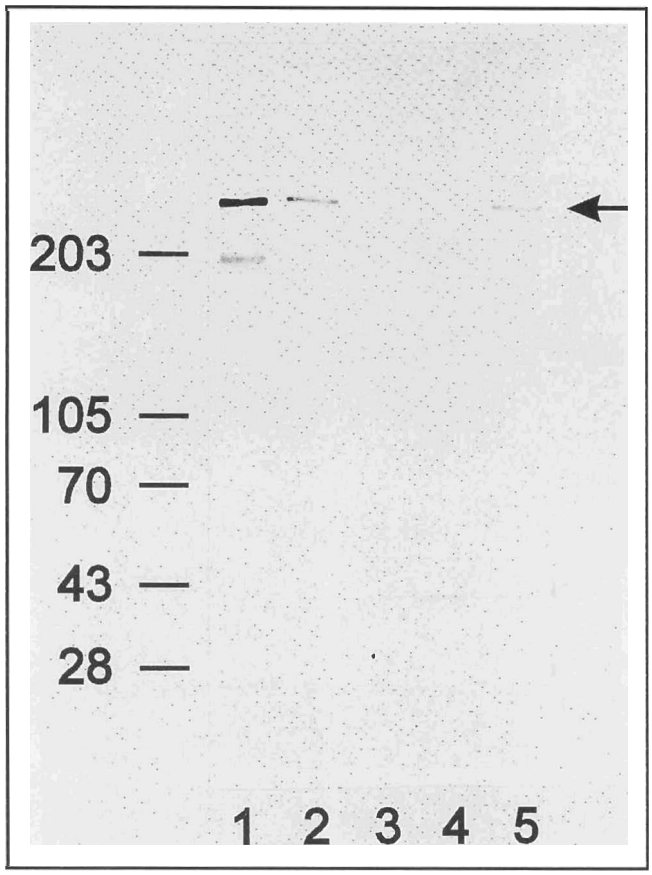

Figure 3. Immunoblot analysis of merosin in skeletal muscle EDTA extracts. Under reducing conditions, the anti-merosin antibody detected the 300-kDa merosin fragment (arrow) in purified human merosin (lane 1), in an adult control (lane 2), and in CMD patient 3 (lane 5), whereas the merosin fragment was undetectable in CMD patients 1 (lane 3) and 2 (lane 4).

tients (shown as CMD 3 in figure 2) of similar age showed normal immunostaining of merosin. Immunofluorescence using antibodies against laminin B1 and B2 chains showed labeling of the basal lamina similar to that seen in controls (figure 2). Although the laminin A chain was weakly expressed at the basal lamina of muscle fibers from control adult and merosin-positive CMD muscle, the expression of A chain was apparently upregulated in the two CMD patients with merosin deficiency (figure 2).

Under reducing conditions, merosin M chain migrates as an N-terminal fragment of 300 kDa and a C-terminal fragment of 80 kDa. A rabbit polyclonal antibody against a recombinant merosin fragment recognized the 300-kDa fragment in control skeletal muscle by immunoblotting. However, the merosin fragment was undetectable in our two CMD patients (figure 3).

Analysis of dystrophin and dystrophin-associated proteins. Immunofluorescence using an anti-dystrophin monoclonal antibody showed homogeneous sarcolemmal labeling of each muscle fiber in CMD patients 1 and 2 (figure 4). Immunolabeling of α-

