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Identification of a Dysferlin Gene Mutation in a Korean Case with Miyoshi Myopathy

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Recent genetic and immunohistochemical analyses have shown that Miyoshi myopathy (MM) is caused by a mutation in the DYSF gene, which induces dysfunction of dysferlin. The author described one patient showing characteristic MM phenotype with deficiency of dysferlin on immunohistochemistry. Direct DNA sequencing of whole exons of DYSF gene revealed one homozygous missense mutation (G1165C) on exon 12, which let to an amino acid substitution from the glutamic acid to glutamine at the 389 of the peptide sequence in this patient. This is the first reported case of MM confirmed by immunohistochemical and genetic analyses in Korea.

Key Words: Miyoshi myopathy (MM), dysferlin, DYSF gene

INTRODUCTION

Miyoshi myopathy (MM) is a subtype of distal myopathy that is characterized by the progressive muscle weakness predominantly involves the calf muscle, an autosomal recessive inheritance, an adolescent or young adulthood onset, and a relatively high serum creatine kinase (CK) level. Recent linkage analysis found that MM is caused by mutation of the dysferlin gene (DYSF, MIM* 603009), which located on chromosome 2p13.3 and it induces either a reduction or a loss of the sarcolemmal protein, named "dysferlin".[1] An identical mutation of the DYSF gene in MM patients or an autosomal recessive limb-girdle muscular dystrophy type 2B (LGMD2B) has also been reported, which suggests that LGMD2B is an allelic variant of the same gene for MM.[2,3] Distal anterior compartment myopathy (DMAT), which is the third muscular dystrophy-phenotype caused by a DYSF gene mutation, was recently described.[4] There was intra- or inter-familial variation in the clinical phenotype, or in the severity of the same DYSF gene mutation. The type of mutation is highly variable, including a missense or nonsense mutation as well as a deletion or insertion. Therefore, there is no mutational hot spot that can be easily detected. Moreover, there is no correlation between the genotype-phenotypical severity and the wide intra- or inter-familial variation in the clinical phenotype.[5,6]

This paper described a Korean patient showing the MM phenotype clinically, along with the results of the immunohistochemical and molecular analyses of the DYSF gene. By screening the entire coding region of the DYSF gene, one homozygous missense mutation was identified on exon 12 of the dysferlin gene.

CASE REPORT

The patient was a 20 year-old male patient who had progressive weakness on both lower extremities for previous 5 years. A neurological examination showed bilateral motor weakness in the legs, which was more severe on the distal part than on the proximal part. Calf muscle atrophy was noted on the bilaterally. The patient could

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walk independently, and stand on his heels but not on his tiptoes. Otherwise, there were not remarkable findings. His parents were not consaguious, and the neurological function of his parents and siblings were normal. The patient's serum creatine kinase (CK) level was elevated to 6400 IU/L (normal; 35-232). Electromyography (EMG) revealed active myopathic changes in the bilateral vastus lateralis, tibialis anterior, and gastrocnemius muscles. The results of nerve conduction study were normal.

A routine pathological examination by a biopsy at biceps femoris muscle showed the following mild dystrophic myopathic changes: increased internal nuclei, fiber size variations without an endomysial or perivascular inflammation, and a rimmed vacuole. Immunohistochemical analyses of the biosed muscle-cryosections were also performed with the antibodies against the various parts of dystrophin (NCL-Dys 1, Dys 2, Dys 3, Novocastra Laboratories, Newcastle upon Tyne, UK), α-sarcoglycan, β-sarcoglycan, γ-sarcoglycan, δ-sarcoglycan (Novocastra Laboratories, Newcastle upon Tyne, UK), dysferlin (Novocastra Laboratories, Newcastle upon Tyne, UK), β-dystroglycan (NCL- β-DG, Novocastra, Newcastle Upon Tyne, UK), and caveolin-3 (Transduction laboratory, Lexington, KY). The expressions of each of the three domains of dystrophin, α, β, γ, δ-sarcoglycan, β-dystroglycan, and caveolin-3 were normal at the periphery of the muscle membrane (Fig. 1). However, a negative immunoreactivity against dysferlin was noted at the sarcolemma when compared with the control (Fig. 2).

