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CLCN1 mutations could lead to atypical myotonic symptoms and abnormities on electromyography

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Abstract

Inactivation of the skeletal muscle chloride channel ClC-1 results in myotonia congenita (MC), which occur with mutations of *CLCN1* gene. However, there is no clear correlation between genotype and phenotype. Clinical data of a patient and his parents with MC were collected retrospectively, including the symptoms and signs, results of blood tests, electromyography, MRI images, and examination results of biceps brachii pathology by histopathology. The patient was diagnosed according to next-generation sequencing. Sanger sequencing was then carried out on his parents' blood samples to verify their mutations. The patient had typical clinical characteristics of Becker myotonia with compound heterozygous mutations of the *CLCN1* gene, inherited from his mother (M560T), who showed only mild symptoms and cold induced myotonic motor unit potentials, and from an unaffected father (c.697-2delA on the intron 5 resulting in exon 6 skipping). In view of the compound heterozygous mutations, he could be classified into Becker myotonia congenita. In conclusion, these results suggested that *CLCN1* mutations could lead to atypical myotonic symptoms and abnormities on electromyography (EMG). EMG after muscle cooling test and exercise tests should be completed in the relatives of patients with MC and some patients with atypical syndrome.

Keywords: Becker myotonia, muscle cooling test, exercise tests, atypical myotonic symptoms, *CLCN1* mutation.

INTRODUCTION

Myotonia congenita (MC) is a genetic, neuromuscular channelopathy that affects skeletal muscles and is characterized by an abnormal delay in muscle relaxation after voluntary or evoked muscle contraction. It is caused by mutations of the skeletal muscle chloride channel gene (*CLCN1*), encoding the skeletal muscle chloride channel ClC-1. Inactivation of ClC-1 results in autosomal dominant MC (Thomsen disease) or autosomal recessive MC (Becker disease).[1]

More than 200 mutations have been reported widely distributed across the 23 exons of *CLCN1*, including nonsense, frame-shifting insertion/deletions or splice-site mutations, but there were no clear correlations between genotype – phenotype.[2] Marked heterogeneity is common in MC even in relatives with the same mutation, while the same mutation can also be inherited in a dominant or recessive manner. The severity of the dominant form is less than the recessive form.[1] In addition, some *CLCN1* mutations correlate with other clinical phenotypes. "EMG disease" is characterized by diffusely increased insertional activity on needle electromyography (EMG) and possibly higher creatine kinase (CK) levels in the absence of a diagnosis of neuromuscular disease.[3,4] An overlap with fibromyalgia syndrome has also been described.[5] There was no consistent evidence for a relationship between these phenotypes and asymptomatic MC.

In this report, our patient was diagnosed with MC by next-generation sequencing (NGS). Sanger sequencing was then carried out to verify the mutations in the patient and his parents. Clinical features of the family were assessed in detail to determine contributions to genotype - phenotype correlation of *CLCN1* mutations. The functional implication of the mutations was further discussed. The clinical features and laboratory data of the patient and his parents were collected retrospectively, and included symptoms and signs, results of blood tests, electromyography, MRI imaging and muscle histopathology of biceps brachii. Informed consent was obtained from all

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subjects. The study was approved by the Ethical Committee of the ZhongDa Hospital Affiliated to Southeastern University. Targeted next-generation sequencing (NGS) and variant identification were conducted by AngPu company in Shanghai (China).

CASE REPORT

The patient was a 21-year-old man, with normal developmental milestones. However, it was noticed that the patient could not open his eyes after sneezing between the ages of 5 and 6 months. Between the ages of 1 and 2 years old, he had difficulty getting into a standing position and displayed stiffness at the beginning of a run or walk, which improved after some motor activities. He walked and ran slower than his peers and fell frequently when he did not take preventative action upon changing posture. At the age of 18, his stiffness increased in the upper limbs and speech difficulties appeared, due to myotonic stiffness. His mother could not relax her hands rapidly after gripping in childhood but has since improved.

On physical examination, he was found to have prominent muscles that were markedly hypertrophied, especially in his legs, an awkward gait, and difficulty running. He arose from a sitting position in a slow and stiff fashion. He had difficulty jumping and climbing stairs, which improved with activity. The dorsiflexion of his feet was limited. Reduced tendon reflexes were seen in the lower limbs, but the reflexes were normal in the upper limbs. He had good muscle strength, normal tone, and flexor plantar responses, as well as intact sensation, coordinated movement, vision, hearing, and cranial nerves. His parents show no abnormal physical signs. The patient's CK was estimated to be 377 IU/L (24-195 IU/L).