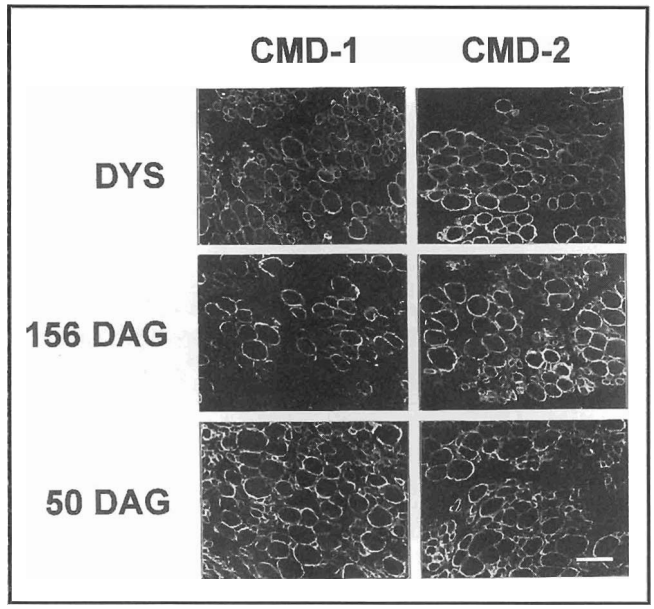

Figure 4. Immunohistochemical analysis of dystrophin and dystrophin-associated proteins in merosin-negative CMD. Immunolabeling of dystrophin (DYS), α-dystroglycan (156 DAG), and adhalin (50 DAG) was normal except for the heterogeneous labeling of α-dystroglycan in CMD patient 1. Bar indicates 50 μm.

dystroglycan (156 DAG), a receptor for merosin, was heterogeneous in CMD patient 1, with relatively large-sized fibers showing normal immunostaining and with some fibers of small or medium diameter showing very weak immunolabeling. In CMD patient 2, α-dystroglycan was homogeneously immunostained in both large and small fibers (figure 4). Other components of DAPs, including adhalin (50 DAG), were apparently normally expressed (figure 4) as compared with control skeletal muscle (data not shown).

Discussion. We reported two cases of CMD in which merosin is completely absent from the basal lamina of skeletal muscle. Both patients showed profound hypotonia and muscle weakness without any detectable signs of intellectual impairment. Nonetheless, the examination was limited, due to the age of these children and their profound weakness. Muscle pathology showed dystrophic changes, including an extensive increase of fatty and fibrous connective tissue in both endomysium and perimysium. The clinical features of both patients were consistent with the diagnosis of classic CMD, implying exclusive involvement of skeletal muscle, in contrast to Fukuyama-type CMD, which is inevitably associated with severe mental retardation.[4,5] However, some occidental CMD cases are merosin-negative and some are merosin-positive. Merosin-negative CMD appears to be a clinically distinct form of CMD and is apparently associated with significant developmental abnormalities of the brain that are detectable by MRI. Merosin-negative CMD has been linked by homozygosity mapping to