For the genetic analysis, the total RNA was isolated from 20 slices of the 20 μm biopsed muscle crystal sections, and 1 μg of the total RNA was reverse-transcribed into the first-strand cDNA. The reverse transcribed-polymerase chain reaction (RT-PCR) using TaKaRa RNA PCR^{TM} kit ver 2.1 (TAKARA bio Inc. Kyoto, Japan) was designed to cover the entire human dysferlin cDNA, as previously described.[7] The amplification reaction mixtures contained 1.5 μl of the template cDNA, 10X PCR buffer, 1.5 mM $MgCL_2$, 10 mM Tris-HCL (pH 9.0), 40 mM KCL, 250 μM dNTPs, 2 μl of each set of primers, and 1 U of Taq DNA polymerase. The amplification profile consisted of an initial denaturation step at 94℃ for 5 min, and 35 cycles of denaturation at 94℃ for 1 min, annealing at either 55℃ or 57℃ for 1 min, and extension at 72℃ for 1 min, and a final extension 72℃ for 5 min using PCR PreMix (Bioneer AccuPower®), Daejeon, Korea). The PCR products were directly sequenced on both strands using an ABI 3700 DNA sequencer (GMI Inc. MN, USA). The genomic DNA was amplified by PCR in the region corresponding to a mutation found in the dysferlin cDNA using the exon-specific intronic primers [forward 5'-CGAAG CTGGGAACTCTTA GAA-3' (Nos. 50569204-50569 223) and reverse 5'-CAGCCTCCTGCAGACCTC-3' (Nos. 50569438-50 569455)][1] The amplification profile consisted of an initial denaturation step at 94℃ for 2 min, and 35 cycles of denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, and extension at 72℃ for 5 min. Each PCR product was directly sequenced described above.

Direct sequencing analysis of the DYSF gene identified one homozygous missense mutation in exon 12. This mutation was a G-to-C transition at the portion 1165 of the coding sequence (G1165C), which let to an amino acid substitution from the glutamic acid [E] to glutamine [Q] at the 389 of the peptide sequence (Fig. 3). The results of gDNA sequencing showed the same nucleotide changes at the portion of 1165 on exon 12. This nucleotide change was not observed in the 30 control DNA samples (60 chromosomes) used in this study.

DISCUSSION

Our case met the characteristic clinical features of MM.[8] Immunohistochemistry showed marked deficiency of dysferlin, and genetic analysis revealed one homozygous missense mutation on exon 12 of DYSF gene. We believe that the G1165C mutation is a causative of disease, since this mutation in our patient was under homozygous status, and it was not found in the 30 healthy controls. Furthermore, one Portuguese with a compound heterozygous G1165C/C509A mutation was reported in the Leiden Muscular Dystrophy Database Page (http://www.dmd.nl/md.html), which supports our observation. To our knowledge, this is the first reported MM case confirmed by immunohistochemical and genetic analysis in Korea.

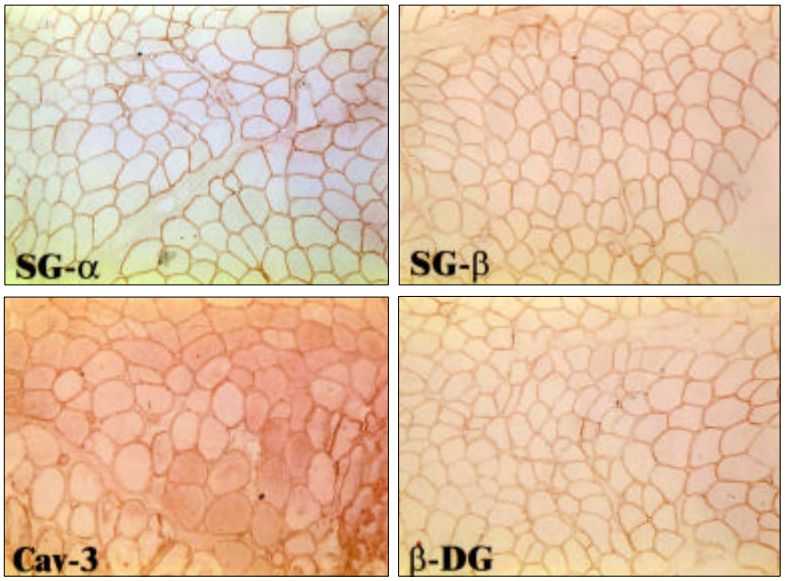

Fig. 1. Immunohistochemistry of SG-α, SG-β, Cav-3 and β-DG using a diaminobenzidine (DAB)-peroxidase reaction technique. Abbreviations: SG-α, α-sarcoglycan; SG-β, β-sarcoglycan; Cav-3, caveolin-3; β-DG, β-dystroglycan ($\times$ 200 magnification).