Electromyography (EMG) tests

The patient underwent both short and prolonged exercise tests and repetitive nerve stimulation tests; his parents underwent the muscle cooling test.

Muscle cooling test: The limb was wrapped in a plastic bag and submerged in ice water for 10 minutes. Then, needle EMG of the extremity is performed.

Exercise Tests: Compound muscle action potentials (CMAP) were recorded over the abductor digiti minimi after supramaximal ulnar nerve stimulation at the wrist. The patient was asked to rest for 5 minutes while CMAPs were recorded every minute to ensure a stable baseline. For the short exercise test, after 10 seconds of maximal voluntary contraction, CMAP was recorded every 10 seconds for 60 seconds. In the prolonged exercise test, after maximal voluntary muscle contraction for 5 minutes, resting every 15 seconds for 4 seconds, CMAPs were recorded every 5 minutes for between 30 minutes to 120 minutes.

Repetitive nerve stimulation: Repetitive nerve stimulation was performed over abductor digiti minimi muscle at 3 Hz and 10 Hz.

EMG showed normal nerve conduction with spontaneous, abundant myotonic discharges in the tibialis anterior, biceps brachi and vastus lateralis muscles (Figure 1A). His mother also showed myotonic discharges in the EMG after muscle cooling to 20°C (Figure 1B). In the short exercise test, an approximately 65% decrease in the amplitude of the abductor digiti minimi muscle, with rapid recovery, was seen (Figure 1C). In a prolonged exercise test, an approximately 50% decrease in the amplitude of the abductor digiti minimi muscle at 60 minutes, with gradual recovery at 90 and 120 minutes. The patient exhibited a >90% amplitude decrement in 10Hz repetitive nerve stimulation test (Figure 1D). In line with the clinical manifestation, the patient had hypertrophy of the thigh and calf muscles in MRI examination, and mild changes in T1w and T2w of the thigh muscles, limited to the sartorius, gracilis and semitendinosus (Figure 2). The muscle biopsy performed on the patient revealed central nuclei and type 2 fibers predominance, with several type 2C fibers.

RT-PCR

We carried out PCR amplification using the synthesized cDNA. The specific primers for CLCN1 were (CLCN1-S 5'-GCAGTTCCTGG TCTGGGTCA-3' and CLCN1-A 5'-GAACAG CAAAGTAGGTGGAGGT-3').

The sequencing analysis revealed compound heterozygous mutations in the patient. One missense mutation was at nucleotide 1679 with a T to C transition in exon 15 of *CLCN1*, resulting in an amino acid substitution p. Met560Thr. Another identified novel mutation was a single base pair deletion in intron 5 (c.697-2delA). The compound heterozygous mutations were further separately proved by Sanger sequencing in his parents (Figure 3A). Additionally, the RNA from RT-PCR were sequenced to investigate the effect of the deletion mutation on intron 5 of *CLCN1* on the expression of RNA. The results showed that