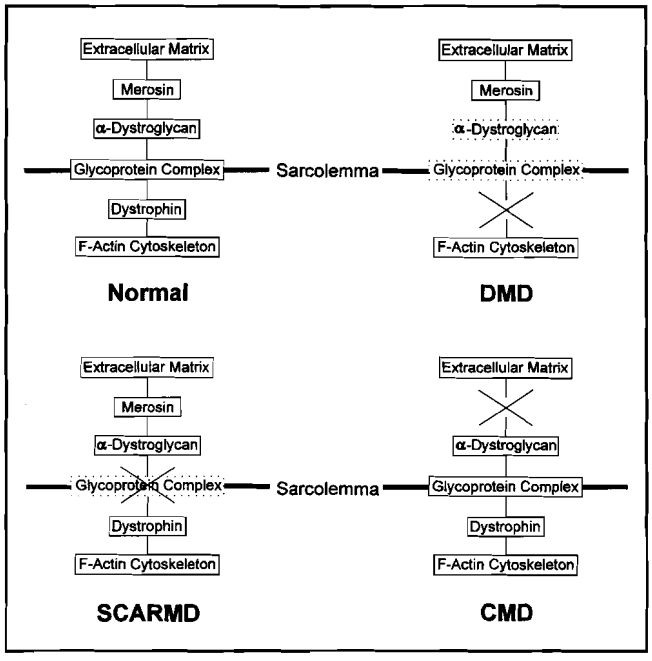

Figure 5. Involvement of the merosin–dystrophin-glycoprotein complex–cytoskeletal linkage in the pathogenesis of muscular dystrophies. Crossed lines (×) indicate primary deficient components. Dotted boxes indicate secondary deficient components. In normal skeletal muscle, α-dystroglycan links the extracellular matrix by binding merosin. Integral membrane glycoprotein complex, including β-dystroglycan and adhalin, links α-dystroglycan and dystrophin. Finally, dystrophin binds F-actin through the N-terminal domain. Disruptions of this critical linkage caused by the deficiency of particular components (dystrophin absence in Duchenne muscular dystrophy [DMD], adhalin deficiency in severe childhood autosomal recessive muscular dystrophy [SCARMD], and merosin deficiency in CMD) lead to muscle cell necrosis in muscular dystrophies.

chromosome 6q2 in the region of the merosin gene and is genetically distinct from merosin-positive CMD, which is not linked to this region.[21]

Merosin is the predominant isoform of laminin A chain in the basal lamina of skeletal muscle.[9-12] Merosin constitutes a cross-shaped heterotrimer molecule, now called laminin 2,[22] together with laminin B1 and B2 chains and has diverse biologic functions: mediating cell attachment and promoting neurite outgrowth and Schwann cell migration.[13] We identified merosin as a native ligand for α-dystroglycan, an extracellular dystrophin-associated protein,[14] suggesting that it is linked to the subsarcolemmal actin-based cytoskeleton via the DGC (figure 5). In Duchenne muscular dystrophy and severe childhood autosomal recessive muscular dystrophy, a disruption of the link between the extracellular matrix and the subsarcolemmal cytoskeleton is caused by the absence of one or more components of the DGC and leads to muscle degeneration[23] (figure 5). An absence of merosin also could disrupt the link and cause muscle degeneration in CMD (figure 5).

On the basis of clinical evaluation of four merosin-negative CMD families, Hillaire et al[21] pointed out that patients present relatively homogeneous clinical pictures: (1) severe neonatal hypotonia associated with joint stiffness and contractures, (2) markedly delayed motor development, (3) elevated serum CK, and (4) changes of the brain white matter detectable by MRI. Both our patients exhibited these clinical features. Additionally, neither patient had clinical or electrophysiologic evidence of cardiac involvement, and as yet neither has had intellectual impairment or clinical seizures, despite the presence of brain abnormalities on MRI. MRI changes consistent with abnormalities of supratentorial white matter myelination are occasionally observed in patients with classic CMD. Both our patients distinctly exhibited such changes; both also showed changes suggesting abnormalities of brain neuronal migration (polymicrogyria and probable areas of migrational arrest). Merosin promotes neurite outgrowth and Schwann cell migration, the most critical events during nervous system development. Therefore, merosin likely has a role in the normal development of the CNS, where its expression recently was identified.[24] Merosin and α-dystroglycan are co-expressed not only in skeletal muscle but also in peripheral nerve.[25] Disrupted interaction of α-dystroglycan with merosin, caused by deficiency of merosin, results in developmental dysmyelination of peripheral nerve in the dystrophic *dy* mouse.[14,26,27] Although the precise localization of merosin in the brain remains unknown, a similar disruption of merosin–dystroglycan interaction could be involved in the pathogenesis of dysmyelination and abnormal neuronal migration in these two patients.

In normal skeletal muscle tissue, laminin A chain is predominantly expressed in the basal laminae of blood vessels, the capsules of muscle spindles, and intramuscular nerve fibers and is only weakly expressed at muscle fibers. However, the expression of laminin A is apparently upregulated in merosin-deficient muscle fibers. Since laminin A chain and merosin have considerable homology and both bind α-dystroglycan, upregulation of laminin A chain may take place to compensate for merosin deficiency. This finding may suggest a potential significance of laminin A chain upregulation as a future therapy for merosin-negative CMD.

Acknowledgments

We thank Dr. Y. Yamada (NIH) for providing an antibody against merosin and Dr. S.L. Roberds for his helpful comments.

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