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Clinical and Genetic Analysis of Korean Patients with Miyoshi Myopathy: Identification of Three Novel Mutations in the *DYSF* Gene

Miyoshi myopathy (MM) is an autosomal recessive distal muscular dystrophy caused by mutations in the dysferlin gene (*DYSF*) on chromosome 2p13. Although MM patients and their mutations in the *DYSF* gene have been found from all over the world, there is only one report of genetically confirmed case of MM in Korea. Recently, we encountered three unrelated Korean patients with MM and two of them have previously been considered as having a type of inflammatory myopathy. The clinical and laboratory evaluation showed typical features of muscle involvement in MM in all patients but one patient initially had moderate proximal muscle involvement and another showed incomplete quadriparesis with rapid progression. Direct sequencing analysis of the *DYSF* gene revealed that each patient had compound heterozygous mutations (Gln832X and Trp992Arg, Gln832X and Trp999Cys, and Lys1103X and Ile1401HisfsX8, respectively) among which three were novel. Although MM has been thought to be quite rare in Korea, it should be considered in a differential diagnosis of patients exhibiting distal myopathy.

Key Words : *Distal Myopathies; Muscular Diseases; DYSF protein, human; Muscular Dystrophies, Limb-Girdle; Mutation*

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INTRODUCTION

Muscular dystrophies with an autosomal recessive mode of inheritance comprise of a genetically heterogeneous group of disorders. Among these, Miyoshi myopathy (MM) is an early-adult onset, autosomal recessive form of distal muscular dystrophy, characterized by predominant involvement in the calf muscles and highly elevated serum creatine kinase (CK) levels (1). MM has been known to be caused by mutations in the dysferlin gene (*DYSF*) on chromosome 2p13 (2). Mutations in the same gene were also identified in patients with limb-girdle muscular dystrophy type 2B (LGMD2B) (3), and distal anterior compartment myopathy (4). These diseases caused by mutations in *DYSF* are known by the term 'dysferlinopathy'.

Although MM patients and their mutations in the *DYSF* gene have been found from all over the world, only a few cases have been reported in Korea (5-8). Considering that there have been many reports of MM among Japanese population (9), the incidence of MM might be underestimated in Korea since both countries are geographically and ethnically related with each other (10, 11). In the present study, we performed clinical and genetic analysis of three Korean patients with MM and found that all patients had compound heterozygous mutations in the *DYSF* gene.

MATERIALS AND METHODS

Subjects

Three unrelated and non-consanguineous patients with clinical features of MM were evaluated (Table 1). All the patients experienced early adulthood onset of slowly progressive muscle weakness, which preferentially involved muscles in the lower extremities. Patients 1 and 2 had a history of steroid medication under the presumptive diagnosis of polymyositis according to the muscle biopsies and both showed transient responses. Patient 3 suffered from diabetes and showed the EMG findings of superimposed peripheral polyneuropathy.

Mutation analysis

The genomic DNA was extracted from the peripheral blood leukocytes using a Wizard Genomic DNA Purification kit according to the manufacturer's instructions (Promega, Madison, WI, U.S.A.). All the coding exons as well as the flanking introns of the *DYSF* gene were amplified using the primer sets designed by the authors (primer sets available on request). A polymerase chain reaction was performed with a thermal cycler (model 9700, Applied Biosystems, Foster City, CA,

U.S.A.) using the following conditions: 32 cycles of denaturation at 94℃ for 30 sec, annealing at 60℃ for 30 sec, and extension at 72℃ for 30 sec. After the amplicon (5 μL) treatment with 10 U shrimp alkaline phosphatase and 2 U exonuclease I (USB Corp., Cleveland, OH, U.S.A.), direct sequencing was performed with the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) using a ABI Prism 3,100 genetic analyzer (Applied Biosystems). All the novel mutations were confirmed by sequencing 100 control chromosomes.

RESULTS

Clinical findings

Patient 1 was a 45 yr-old male patient who had presented with progressive weakness in both lower extremities for the previous 17 yr. He noticed difficulty in running and atrophy of the lower leg muscles at 27 yr of age. The muscle weakness and atrophy progressed. A neurological examination showed bilateral motor weakness and symmetric atrophy of the entire lower extremity muscles, which were more severe on the distal part than on the proximal part. Marked atrophy of the lower leg muscles with relatively spared pelvic girdle and upper thigh muscles resulted in a stalk-leg appearance. He required a cane for level walking and also complained of decreased pinch strength. He had been diagnosed of having a polymyositis at the age of 28 yr as a result of a muscle biopsy of the medial gastrocnemius showing myopathic changes with inflammatory cell infiltration. Immunohistochemical stain was not performed on the biopsy specimen. His 43 yr-old younger sister was affected in the same way in her early thirties and found it difficult to walk up and downhills. Since then, the muscle weakness worsened so that walking was still possible but only for short distances using a cane. She also