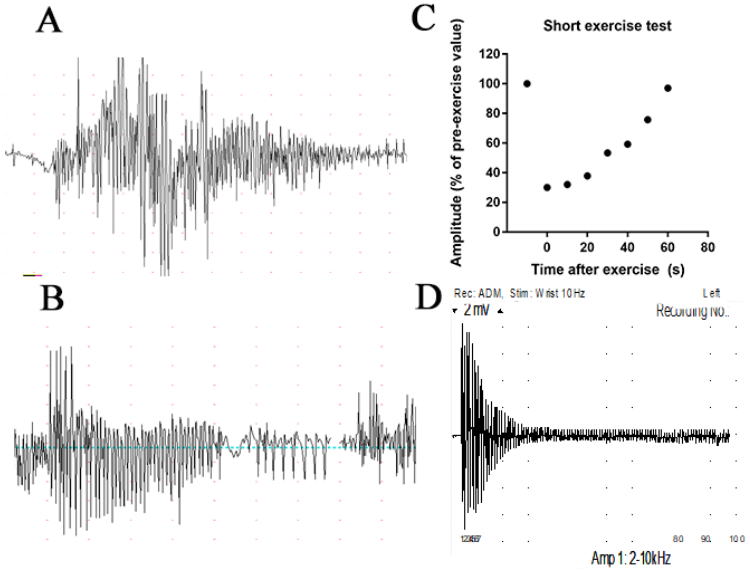

Figure 1. The EMG characteristics of the patient and his mother. The myotonic discharges were found in the patient (A) and his mother after muscle cooling to 20°C (B). In the short exercise test, an approximately 65% decrease in the amplitude of the abductor digiti minimi muscle was seen, with rapid recovery (C). The patient exhibited a decrement in high frequency repeated nerve stimulation (D).

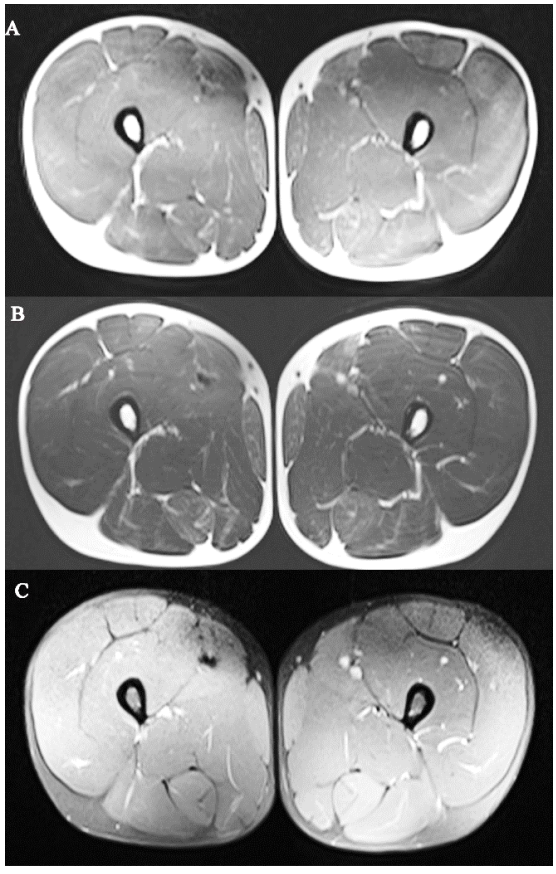

Figure 2. The MRI examination of the thigh muscles of the patient in T1W (A) and T2W (B) images, showing slight changes in the sartorius, gracilis and semitendinosus, and no changes in the STIR images (C).

the exon 6 was skipped in the mRNA Transcript (Figure 3B, 3C).

DISCUSSION

The patient with compound heterozygous mutations of the CLCN1 gene had typical clinical characteristics of Becker myotonia. The c.697-2delA was novel and had not been reported previously. In addition to electrophysiological myotonic discharges, he also showed a Fournier pattern II on short exercise test, which are displayed in most MC patients with chloride channelopathy, particularly the recessive form.[6] The decrement of CMAP amplitude both in 3Hz and 10Hz repetitive nerve stimulation correlated with the presence of transient weakness in MC[7,8] and is used for assessing the anti-myotonic effect of drugs.[8] However, the patterns of response to Repetitive nerve stimulation were not specific to individual myotonic syndromes. The changes of lower limb muscle MRI were consistent with recessive MC as described in earlier reports.[9,10] The number of internal nuclei in the muscles was increased. Interestingly, type 2A fibers were predominant, while fibers of intermediate type 1C and 2C were found in the biceps brachii.

It is well established that the dysfunction of chloride channels by mutations modifies the cycle of excitability of the muscle membrane, shifting it toward hyperexcitability by slowing down the return of the membrane to the resting potential after depolarization, and resulting in delayed skeletal muscle relaxation after voluntary contraction. However, heterozygotes for the *CLCN1* mutation can have a broad phenotypic spectrum. The heterozygous M560T mutation has been commonly reported in East Asian populations.[4,5,11] As earlier reported, patients with the same mutation show mild myotonia[11,12], while in the first-degree relative, there are no myotonic associated clinical symptoms or myotonia shown by EMG.[13] Instead of myotonia, a patient carrying the same mutation may clinically display fibromyalgia syndrome and diffusely increased insertional activity on EMG.[4,5,13] In our report, the mother with the heterozygous M560T mutation was not aware of her clinical symptoms at the first visit, which included delayed relaxation of handgrip in her childhood. She only showed cold induced myotonia on EMG. His mother could be diagnosed as Thomson type MC. It is suggested that some people may be carriers of the mutations with atypical symptoms and abnormalities on

