CASE REPORT

Novel Mutations in the CLCN1 Gene of Myotonia Congenita: 2 Case Reports

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Introduction: Myotonia Congenita is an inherited myotonia that is due to a mutation in the skeletal muscle chloride channel CLCN1. These mutations lead to reduced sarcolemmal chloride conductance, causing delayed muscle relaxation that is evident as clinical and electrical myotonia.

Methods: We report the clinical presentations of two individuals with Myotonia Congenita (MC†).

Results: Patient 1 has been diagnosed with the recessive form of MC, known as the Becker variant, and Patient 2 has been diagnosed with the dominant form of MC, known as the Thomsen variant. In both patients, the diagnosis was made based on the clinical presentation, EMG and CLCN1 gene sequencing. Patient 1 also had a muscle biopsy.

Conclusions: Genetic testing in both patients reveals previously unidentified mutations in the CLCN1 gene specific to Myotonia Congenita. We report the salient clinical features of each patient and discuss the effects and common types of CLCN1 mutations and review the literature.

INTRODUCTION

Myotonia refers to impaired muscle relaxation following a voluntary forceful contraction. It is found in several clinical disorders with different etiologies. One of these disorders, Myotonia Congenita (MC), is an inherited myotonia due to a mutation in the skeletal muscle chloride channel CLCN1. A defect in CLCN-1 (a gene coding for the chloride channel ClC-1) leads to reduced sarcolemmal chloride conduc-

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†Abbreviations: MC, Myotonia Congenita; EMG, electromyography.

tance, which in turn allows the muscle to be hyperpolarized, causing delayed relaxation evident as clinical and electrical myotonia. Affected patients will describe muscle stiffness after initiating a forceful movement [1]. MC is found in dominant and recessive forms. The recessive form is usually more severe and is known as Becker Myotonia Congenita or the Becker variant. The dominant form varies in severity from asymptomatic to moderately severe and is known as Thomsen Myotonia Congenita or the Thomsen variant. These two forms may be distinguished by clinical presentation, inheritance pattern, and time of onset. Patients affected by the Thomsen variant do not characteristically show any significant muscle hypertrophy, but it can present in infancy or adulthood [2,8]. Patients with the Becker variant usually present during childhood, show pronounced muscle hypertrophy, and typically have transient (and occasionally permanent) weakness [2,8].

CASE REPORTS

Patient 1: Becker variant

DM is a 5-year-old male who first presented to the Yale-New Haven Pediatric Clinic between the ages of 2 and 3 years old with delayed gross motor milestones and difficulty getting into standing position. There was no history of any neurologic, developmental, or muscle diseases in his family. There was no evidence of consanguinity in the family. His pregnancy and birth history were unremarkable. On physical examination, he was found to have prominent calves and forearms, an awkward gait, and he ran poorly. He arose from the floor in a slow, stiff fashion, but without the classical Gower's sign. He was able to jump, climb stairs, and climb into a chair without difficulty. He had good muscle strength, normal tone, brisk reflexes, and flexor plantar responses, as well as intact sensation, fine motor, vision, hearing, and cranial nerves. CK was measured at 175 (24-195). A muscle biopsy was performed (Figures 1, 2, and 3) that showed scattered areas of atrophic and
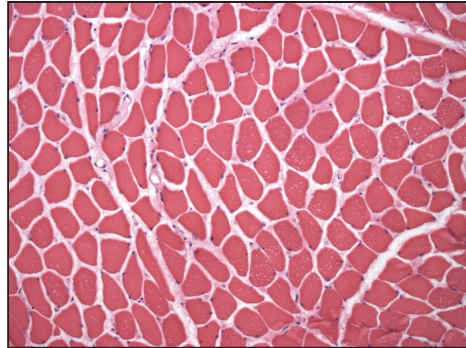

Figure 1. H&E (20x): Mild fiber atrophy and mild increase of endomysial fibrous tissue.
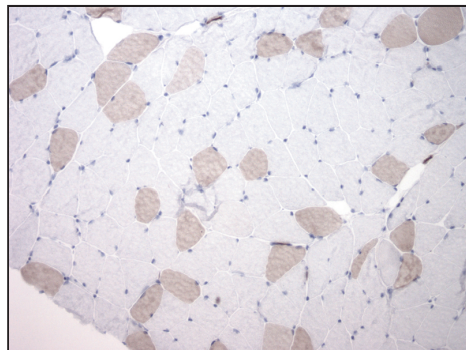

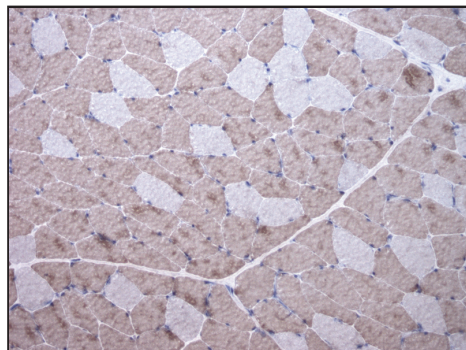**Figure 2.** ATPase 4.3 (20x): Predominance of type 2 fibers.