Table 1. Summary of the clinical and laboratory findings of the patients

Patient	Sex/ age (yr)	Age at onset (yr)	Muscle biopsy finding	EMG	CK level (IU/L)	Family history	Distribution
1	M/44	28	Inflammatory myopathy	myopathic	5,400	+	Thigh, Lower leg, Fore-arm
2	F/30	26	Inflammatory myopathy	myopathic	5,152	–	Upper arm, Thigh, Lower leg
3	M/37	18	Not available	myopathic	2,100	+	Upper arm, Fore-arm, Thigh, Lower leg

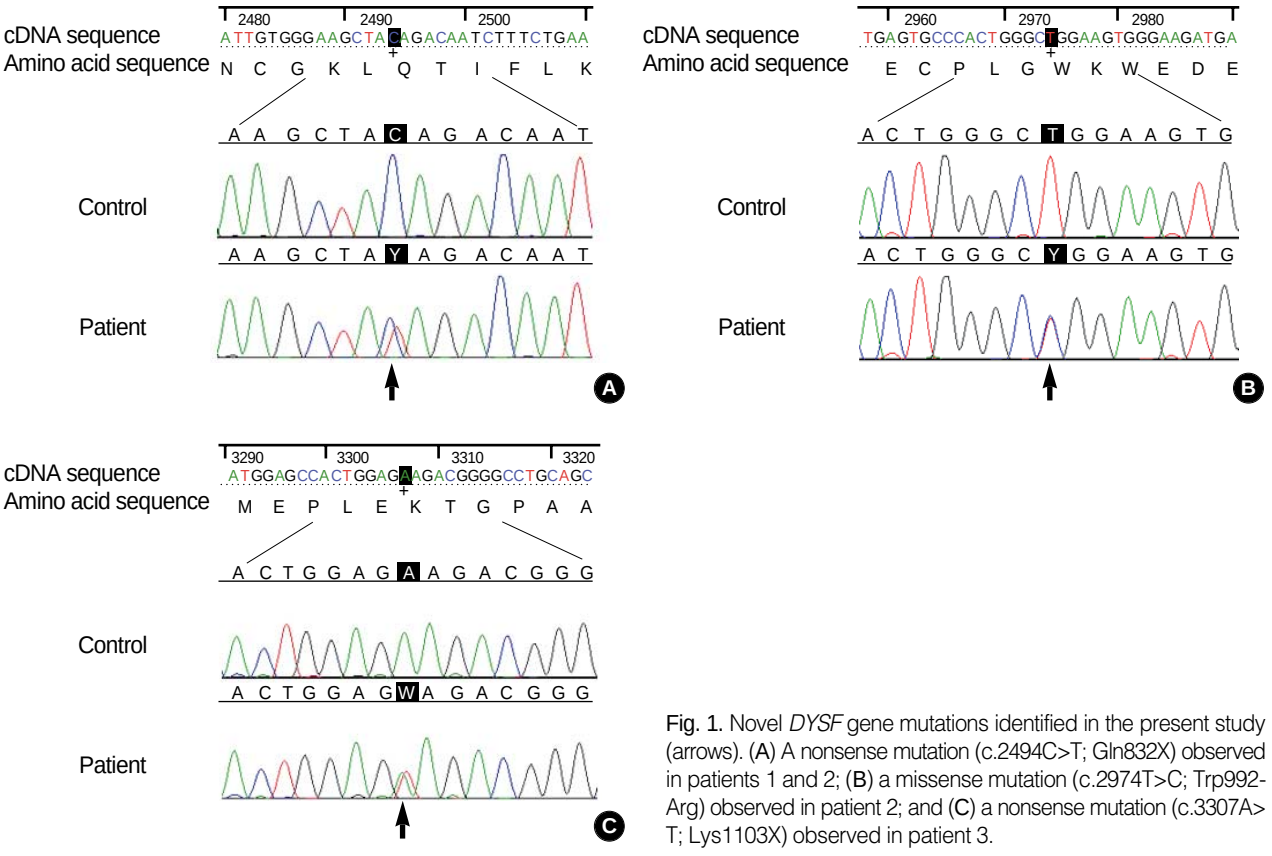

Fig. 1. Novel *DYSF* gene mutations identified in the present study (arrows). (A) A nonsense mutation (c.2494C>T; Gln832X) observed in patients 1 and 2; (B) a missense mutation (c.2974T>C; Trp992-Arg) observed in patient 2; and (C) a nonsense mutation (c.3307A>T; Lys1103X) observed in patient 3.

had difficulty in holding her baby due to extended weakness of her upper extremity muscles. Standing on their tip-toe or heels were impossible in both patients. They could stand from sitting position only with the aid of their hands.

