Mutation at a new allele of the dysferlin gene causes Miyoshi myopathy: A case report

Hai-Yan Yan¹, Ya-Nan Xie², Jing-Zhe Han¹, Xue-Qin Song³,⁴,⁵

¹Department of Neurology, Harrison International Peace Hospital, Hebei, China; ²Department of Angiocardiopathy, The Second Hospital of Hebei Medical University, Hebei, China; ³Department of Neurology, The Second Hospital of Hebei Medical University, Hebei, China; ⁴Institute of Cardiocerebrovascular Disease, Hebei, China; ⁵Neurological Laboratory of Hebei Province, Hebei, China

Abstract

Miyoshi myopathy (MM) is a rare autosomal recessive disorder caused by dysferlin (DYSF) gene mutation. Miyoshi myopathy-inducing mutation sites in the DYSF gene have been discovered worldwide. In the present study, a patient with progressive lower extremity weakness is reported, for which MM was diagnosed according to clinical manifestations, muscle biopsy, and immunohistochemistry. In addition, the DYSF gene of the patient and his parents was sequenced and analyzed and two heterozygous mutations of the DYSF gene (c.4756C> T and c.5316dupC) were discovered. The first mutation correlated with MM while the second was a new mutation. The patient was diagnosed with a compound heterozygous mutation. The mutation site is a new member of pathogenic MM gene mutations.

Keywords: Dysferlin, Gene Mutation, Miyoshi Myopathy, Muscle, Recessive Inheritance

Introduction

Miyoshi myopathy (MM) is a rare autosomal recessive inherited distal myopathy caused by mutations in the dysferlin (DYFS) gene. It is primarily characterized by symmetrical involvement of the distal lower limbs, primarily affects the gastrocnemius and soleus muscles, and can affect the proximal muscles and muscles of the upper limbs in the late stage of the condition¹. In the present study, a sequencing analysis of the DYSF gene was performed on a patient who had been clinically diagnosed with MM. The results detected two DYSF gene heterozygous mutation sites (c.4756C> T and c.5316dupC), one of which was a new mutation.

Case presentation

A 22-year-old male patient was admitted to our hospital mainly due to progressive aggravation of lower limb weakness over a period of eight years. The patient had lower limb adynia eight years previously that was most severe in the distal end. Furthermore, the patient had difficulty with toe walking and easily became fatigued when walking. The patient’s symptoms slowly became aggravated. The patient gradually developed strenuosity while walking and had difficulty walking up the stairs. The patient was then admitted to our hospital. His creatine kinase (CK) was 6,679 U/L (reference range: 18.0-198.0 U/L), and electromyography revealed myogenic abnormal results in this regard. The patient reported no history of other diseases and denied a similar family medical history.

Physical examination results

In the distal lower limbs, the muscle force was level 4⁻ and the proximal muscle force level was 4⁺; in the upper limbs, the muscle force was level 5. The bilateral gastrocnemius muscle and soleus muscle presented with atrophy. The lower limb tendon reflex was attenuated. The bilateral Babinski sign was

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Corresponding author: Jing-Zhe Han M.D., Department of Neurology, Harrison International Peace Hospital, No. 2 Renmin Zhong Road, Taocheng District, Hengshui 053000, China
E-mail: hanjingzhe1915@163.com

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negative. No abnormalities were found in other neurological examinations. No abnormalities were found in the antinuclear antibodies, autoantibody profile, or thyroid function. Electromyography revealed that the bilateral anterior tibial muscles, gastrocnemius muscle, quadriceps muscle of the thigh, and right short abductor muscle of the thumb all presented with obvious short-spined polyphasic waves, and a pathological interference pattern was present during re-contraction, suggesting myogenic damage. After treatment, a re-examination of the creatase revealed a CK level of 3,455 μ/l. Furthermore, the gastrocnemius muscle presented with obvious atrophy. Therefore, a biopsy was performed in the right quadriceps muscle of the thigh, in which the transverse section presented with several muscle fiber bundles with clear boundaries. The hematoxylin and eosin staining revealed muscle fibers that were closely arranged in muscle bundles of various sizes; some atrophic muscle fibers had a circular or angular appearance, and degeneration, necrosis, and muscle regeneration could be observed. Inflammatory cells were scattered among the muscle fibers, and muscle division and nuclear ingression were occasionally observed. Modified Gomori trichrome staining revealed no ragged red fibers. Nicotinamide adenine dinucleotide-tetrazolium reductase staining and succinate dehydrogenase staining revealed that the distribution of oxidase was uneven in some muscle fibers, while a few atrophic muscle fibers were deeply stained. Acid phosphatase staining revealed that the enzyme activity was

Figure 1. The H&E staining revealed that the muscle fibers were closely arranged in the muscle bundles in various sizes, some atrophic muscle fibers had a circular or angular appearance, and the degeneration, necrosis and muscle regeneration could be observed. The inflammatory cells were scattered among the muscle fibers, and muscle division and nuclear ingression were occasionally observed. The MGT staining revealed no ragged red fibers. The NADH-TR staining revealed that the distribution of oxidase was uneven in some muscle fibers, and few atrophic muscle fibers were deeply stained. The ATP enzyme staining revealed that type II muscle fiber distribution was slightly dominant, and the atrophy affected two types.

