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Clinical Notes

Duchenne muscular dystrophy diagnosis using fibroblast-derived myotube cells

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Duchenne muscular dystrophy (DMD) is one of the most common and severe forms of muscular dystrophy with an onset between ages 2 to 4 years.[1,2] With disease progression, movement is gradually lost, eventually leading to motor paralysis, cardiomyopathy, and respiratory disorders with life expectancy reduced to the 30s in general.[1] Patients with Becker muscular dystrophy (BMD), a milder form of DMD, may continue to walk well into their fourth decade or later and can have a normal life expectancy.[1] Duchenne muscular dystrophy and BMD are both caused by alterations in the dystrophin gene (*DMD*), a large gene of 79 exons located at Xp21.2.[1] Mutations in *DMD* that keep the reading frame intact and generate shorter but partly functional dystrophins are associated with the less severe BMD.[1] The multiplex ligation-dependent probe amplification (MLPA) method, a relatively easy blood-based test, can identify deletions and duplications in the exons in about 60–80% of dystrophin-related muscular dystrophy patients.[1] However, point mutations and microdeletions in the remaining 30% of the patients remain unidentified.[1] Here, we report on an advanced-staged case of DMD diagnosis confirmed through *in vitro* myotube cell differentiation using fibroblasts isolated from the patient.

We were presented with the case of a 57-year-old man with severely impaired general and upper limb motor function and a limited range of motion throughout the body, necessitating assistance with daily living activities. The patient required non-invasive positive pressure ventilation all day. There was no information before admission and it was unclear how the DMD diagnosis was made. Serum creatine kinase and lactate dehydrogenase levels were within the normal range. Some DMD patients live to the age of more than 50 years,[1] so there was a possibility of the patient suffering from BMD or some other muscle disease. Therefore, the diagnosis of DMD was reviewed. First, for a definitive diagnosis, *DMD* was analyzed using the MLPA method but no deletions or duplications were observed. Next, to ascertain the dystrophin protein abnormality, the clinical significance of this variant, and to identify the muscular dystrophy type, a muscle biopsy was imperative; however, given the high level of muscle atrophy in the patient (Fig. 1a), it was difficult to obtain the sample for biopsy. We, therefore, used skin-derived fibroblasts to facilitate *in vitro* myotube cell differentiation. Briefly, a retroviral vector carrying the *MyoD* gene (the master regulatory gene of skeletal myogenesis) was transfected into the cutaneous fibroblasts isolated from the patient and cultured for 3 weeks to allow myotube differentiation (as previously described).[2,3] Figure 1b shows the immunostaining image of the dystrophin protein in the *in vitro* differentiated myotube cells; the red part of the image on the left represents the myosin heavy chain, indicating that the *MyoD* transfected fibroblast cells have been replaced with the myotube cells.[3] The dystrophin protein was expressed in the fibroblast-derived myotube cells of the healthy control but not in those derived from the patient's fibroblast.[3]

The defective expression of dystrophin protein in the myotube cells regenerated from the patient's fibroblasts suggested a case of DMD rather than BMD. To further investigate the pathogenesis, the *DMD* exon was sequenced. Two rare variants, c.5878G>T causing p. Glu1960* and c.2729A>T causing p. Asp910Val were detected. The ClinVar archives do not contain the p. Glu1960* variant, however, they include the p. Asp910Val variant with uncertain significance. It was assumed that this nonsense mutation (c.5878G>T (p. Glu1960*)) caused the defective expression of the dystrophin protein resulting in the DMD condition.

This mutation was a nonsense mutation, but had it been a missense mutation, a muscle biopsy would have been required for a definite DMD diagnosis. Some reports suggest attempts to make a definitive diagnosis with melanocytes, but there is an associated limitation because the method does not use muscle samples.[4] In this study, we used *in vitro* differentiation of the fibroblasts into myotube cells to confirm DMD diagnosis. This method using skin biopsy is a far less invasive alternative

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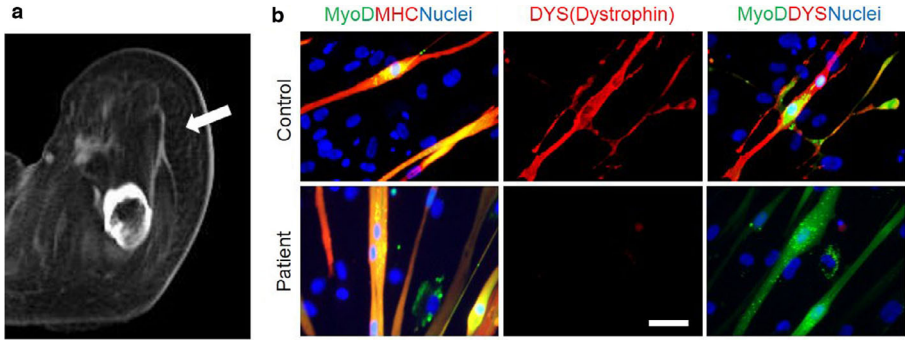

Fig. 1 (a) Computed tomography scan of the region from the buttocks to the thigh indicates atrophy of the quadriceps (arrow). (b) Immunostaining of the dystrophin protein after myogenic differentiation. The red part of the image on the left represents the myosin heavy chain (MHC) indicating that *MyoD* transfected cells have been replaced with myotube cells. The red part in the middle and right images indicates the dystrophin protein. The myotubes from *MyoD* transfected patient fibroblasts showed defective expression of the dystrophin protein compared to those from the healthy control.

to diagnose DMD. This method might prove helpful in definite diagnosis and genetic support for patients with DMD.

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Disclosure

The authors declare no conflict of interest.

Author contributions

Y.O. wrote the initial manuscript, and acquired and analyzed data. M.F. contributed to the conception and design of the study, and revised the manuscript. S.T. provided the study materials. H.K. revised the manuscript. All authors approved the final manuscript.

Ethics

The study was approved by the institutional review board of the National Hospital Organization Nagara Medical Center, Japan. Informed consent for the publishing this study was obtained from the patient.

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