Patient 2 was a 30 yr-old female who presented with difficulty in stair walking and pain in her low back and knee. Difficulty in climbing stairs developed at 26 yr of age and this difficulty in walking gradually worsened so that she could not climb stairs without the support of her upper extremities. A neurological examination showed atrophy of both calf muscles, decreased strength of the lower extremity muscles, and an absent tendon reflex. The Gowers' sign was positive. She could not stand on her tip-toe, but standing on her heels was possible. The EMG revealed the myopathic changes in gastrocnemius, rectus femoris, and biceps brachii muscles. A muscle biopsy without immunohistochemial stain from the biceps brachii suggested a diagnosis of inflammatory myopathy. Magnetic resonance imaging of her lower leg disclosed symmetric fatty degeneration and decreased muscle volume of the posterior compartment muscles in both lower legs. The anterior and lateral compartment muscles had either a normal or a relatively normal signal intensity and morphology. The patient denied a family history of neuromuscular disease.

Patient 3 was a 37-yr-old man with quadriparesis who first became aware of periodic weakness in his lower extremities at the age of 18 yr. He noticed difficulties in standing and climbing stairs at the age of 21 and could not walk by himself by the age of 33. An examination at the age of 37 showed incomplete quadriparesis and he could walk with a four-point crutch. His older sister developed lower extremity weakness and atrophy at the age of 18 yr and could not walk by the age of 36.

Mutation analysis

Direct sequencing analysis identified five heterozygous mutations in the *DYSF* gene including three novel mutations (Fig. 1). Patient 1 and his sister had compound heterozygous mutations of a novel C to T transition (c.2494C>T) in exon 24 resulting in a nonsense mutation (Gln832X) along with a previously reported missense mutation (c.2997G>T; Trp999Cys). The Gln832X mutation was also found in the patient 2 with a novel missense mutation (c.2974C>T; Trp992Arg). Patient 3 had two mutations including a novel A to T transversion (c.3307A>T) in exon 30, which resulted in a nonsense mutation (Lys1103X), and a previously reported insertion mutation (c.4200dupC) in exon 39 resulting in a frame shift mutation (Ile1401HisfsX8). Three novel mutations (Gln832X, Trp992Arg, and Lys1103X) were not detected in 100 control chromosomes.

DISCUSSION

This study identified five different mutations in three Korean MM patients, and all patients had compound heterozygous mutation in the *DYSF* gene. The c.2997G>T (Trp999Cys) mutation is reported to be one of the most frequent mutations found in Japanese MM patients, and has been associated with a significantly later onset and a relatively mild form of MM (9). The age at onset in patient 2 with the c.2997G>T mutation was delayed compared with the other patients, but was earlier than the mean age at onset reported elsewhere (9). The c.4200dupC mutation (Ile1401HisfsX8), which was found in two patients with MM or LGMD2B, appeared in the Dysferlin sequence variations in the Leiden Muscular Dystrophy pages. The c.2494C>T (Gln832X) mutation was novel and was found in 2 unrelated patients, which might be due to a possible founder effect in the Korean population. However, it will be necessary to perform haplotype analysis and collect more data on MM patients in Korea to address this issue.

Some patients with MM can be easily misdiagnosed as having an inflammatory myopathy because inflammation is frequently observed in patients with MM and the inflammatory changes found in patients with MM are indistinguishable from those observed in patients with inflammatory myopathy (12). Indeed, two patients in this study had previously been suspected of having a form of inflammatory myopathy based on the muscle biopsy findings. McNally et al. (13) reported that the presence of inflammation is not an exclusion criterion in muscular dystrophy related to a dysferlin deficiency, while the muscle biopsies of MM or LGMD2B patients generally demonstrates signs of a dystrophy with a dysferlin deficiency.

It is possible that some MM patients have been erroneously diagnosed with polymyositis based on the absence of dystrophic features and the presence of inflammation in the muscle biopsies. Consequently, MM might has been considered to be quite rare in Korea. Therefore, MM should be considered when making a differential diagnosis of patients exhibiting distal myopathy and muscular inflammation on muscle biopsies, and *DYSF* gene analysis should also be considered.

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