Abnormal expression of dystrophin-associated proteins in Fukuyama-type congenital muscular dystrophy

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The absence of dystrophin causes Duchenne muscular dystrophy. Dystrophin is associated with a large complex of sarcolemmal glycoproteins which provides a linkage to the extracellular matrix component, laminin, and when dystrophin is absent all the dystrophin-associated proteins are much reduced. We report here that dystrophin-associated proteins have abnormally low expression in Fukuyama-type congenital muscular dystrophy (FCMD), despite near-normal expression of dystrophin. An abnormality of dystrophin-associated proteins in the sarcolemma seems to be a common denominator in the pathological processes leading to muscle cell necrosis in three forms of severe muscular dystrophy (Duchenne, Japanese Fukuyama-type, and north African Duchenne-like autosomal recessive).

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Dystrophin, a membrane cytoskeletal protein encoded by the Duchenne muscular dystrophy (DMD) gene, is associated with a large oligomeric complex of sarcolemmal glycoproteins.[1] One of these dystrophin-associated proteins (DAPs) is dystroglycan (156 DAG), which provides a linkage to the extracellular matrix component, laminin.[2] The absence of dystrophin causes a drastic reduction in all DAPs.[3] The structure of the dystrophin-glycoprotein complex linking subsarcolemmal cytoskeleton to extracellular matrix suggests that its disruption or dysfunction could lead to severe instability of the sarcolemma and, in turn, muscle cell necrosis.[1,3] An intriguing possibility is that a primary defect in the DAPs could be the cause of hereditary neuromuscular disease with DMD-like phenotype. We have demonstrated deficiency of the 50 DAG in one such DMD-like condition—namely, severe childhood autosomal recessive muscular dystrophy (SCARMD), which is common in north Africa.[4] We report here on the status of the dystrophin-glycoprotein complex in another severe autosomal recessive muscular dystrophy of childhood, Fukuyama-type congenital muscular dystrophy (FCMD).[5] In Japan this is the most common form of childhood myopathy apart from DMD. The phenotype consists of a combination of severe muscular dystrophy and an anomaly of the brain.[5]

Immunohistochemical analysis of muscle biopsy specimens from twelve FCMD patients (seven males, five females; age 4–20 months) were done with nine antibodies:

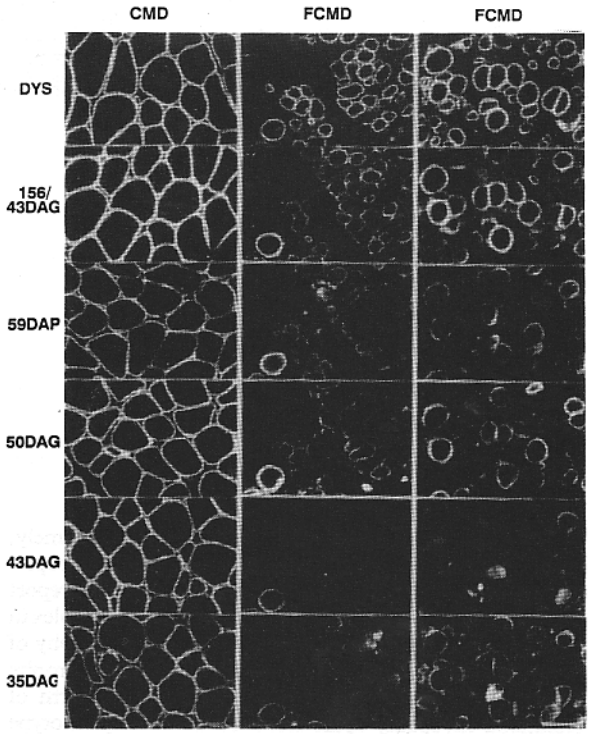
Antibody	*Directed against*
Monoclonal	
(1) $VIA4_2$	Dystrophin
(5) $IVD3_1$	50 DAG
Polyclonal (sheep, affinity purified)	
(2) FP-D	156 DAG fusion protein
(3) anti-59 DAP	59 DAP
(4) anti-50 DAG	50 DAG
(6) anti-43 DAG	43 DAG
(7) FP-A	43 DAG fusion protein
(8) FP-B	156/43 DAG fusion protein
(9) anti-35 DAG	35 DAG

DAG = dystrophin-associated glycoprotein. DAP = dystrophin-associated protein.

So far we have found no abnormality of the components of this complex in limb-girdle, myotonic, facioscapulohumeral, oculopharyngeal, or non-Fukuyama congenital muscular dystrophies, in congenital fibre type disproportion, in spinal muscular atrophy, or in amyotrophic lateral sclerosis (age range 5 months to 70 years).

In FCMD patients staining for dystrophin was well preserved in the sarcolemma except in a few muscle fibres with reduced and patchy staining.[6,7] In contrast, staining for the DAPs (figure) was greatly reduced in the sarcolemma of most muscle fibres, and this was especially true for 43DAG (figure).* A few muscle fibres had abnormally intense staining in the sarcolemma or had diffuse cytoplasmic staining (figure); these abnormalities were common to all patients but the severity varied and did not correlate with age or sex. The reduction in FCMD was confirmed by the immunoblot analysis of skeletal muscle biopsy extracts (not shown). These findings in FCMD contrast sharply with those in DMD, where the absence of dystrophin causes a

*Composite panels showing 36 slides of FCMD and control material immunostained with various antibodies are available from K. P. C.



Immunohistochemical analysis of components of dystrophin-glycoprotein complex in skeletal muscle.

Transverse cryosections (7 μm) of skeletal muscle biopsy material from a patient with non-Fukuyama congenital muscular dystrophy (CMD) and two patients with FCMD were immunostained with antibodies against dystrophin (DYS) and the dystrophin-associated proteins. Bar = 50 μm.

reduction in all DAPs,[3] or in SCARMD where 50 DAG deficiency causes a secondary reduction of 35 DAG.[4]

This abnormal expression of DAPs despite near-normal expression of dystrophin is pathognomonic and immunochemical analysis should considerably assist in the diagnosis of FCMD. The finding that dystrophin remained localised to the sarcolemma supports our hypothesis that dystrophin is linked to both the subsarcolemmal cytoskeleton and DAPs.[1,2] In the absence of DAPs, dystrophin could still be associated with cytoskeletal components such as γ-actin and be properly localised to sarcolemma.

An abnormality of DAPs in the sarcolemma seems to be the common denominator to the pathological process that leads to muscle cell necrosis in three major forms of severe childhood muscular dystrophy (DMD, SCARMD, and FCMD). We propose that disruption/dysfunction of the dystrophin-glycoprotein complex interrupts the link between subsarcolemmal cytoskeleton and extracellular matrix, resulting in cascade of events leading to muscle cell necrosis. The cause of the abnormal expression of DAPs in FCMD is unknown. One possibility is a defect in the structure or expression of a gene for a DAP, which would be consistent with the recent observation of a possible interaction between dystrophin and the putative FCMD gene product.[8] On clinical grounds the FCMD gene product should be expressed in both muscle and brain. We have identified a single gene which encodes both 156DAG and 43DAG, and the transcript is expressed in these two tissues.[2] Characterisation of this 156DAG/43DAG gene from FCMD patients is underway in our laboratory.

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