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Merosin 결핍 선천성근이영양증 1예

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Merosin Deficient Congenital Muscular Dystrophy: A Case with Immunocytochemical Analysis

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Primary merosin (laminin α2 chain)-deficient congenital muscular dystrophy (CMD) is a uncommon and severe form of CMD, which is caused by the mutations in the laminin α2 chain gene. It is an autosomal recessively inherited form of muscular dystrophy that is associated with severe neonatal hypotonia, a high serum creatine kinase level, and abnormal brain imaging without intellectual dysfunction. We report a case of merosin-deficient CMD confirmed by the immunocytochemical analysis of the frozen muscle biopsy. This is the first case of merosin-deficient CMD in Korea.
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Key Words: Merosin-deficient congenital muscular dystrophy, Laminin α2

Congenital muscular dystrophies (CMD) are a heterogeneous group of autosomal recessive neuromuscular disorders which is characterized by the early onset of muscle weakness starting at newborn or infant, high serum creatine kinase level, and dystrophic pattern on the muscle biopsy.[1,2] CMD is classified into several forms according to the clinical features and the primary gene defects.[2] Among them, the primary deficiency of merosin is caused by the mutations in the laminin α2 (formerly named merosin) chain gene (LAMA2), which is located at chromosome 6q2. Merosin-deficient CMD is the most common form of CMD in Europeans, while it is rare in Japan and has not reported in Korea.[3] We report a case of merosin-deficient CMD with immunocytochemical analysis of the cytoskeletal proteins.

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Case

A 2-year-old girl presented with a neonatal developmental delay. She was floppy at birth, and the motor milestones were delayed. She had difficulties in swallowing and walking, and was unable to control her head. A neurological examination showed generalized hypotonia with joint contractures in the knees and ankles, and proximal muscle weakness without muscle hypertrophy. There were no sensory abnormalities, and the deep tendon reflexes were preserved. There was no spasticity, Babinski signs, or ankle clonus. Her intellectual and speech development was normal. She had no history of neuromuscular diseases in her family. Serum creatine kinase level was moderately elevated to 914 IU/l (normal <200 IU/l). The electrocardiogram showed sinus tachycardia

(152 beat/min). The motor and sensory nerve conduction studies were normal. A needle electromyography (EMG) revealed a small and polyphasic motor unit action potentials in the left adductor longus and vastus medialis muscles. A muscle biopsy was performed in the left vastus lateralis muscle. There were dystrophic myopathic changes with degenerating and regenerating fibers, increased fiber size variations, perimysial fibrosis and fatty infiltration (Fig. 1-A). Gomori trichrome staining showed no ragged red fibers or nemaline bodies (Fig. 1-B). On ATPase staining, there was no fiber type grouping (figure not shown). Immunohistochemistry using antibodies against C-terminal of dystrophin (NCL-DYS2, Novocastra), N-terminal of dystrophin (NCL-DYS1, Novocastra), rod-domain of dystrophin (NCL-DYS3, Novocastra), laminin α2 (NCL-MEROSIN, Novocastra), and α-sarcoglycan (NCL-a-SARC, Novocastra), β-sarcoglycan (NCL-b-SARC, Novocastra), γ-sarcoglycan (NCL-g-SARC, Novocastra), δ-sarcoglycan (NCL-d-SARC, Novocastra), α-dystrophin (VIA4-1, Upstate), β-dystrophin (NCL-b-DG, Novocastra) and dysferin (NCL-Hamlet, Novocastra) was perform. The immunoreactivity against the laminin α2 chain was completely lost (Fig. 1-D) and α-dystroglycan was not expressed on some of the muscle membranes (Fig. 2). However, dystrophin, α-,β-,γ-,δ- sarcoglycan, dysferin and β-dystroglycan were normally expressed (Fig. 2).

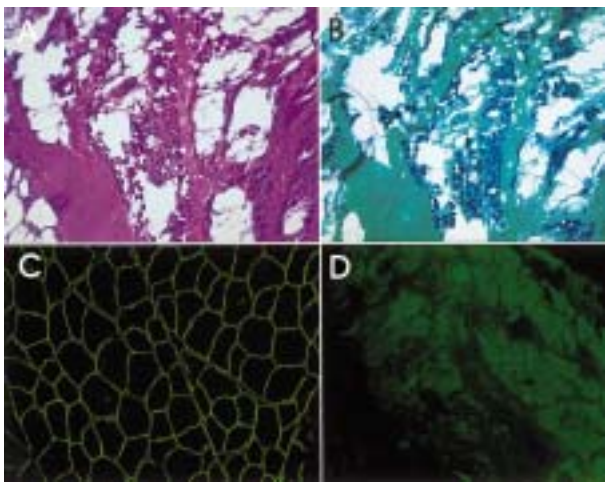

Figure 1. Pathologic and immunocytochemical findings of patient (A, B, D) and normal control (C): There are dystrophic myopathic changes with degenerating and regenerating fibers, increased fiber size variations, perimysial fibrosis and fatty infiltration (A: H&E, magnification ×100, B: trichrome gomori, magnification ×100). All muscle fibers stained positively to anti-merosin antibodies on the muscle surface membrane in the normal controls (C, magnification ×100). In our patient, staining of the laminin α2 chain is negative (D, magnification × 100).