Figure 3. ATPase 9.4 (20x): Predominance of type 2 fibers.

hypotrophic fibers with relative increase in endomysial connective tissue on H & E staining and predominance of type 2 fibers on ATPase staining with hypotrophic fibers being either type 1 or type 2 but the occasional hypertrophic / hypercontracted fiber being predominantly type 2.

By the time the patient was 4 years and 6 months of age, it was reported that he "gets stiffer after immobility but can walk it off." Cold temperatures did not seem to
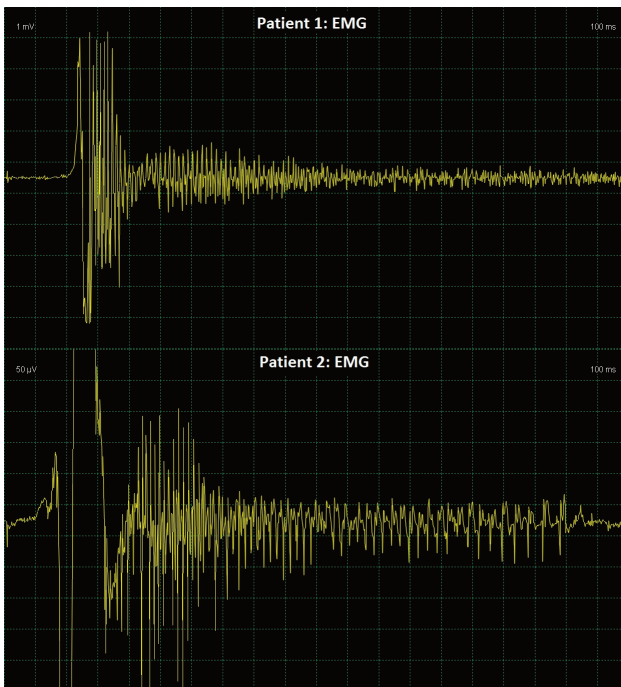
Figure 4. At left: EMG Representation. Myotonia is characterized by increased insertional activity and continued high frequency discharges. The motor unit response is much greater than that of a healthy subject by several seconds that can be incited by tapping, needle movement, or voluntary contraction. Audio feedback during EMG can be described as similar to sounds produced by propeller dive bomber in World War II. The length of time of repetitive discharged correlated with delayed muscle relaxation. This is an electrophysiological hallmark of MC.

Figure 5. Below: Photo of patient 1 (DM) hypertrophied muscles.

worsen or alter these symptoms. Physical exam revealed muscle prominence and grip myotonia followed by transient weakness. Eyelid myotonia was not clearly present. An EMG (Figure 4) was performed and showed multiple runs of spontaneous positive wave discharges that varied in both frequency and amplitude and lasted approximately 2 to 5 seconds each. Subsequent CLCN1 DNA sequencing showed a homozygous transversion in nucleotide position 936 (codon 312) of C to G, resulting in an amino acid change of serine to arginine.

At 5 years of age, he complained of difficulty writing and repeated falls as well as periods where his legs got stiff and he was unable to walk. He denied injuries but reported bruises on his shins. Further review of systems was unremarkable. He was now able to run and go up stairs without difficulty. Physical exam revealed muscle prominence in calves, thighs, biceps, triceps, and neck muscles (Figure 5). Grip myotonia but no eyelid myotonia was elicited.

Patient 2: Thomsen variant

DT is a 59-year-old male with a self-reported history of Thomsen variant Myotonia Congenita who presented to the Yale-New

Haven Clinic for further evaluation. The patient reported muscle cramping starting at a younger age and was diagnosed with MC while in the military. The patient had re-

portedly managed his symptoms of cramping by simply keeping "warm and dry." On presentation, the patient denied lid myotonia, diplopia, dysarthria, dysphagia, numbness, or tingling. Physical exam revealed normal cranial nerve function without eyelid myotonia. There was full strength throughout with mild grip and percussion myotonia. Reflexes, sensation, and cerebellar testing were unremarkable. Past medical history was significant for broken teeth secondary to the myotonia. Family history revealed similar symptoms of "cramping" in his father and a daughter (both without confirmed genetic diagnosis). EMG showed myotonic discharges (Figure 4) with multiple runs of spontaneous positive wave discharges that varied in both frequency and amplitude and lasted approximately 2 to 3 seconds. The patient had been put on quinine and phenytoin to improve his myotonia symptoms in the past; however, both medications were stopped secondary to cognitive side effects. CLCN1 DNA sequencing showed a heterozygous transition in nucleotide position 593 (codon 198) of T to C, resulting in an amino acid change of leucine to proline. DMPK (DM1), ZNF9 (DM2) and SCN4A genes showed no DNA sequence variants.