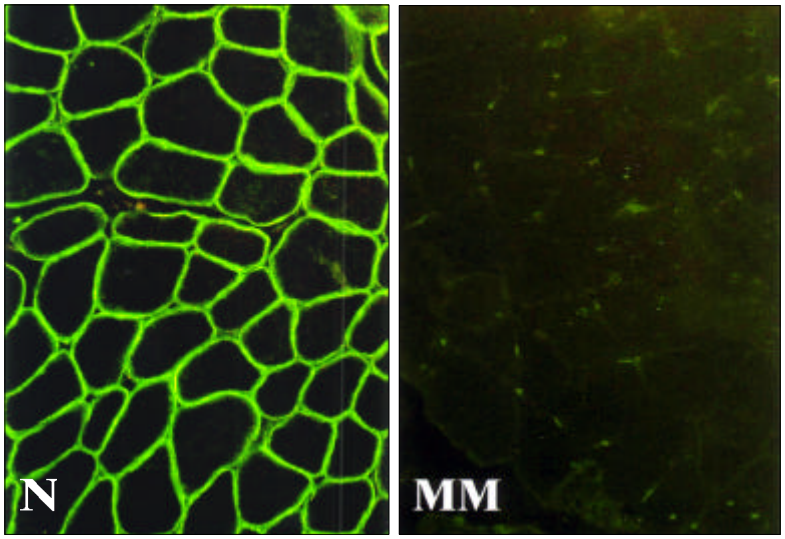

Fig. 2. Immunohistochemistry using a fluorescent isothiocyanate (FITC)-avidin D technique in patient and normal control (N). ($\times$ 200 magnification) MM, Miyoshi myopathy.

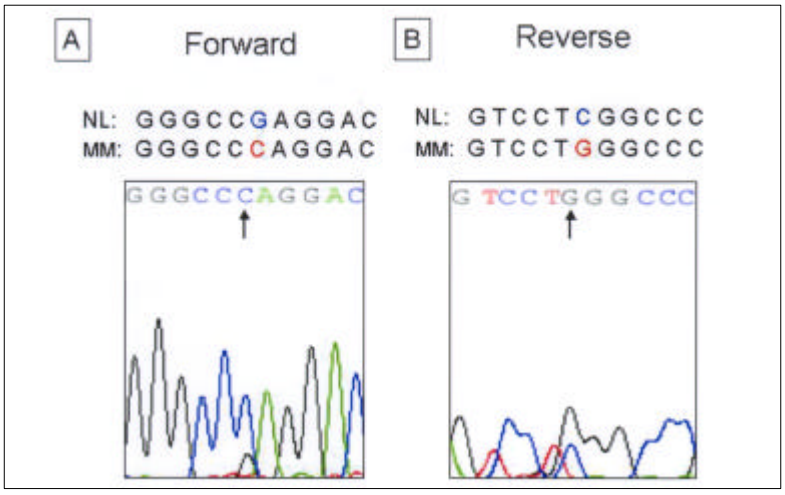

Fig. 3. cDNA sequencing analysis of exon 12 of in the DYSF gene. There was a G-to-C transition at portion 1165 of the coding sequence from the forward (A) and reverse (B) strands. G, guanine; A, adenine; T, thymine; C, cytosine; NL, normal control; MM,

Dysferlin is composed of 2080 amino acids with six putative C2 domains, and one transmembrane domain near the C-terminus. The C2 motif appears to bind calcium and mediate the calcium-dependent signaling process. Dysferlin is homologous (27% identity, 57% similarity) to the nematode (Caenorhabditis elegans) spermatogenesis factor FER-1, which has been implicated in membrane fusion.[9] A recent study reported that dysferlin plays a role in the calcium-dependent membrane fusion step of the sarcolemmal repair process,[10] and the region located at either the C2 domain or the transmembrane domain were believed to be essential for maintaining the function of dysferlin.[7,11] Although the mutation in our cases was located outside the C2 domain or transmembrane domain, it may be assumed that even a single missense nucleotide change in the DYSF gene results in a conformational change or increased degradation of the mutant dysferlin protein because there was negative reactivity against the dysferlin protein on immunohistochemistry.