Figure 2. Immunohistochemistry: The dysferlin protein staining revealed the complete absence of dysferlin protein in the myocutaneous membrane (A: control; B: absence).
The DYSF protein staining revealed the complete absence of DYSF protein in the myocutaneous membrane (Figure 2). The pathological diagnosis of the muscle was myogenic damage, suggesting dysferlinopathy. The gene sequencing revealed that the patient had compound heterozygous mutations in the DYSF gene, among which c.4756C>T was a mutation in which the 4,756th nucleotide in the coding region was altered from cytosine into thymine, causing amino acid change. In this alteration, p.R1586X (nonsense mutation) was derived from the father, c.5316dupC was a mutation wherein a cytosine had been inserted at the 5,316th nucleotide in the coding region (causing amino acid change), and p.S1772fs (frameshift mutation) was derived from the mother (Figure 3). The final diagnosis was MM.

**Immunohistochemistry**

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**Discussion**

Miyoshi myopathy is a rare distal myopathy that is derived from autosomal recessive inheritance. Miyoshi et al. first reported its pathogenic gene as being the DYSF gene located in 2p13 in 1967. The encoding product of the gene is the DYSF protein. The molecular weight of the protein is approximately 230,000, and it is primarily expressed in skeletal muscles and myocardia. Its function is correlated with the repair of the myocyte membrane. The C-terminal of DYSF protein is a transmembrane domain, and the N-terminal and other protein structures are located in the cytoplasm. It is involved in the process of muscle contraction, vesicle aggregation, and membrane fusion and is related to the regeneration and repair of muscle cell membranes. The process of DYSF protein participating in repairing damaged plasma membrane is completed by exocytosis of various intracellular vesicles triggered by calcium ions and contributed to by lysosomes. Following the vesicle-vesicle, and vesicle-serosa fusion processes, the large vesicles quickly gather at the injured area and transport a membrane patch to repair the damaged membrane structure. Mutations in the DYSF gene will, accordingly, cause the loss of function of the DYSF protein, while the damaged myocyte membranes will cause degeneration or necrosis due to ineffective repair. The muscle diseases caused by DYSF protein deficiency have a variety of clinical manifestations, which can be broadly divided into MM, LGMD 2B, distal anterior sylmoth myopathy, and others. The early involved muscles determine the clinical phenotype of dysferlinopathy. The typical clinical manifestations of MM are progressive adynamia and atrophy of the distal muscles of the extremities, which mainly affect the muscle groups of the posterior legs, such as the gastrocnemius and soleus in the early stage; it can also affect the proximal muscles, such as the biceps muscle of the thigh, the quadriceps muscle of the thigh, and the iliopsoas muscles in the late stage. In the early stage, the CK can significantly increase. The typical clinical manifestations and DYSF protein deletion or expression reduction are highly indicative of MM, and DYSF gene mutations can make a clear diagnosis.

The patient included in this case study developed the disease in adolescence. The main manifestation was adynamia of the distal lower limbs. Furthermore, his serum CK level was significantly increased. Electromyography suggested myogenic damage. All of the above manifestations suggested that muscle disease was more likely. Muscle biopsy revealed muscular dystrophy manifestations. The DYSF protein staining revealed the complete absence of DYSF protein in the myocutaneous membrane. The results of the gene sequencing revealed the presence of two heterozygous mutations in the DYSF gene. The patient was diagnosed with MM. Gene c.4756C>T is related to MM, and the patient’s father was the carrier of this mutant DYSF gene. The patient’s sister did not exhibit the mutation site and did not develop the disease. Therefore, combined with the patient’s clinical manifestations, muscle biopsy, and pedigree verification, gene c.5316dupC was confirmed as being a new pathogenic mutation. It was derived from the patient’s mother, who was also a carrier of the mutant gene.

The patient’s symptoms were characterized by muscle weakness. His creatase was observably increased, and the pathology showed soaked inflammatory cells. The patient was previously misdiagnosed with myopathy and was treated with hormone and immunosuppressant treatment, for which the side-effects were obvious. This highlights the need for vigilance among clinicians. Proper diagnosis of MM allows patients to avoid receiving incorrect treatment due to misdiagnosis. There is no special treatment for this disease, and patients are primarily treated via symptomatic management. The disease develops slowly. Based on understanding the pathogenesis of...
dysferlinopathy, researchers have attempted gene therapy, cell therapy, molecular drug therapy, and corticosteroid and immunosuppression therapy in animal models. Although there is currently no effective clinical treatment, the correct diagnosis can prevent patients from receiving incorrect treatment due to misdiagnosis\textsuperscript{10,11}.

A detailed medical history, muscle histology, immunohistochemistry staining, and gene testing are of significant importance for the diagnosis of muscle diseases, particularly MM. The mutation site discovered in this study represents a new member of pathogenic MM gene mutations. The discovery of this new mutation site may be helpful for the future gene diagnosis of MM.

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