DISCUSSION

Myotonia Congenita (MC) is a musculoskeletal disorder whose identity in the research world first emerged with the seminal studies by Bryant, Lipicky, and colleagues in the early 1960s, which described deficient muscle chloride conductance [9,10]. The first CLCN1 mutations for both recessive and autosomal dominant MC were discovered in the early 1990s [11,12]. Although this specific form of myotonia continues to be rare, researchers have come to know much more about the pathophysiologic, molecular, and genetic basis through various studies performed over the years. While it is well established that the basis of MC pathology is a mutation in the CLCN1 muscle chloride channel, multiple studies show that there is much variability in the way this mutation may present. In 2008, Lossin et al. re-

ported as many as 130 mutations in this gene [1]. Different mutations have resulted in either dominant or recessive forms of myotonia, yet no two individuals with dominant or recessive inheritance will necessarily present the same way. In fact, presentation even varies greatly in one family line or even from day to day in one person [1]. It has been hypothesized that this variability may be attributed to variable expressivity, incomplete penetrance, impact of mutants alleles on wild type channel proteins, allelic expression, and intrinsic variability of channel dysfunction [13]. This variability has not only made it hard for physicians to diagnose MC in certain cases, but has also led to an inability to support genetic, physiologic, and molecular hypotheses surrounding MC, all of which could potentially result in better treatment methods. The most common reported mutation in North America associated with the Thomsen variant is a nonconservative substitution of glycine-230 with glutamic acid (G230E) [11,14]. The two most frequently discovered alleles in patients with the Becker variant are the missense mutation F413C and a 14-bp deletion leading to a frameshift [12,15]. Today, the number of different mutations and secondarily phenotypic presentations continue to multiply as evidenced by our case report.

As mentioned previously, myotonia as a symptom has many different etiologies. Clinical history, inheritance pattern, and review of systems are all important to distinguish one cause of myotonia from another. Temperature effects, age of onset, and symptoms of predominant or transient weakness help in working through the differential etiologies [2]. Diagnostic studies such as electromyography (EMG) and molecular genetic testing usually allow an easy diagnosis of MC. In this way, we were able to confirm the diagnoses of both patients in this case report. Patient 1 (DM) has the classically described phenotype of the Becker variant and evidence clinically of muscle hypertrophy and grip myotonia, which was confirmed with electromyography. Additionally, Patient 1's muscle biopsy showed a predominance of type 2 fibers, which is consistent with previ-

ous reports of muscle biopsies of patients with myotonia congenita [8]. Patient 2 (DT) has a family history consistent with an autosomal dominant pattern of inheritance, and both grip and percussion myotonia were confirmed with electromyography. Additionally, Patient 2 had normal sequencing of the other myotonia associated genes, DMPK (DM1), ZNF9 (DM2), and SCN4A. Finally, the described mutations in the CLCN1 gene, homozygous C936G (Ser312Arg) mutation in Patient 1 and the heterozygous T593C (Leu198Pro) mutation in Patient 2, are compatible with the known recessive and dominant inheritance patterns of myotonia congenita, respectively.

Though a small subtype of all myotonias, MC research has focused on the clinical presentation, molecular physiology, and genetics. One of the recurrent themes in the research is that MC has variable expressivity affecting the phenotypic presentation. Though many hypotheses have been suggested to explain this, they are not supported by scientific evidence. The trend seems to be that one explanation cannot be used for all mutations. Recent studies investigating the pathology of the CLCN1 channel continue to show this pattern. In 2011, Tang et al. suggested that mutations are scattered throughout the entire protein sequence and no clear relationship exists between the inheritance pattern of the mutation and the location of the mutation in the channel protein [6]. The inheritance pattern of some mutants can be explained by "dominant negative" effect on the channel gate; however, this does not apply to all mutants [6].