Immunohistochemistry in our case revealed that the immunoreactivity of caveolin-3 was normal. Caveolin-3 is another sarcolemmal protein that is important in the formation of caveolae, and is the first-proven interacting partner of dysferlin.[12] A mutation of CAV3 causes autosomal dominant limb-girdle muscular dystrophy type 1C (LGMD1C). Amino acid sequence analysis of the dysferlin protein revealed 7 sites corresponding to the caveolin-3 scaffold-binding motifs, and 1 site that is a potential target for binding the WW domain of the caveolin-3.[12] Although the mutation found in this study was not associated with the secondary reduced expression level of caveoiln-3, lower caveolin-3 expression levels have been reported in some MM patients, and vice versa.[13,14] This underlines the importance of the genetic analysis of DYSF gene or CAV3 for the detection of secondary changes in these proteins when a combined deficiency of dysferlin and caveolin-3 are observed on immunohistochemistry.

In conclusion, we identified one homozygous missense mutation of DYSF gene with deficient dysferlin on immunohistochemistry in a Korean MM patient. Further studies will be needed to clarify the clinical and pathological characteristics of MM in this type of mutation.

REFERENCES

1. Liu J, Aoki M, Illa I, Wu C, Fardeau M, Angelini C, et al. Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. Nat Genet 1998;20:31-6.
2. Illarioshkin SN, Ivanova-Smolenskaya IA, Greenberg CR, Nylen E, Sukhorukov VS, Poleshchuk VV, et al. Identical dysferlin mutation in limb-girdle muscular dystrophy type 2B and distal myopathy. Neurology 2000;55:1931-3.
3. Weiler T, Bashir R, Anderson LV, Davison K, Moss JA, Britton S, et al. Identical mutation in patients with limb girdle muscular dystrophy type 2B or Miyoshi myopathy suggests a role for modifier gene(s). Hum Mol Genet 1999;8:871-7.
4. Illa I, Serrano-Munuera C, Gallardo E, Lasa A, Rojas-Garcia R, Palmer J, et al. Distal anterior compartment myopathy: a dysferlin mutation causing a new muscular dystrophy phenotype. Ann Neurol 2001;49:130-4.
5. Aoki M, Liu J, Richard I, Bashir R, Britton S, Keers SM, et al. Genomic organization of the dysferlin gene and novel mutations in Miyoshi myopathy. Neurology 2001;57:271-8.
6. Takahashi T, Aoki M, Tateyama M, Kondo E, Mizuno T, Onodera Y, et al. Dysferlin mutations in Japanese Miyoshi myopathy: relationship to phenotype. Neurology 2003;60:1799-804.
7. Ueyama H, Kumamoto T, Nagao S, Masuda T, Horinouchi H, Fujimoto S, et al. A new dysferlin gene mutation in two Japanese families with limb-girdle muscular dystrophy 2B and Miyoshi myopathy. Neuromuscul Disord 2001;11:139-45.
8. Bashir R, Strachan T, Keers S, Stephenson A, Mahjneh I, Marconi G, et al. A gene for autosomal recessive limb-girdle muscular dystrophy maps to chromosome 2p. Hum Mol Genet 1994;3:455-7.
9. Argon Y, Ward S. Caenorhabditis elegans fertilization-defective mutants with abnormal sperm. Genetics 1980; 96:413-3.
10. Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, Williamson R, et al. Defective membrane repair in dysferlin-deficient muscular dystrophy. Nature 2003; 423:168-72.
11. Rizo J, Sudhof TC. C2-domains, structure and function of a universal Ca^{2+}-binding domain. J Biol Chem 1988; 273:15879-82.
12. Matsuda C, Hayashi YK, Ogawa M, Aoki M, Murayama K, Nishino I, et al. The sarcolemmal proteins dysferlin and caveolin-3 interact in skeletal muscle. Hum Mol Genet 2001;10:1761-6.
13. Walter MC, Braun C, Vorgerd M, Poppe M, Thirion C, Schmidt C, et al. Variable reduction of caveolin-3 in patients with LGMD2B/MM. J Neurol 2003;250:1431-8.
14. Figarella-Branger D, Pouget J, Bernard R, Krahn M, Fernandez C, Levy N, et al. Limb-girdle muscular dystrophy in a 71-year-old woman with an R27Q mutation in the CAV3 gene. Neurology 2003;61:562-4.