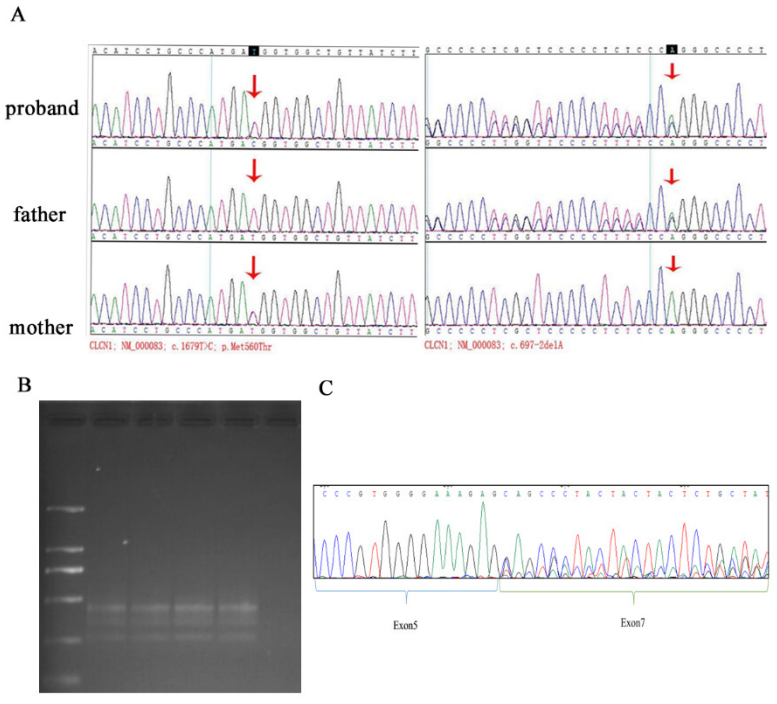

Figure 3. The compound heterozygous mutations were proven by Sanger sequencing of the patient and his parents (A). Furthermore, the RNA from RT-PCR was sequenced to investigate the effect of the deletion mutation in intron 5 of CLCN1 on the expression of RNA. The results showed that exon 6 was skipped in the mRNA Transcript (B, C).

EMG and mild characteristics of MC, and their offspring may inherit homozygote or compound heterozygous mutations. The cold-induced test and the short and prolonged exercise tests should be considered when encountering the aforementioned atypical symptoms, especially when it fails to respond to conventional treatment. In addition to the M560T mutation, the patient also had another heterozygous mutation (c.697-2delA), located on intron 5 and resulting in Exon 6 skipping. This mutation was inherited from his father who had no myotonic associated clinical symptoms or myotonia on EMG. Exon 6 skipping could lead to a domain 4 deficient in clc-1 ion channel; domain 4 is not a trans-membrane region.[15] An earlier report showed that the *CLCN1* exon deletions or duplications are an important genetic mechanism in patients with recessive MC.[16] As this is a deletion, it is likely to be a significant mutation and not a polymorphism. Hence, we speculated that the combined effect of both mutations might have had a severe impact on the expression and function of CLC-1, thus resulting in the clinical symptoms of myotonia. In Becker and Thomson forms of myotonia congenita, mutations of the *CLCN1* gene are thought to affect ClC-1 channel gating, resulting in decreased chloride conductance. Pharmacological experiments indicate that mor than 50% of the chloride conductance must be inhibited in order to cause myotonia.[17] Therefore, the severity of MC symptoms was depended on the effect of mutation of *CLCN1* on chloride conductance. Furthermore, earlier reports showed that some mutations in *CLCN1* might indeed function recessively or dominantly with incomplete penetrance.[18,19]

In conclusion, the case with compound heterozygous *CLCN1* mutations could not be classified into Thomsen or Becker myotonia basing on the myotonic symptoms and family history. Attention should be paid to the mutations leading to atypical symptoms and abnormities on EMG, such as "EMG disease" and "fibromyalgia". An EMG after muscle cooling and exercise tests should be completed in the relatives of patients with MC.

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DISCLOSURE

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