Discussion

Merosin (laminin-2 and -4), which contains the laminin α2 chains, is a major component of basal lamina (BL) of the skeletal muscle fiber. The BL surrounds each muscle fiber and is believed to have a critical role in maintaining the cytoarchitecture and homeostasis of the mature muscle fibers, along with the proper migration and proliferation of myogenic cells during myogenesis.[4,5] Laminin α2 is expressed in numerous tissues including the skeletal muscle fibers, Schwann cells, the synaptic basal lamina of the peripheral nerves, the heart, trophoblast and skin. In skeletal muscle, the laminin (2 is located at the extracellular matrix and provides a link between the extracellular matrix and α-dystroglycan. Although laminin α2 deficiency is caused by merosin-deficient CMD, secondary reduction of laminin α2 had also been reported in other forms of CMD.[6,7] In our case, the partial loss of α-dystroglycan is a secondary change caused by the selective loss of laminin (2, which is directly linked to α-dystroglycan.

The prevalence of CMD was estimated to be 0.7/100000 in a sample from north-east Italy.[8] While merosin deficient CMD accounts for approximately 30% of the CMD cases in European countries, it occupies only 6% in Japan.[3] Tome et al. first demonstrated merosin deficient CMD in 13 out of 20 patients with non-Fukuyama CMD.[1]

The human LAMA2 gene is located on 6q22-23. It spans over 260kb and consists of 64 exons.[3] An analysis of the LAMA2 gene showed that nucleotide substitutions, small deletions, or insertions induce a complete merosin deficiency and a severe phenotype. Partial merosin deficient CMD is caused by a homozygous missense mutation, a missense mutation associated with a nonsense mutation, in-frame deletions in the LAMA2 gene, or nonsense mutations in the last exons of the G domain (exon58 to 64).[9]

The clinical features have been described as severe neonatal hypotonia, joint contracture, inability to walk, highly elevated serum creatine kinase, alterations in the somatosensory and visual evoked potentials, and abnormalities in the brain images. Most patients have a normal intelligence. To date, treatment is not available but the conditions of life can be improved by

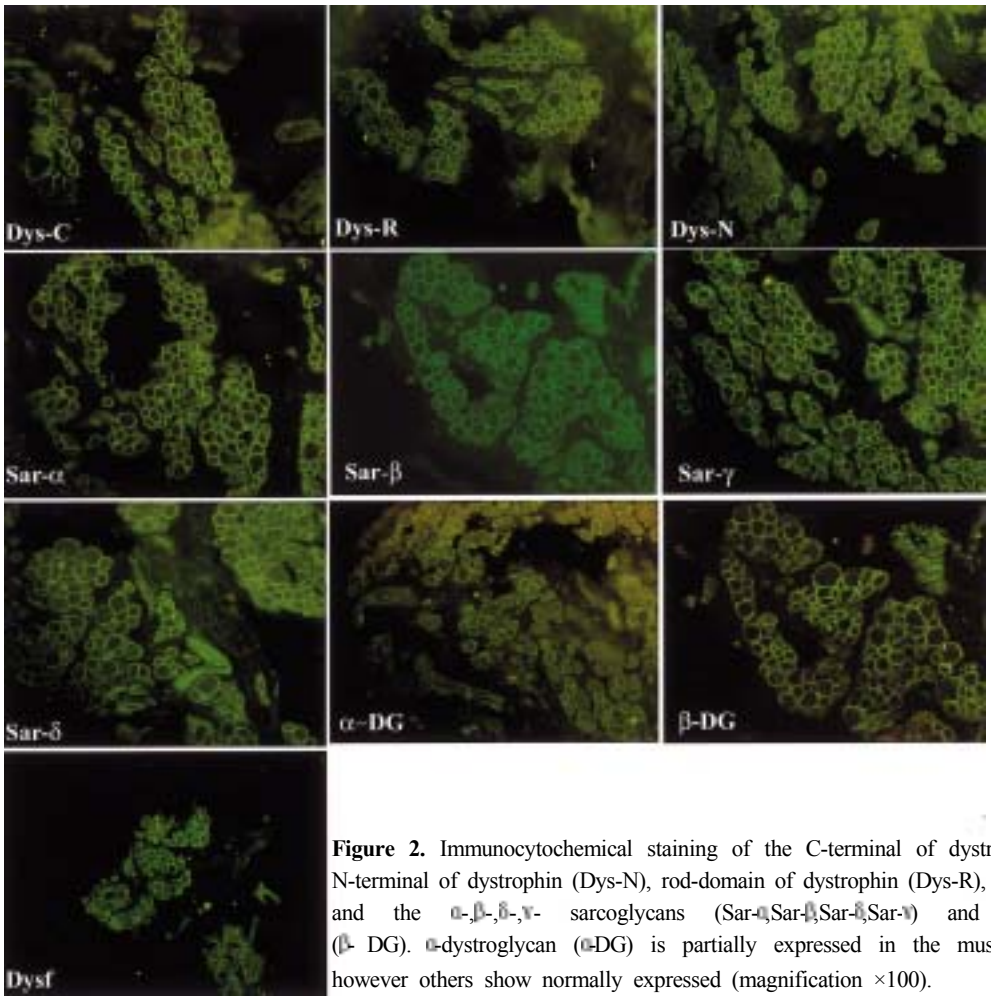

Figure 2. Immunocytochemical staining of the C-terminal of dystrophin (Dys-C), N-terminal of dystrophin (Dys-N), rod-domain of dystrophin (Dys-R), dysferin (Dysf) and the α-,β-,δ-,γ- sarcoglycans (Sar-α,Sar-β,Sar-δ,Sar-γ) and β-dystroglycan (β- DG). α-dystroglycan (α-DG) is partially expressed in the muscle membrane, however others show normally expressed (magnification ×100).

physiotherapy to reduce the contractures and arthrodesis in order to limit deformation.

We described a Korean patient with a merosin deficient CMD, who showed typical clinical features. On immunocytochemistry, there was complete absence of laminin α2 with partial loss of α-dystroglycan. However, the other cytoskeletal proteins were present in the muscle membrane. This is the first case of Korean merosin-deficient CMD confirmed by immunocytochemical staining.

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