Further attempts to explain these findings have led to studies of channelopathies as a whole. In a study of severity of muscle channelopathies, Duno et al. hypothesized that homozygotes show more severe clinical features and compound muscle action potential changes; the presence of 100 percent defective ion channels in homozygotes accounts for the most severe phenotype when compared to heterozygotes of the same mutations [4]. This is supported by the known fact that recessive MC is associated with more severe muscle stiffness than the dom-

inant form [3]. However, this information is not specific enough or useful to determine better treatment methods or allow earlier diagnoses in patients affected by MC.

Although EMG is very helpful as a diagnostic tool, it is not capable of differentiating MC from other myotonias on its own [1]. Even molecular genetic testing, the emerging diagnostic tool, has its limitations, and in the case of certain mutations, it cannot completely rule out MC. Several protocols using EMG to enhance the diagnostic sensitivity and specificity of the myotonic disorders have been suggested. Long and short exercise tests at room temperature after cooling and re-warming have been described, and the short exercise tests aided in accurate DNA based diagnosis [5]. Another proposed method involved repetitive nerve stimulation at 3Hz, which, in an Italian cohort of patients affected by recessive MC, showed high sensitivity and reproducibility in detecting recessive cases. All dominant cases and patients affected by myotonia not due to the CLCN1 mutation showed negative results [7]. Although not discussed in detail in this case report, histological muscle biopsy features also have a role and may aid in diagnosis.

It is our hope that these two previously undescribed mutations in the CLCN1 genes will add to the growing database of CLCN1 variants linked to myotonia congenita and help elucidate some of the polymorphisms associated with this disease and these specific mutations.

REFERENCES

1. Lossin C, Geroge AL Jr. Advances in Medicine. 1st edition. San Diego: Elsevier; 2008. p. 25-55.
2. Pestronk A. Neuromuscular disease center [Internet]. Available from: http://neuromuscular.wustl.edu/.
3. Duno M, Colding-Jorgensen E. Myotonia Congenita. Gene Reviews [Internet]. 2005. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1355/.
4. Arzel-Hezode M, Sternberg D, Tabti N, Vicart S, Goziet C, Eymard B, et al. Homozygosity for dominant mutations increases severity of muscle channelopathies. Muscle Nerve. 2010;41(4):470-7.
5. Tan SV, Matthews E, Barber M, Burge JA, Rajakulendran S, Fialho D, et al. Refined exercise testing can aid DNA-based diagnosis

in muscle channelopathies. Ann Neurol. 2011;69(2):328-40.

6. Tang CY, Chen TY. Physiology and Pathophysiology of CLC-1: Mechanisms of a Chloride Channel Disease, Myotonia. J Biomed Biotechnol. Epub 2011 Dec 1.

7. Modoni A, D'Amico A, Dallapiccola B, Mereu ML, Merlini L, Pagliarani S, et al. Low-rate repetitive nerve stimulation protocol in an Italian cohort of patients affected by recessive myotonia congenita. J Clin Neurophysiol. 2011;28(1):39-44.

8. Crews J, Kaiser KK, Brooke MH. Muscle pathology of myotonia congenita. J Neurol Sci. 1976;28(4):449-57.

9. Bryant SH. Muscle membrane of normal and myotonic goats in normal and low external chloride. Fed Proc. 1962;21:312.

10. Bryant SH, Morales-Aguilera A. Chloride conductance in normal and myotonic muscle fibres and the action of monocarboxylic aromatic acids. J Physiol. 1971;219(2):367-83.

11. George AL, Jr., Crackower MA, Abdalla JA, Hudson AJ, Ebers GC. Molecular basis of Thomsen's disease (autosomal dominant myotonia congenita). Nat Genet. 1993;3(4):305-10.

12. Koch MC, Steinmeyer K, Lorenz C, Ricker K, Wolf F, Otto M, et al. The skeletal muscle chloride channel in dominant and recessive human myotonia. Science. 1992;257:797-800.

13. Duno M, Colding-Jorgensen E, Grunnet M, Jespersen T, Vissing J, Schwartz M. Difference in allelic expression of the CLCN1 gene and the possible influence on the myotonia congenita phenotype. Eur J Hum Genet. 2004;12:738-43.

14. Koty PP, Pegoraro E, Hobson G, Marks HG, Turel A, Flagler D, et al. Myotonia and the muscle chloride channel: Dominant mutations show variable penetrance and founder effect. Neurology. 1996;47:963-8.

15. Meyer-Kleine C, Ricker K, Otto M, Koch MC. A recurrent 14 bp deletion in the CLCN1 gene associated with generalized myotonia (Becker). Hum Mol Genet. 1994:3:1015-6.