Short Report

Myotonia congenita and myotonic dystrophy in the same family: coexistence of a CLCN1 mutation and expansion in the CNBP (ZNF9) gene


Myotonia is characterized by hyperexcitability of the muscle cell membrane. Myotonic disorders are divided into two main categories: non-dystrophic and dystrophic myotonias. The non-dystrophic myotonias involve solely the muscle system, whereas the dystrophic myotonias are characterized by multisystem involvement and additional muscle weakness. Each category is further subdivided into different groups according to additional clinical features or/and underlying genetic defects. However, the phenotypes and the pathological mechanisms of these myotonic disorders are still not entirely understood. Currently, four genes are identified to be involved in myotonia: the muscle voltage-gated sodium and chloride channel genes SCN4A and CLCN1, the myotonic dystrophy protein kinase (DMPK) gene, and the CCHC-type zinc finger, nucleic acid binding protein gene CNBP. Additional gene(s) and/or modifying factor(s) remain to be identified. In this study, we investigated a large Norwegian family with clinically different presentations of myotonic disorders. Molecular analysis revealed CCTG repeat expansions in the CNBP gene in all affected members, confirming that they have myotonic dystrophy type 2. However, a CLCN1 mutation c.1238C>G, causing p.Phe413Cys, was also identified in several affected family members. Heterozygosity for p.Phe413Cys seems to exaggerate the severity of myotonia and thereby, to some degree, contributing to the pronounced variability in the myotonic phenotype in this family.

Conflict of interest

Authors declare no conflicts of interest.
There are two main categories of myotonic disorders: non-dystrophic and dystrophic myotonias. Non-dystrophic myotonias are caused by mutations in genes CLCN1 and SCN4A which encode the voltage-gated skeletal muscle chloride channel (ClC-1) and the α-subunit of the voltage-gated skeletal muscle sodium channel (SCN4), respectively (1, 2, 3). These non-dystrophic myotonic disorders involve solely the muscle system.

Myotonic dystrophy (DM) is classified into type 1 (DM1) and type 2 [DM2/proximal myotonic dystrophy (PDM)/proximal myotonic myopathy (PROMM)] (4–6). DM1 is caused by a (CTG)n expansion in the 3′-untranslated region of the dystrophia myotonica protein kinase gene, DMPK (7). DM2 is caused by a (CCTG)n expansion in intron 1 of the CCHC-type zinc finger protein gene, CNBP, previously called ZNF9 (8, 9). Both are multisystemic disorders characterized by myotonia, muscle weakness and wasting, cataracts, and other system involvements including the heart and the central nervous system (4, 5). DM2 differs from DM1 by a predominant proximal muscle weakness and atrophy at onset, doubtful congenital form and anticipation. Both DMs are thought to be RNA-mediated diseases caused by a dominant negative effect on the expression of other genes (10, 11), including CLCN1 (12–14).

The myotonic disorders are clinically highly heterogeneous with considerable high inter- and intra-generational variations within and between affected families.

In this study, we present the clinical and molecular findings in a Norwegian family presenting with the clinical coexistence of dystrophic and non-dystrophic myotonias. Expansions of the CNBP gene were identified in all affected family members. In addition, a CLCN1 mutation causing p.Phe413Cys was also detected in several patients (15). We investigated if heterozygosity for p.Phe413Cys has a modifying effect on the DM2 phenotype and thereby causing the unusual myotonic phenotype in this family.

Materials and methods

Patient ascertainment

The family pedigree is shown in Fig. 1. Informed consent was obtained and the study was approved by the Regional Committee for Medical Research Ethics.

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**Fig. 1.** Pedigree of the investigated family. Individuals with positive electrical myotonia on EMG are defined as affected. Family members who had clinical and EMG examinations are indicated with EMG +/−. Results from the molecular analysis of the CLCN1 and CNBP genes are depicted beneath each investigated individual. The presence or absence of c.1238C>G resulting in p.Phe413Cys in the CLCN1 gene is indicated as p.Phe413Cys and −, respectively. Note that not all affected family members are heterozygous carriers of the p.Phe413Cys mutation. Non-expanded CNBP alleles are depicted by ‘−’ whereas abnormally expanded alleles are denoted by ‘exp’. Expanded alleles (exp) range is size from 10 to 15 kb except for patient III:11 who displayed a repeat-size-expansion of approximately 3–4 kb. It should be noted, however, that repeat-sizes in the pathogenic range does not correlate with disease severity.
A neurologic examination was performed, including tests for active and percussion myotonia and for muscle weakness. Electromyography (EMG) was performed by concentric needle electrode using Keypoint EMG equipment (Medtronic Copenhagen, Denmark). The extensor digitorum communis and the anterior tibial muscles were examined. Myotonic discharges were recorded and the motor unit potentials were analyzed for low amplitude, short durations and early recruitment. All examinations were performed by one neurologist (T. T.). Individuals were classified as affected if ‘myotonic runs’ were present on EMG. The clinical and EMG myotonia were further classified in four categories (+/−, +, ++, and ++++) depending on the degree of affection (suspected, mild, moderate, and pronounced).

Blood samples were investigated for the creatine kinase (CK), gamma-glutamyl transpeptidase (γ-GT), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and lactate dehydrogenase (LD). Muscle biopsy, ECG (electrocardiography), and cerebral computed tomography (CT) scan were performed occasionally.

Genotyping and sequencing analysis

Genomic DNA was extracted from peripheral blood cells using an automated DNA extractor (Genepure 341 Nucleic Acid Purification System, Applied Biosystems Inc., Foster City, CA, USA). Haplotype analysis of the CLCN1 gene was performed with chromosome 7 microsatellite markers; cen-D7S1804, D7S1824, D7S2513, and D7S661-tel, spanning a 11.3 Mb interval that harbors the CLCN1 gene (D7S2513 – CLCN1 – D7S661). Sequencing of the entire CLCN1 gene was performed in the probands. Identification of c.1238C>G in additional family members was attained as explained in Sun et al. (15).

Detection of (CCTG)n repeat-sizes in the CNBP gene was done according to Liquori et al. (9), with minor modifications. A fluorescent (FAM) labeled CL3N58-D R primer was used. Polymerase chain reactions (PCRs) were cycled 30 times 94°C for 30 s, 62°C for 30 s and 72°C for 30 s. PCR products were analyzed using an ABI 3100 DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA). Individuals heterozygous for alleles in the normal range (104–176 bp) are non-carriers.

Results

Clinical overview

The subjective symptoms and the results of clinical investigations of the patients are summarized in Table 1. Symptoms and clinical signs in the muscle system are described below. The results of various analyses and abnormalities of non-muscle organs are mainly presented in Table S1, supporting information.

The proband, II:2, has suffered from generalized myalgia during his entire adult life. He experienced attacks of central chest pain that exacerbated by cold. Cardiomyopathy was diagnosed by echocardiography. The left ventricle was greatly dilated with severely reduced function. However, instead of a restrictive filling over mitral ostium, the pattern was described to be typical for an abnormal relaxation of the myocardium. On clinical examination, he had normal cognitive function and facial expression. There was possibly marginal atrophy of the bilateral sternocleidomastoid muscles. Foot dorsi- and flexion, and the Achilles reflex were mildly reduced. There was slight action myotonia. EMG showed myopathic changes and myotonic runs, consistent with DM1.

Another proband, II:5 was first examined at the age of 74. He had daily myalgia and complained of weakness in both legs. Mild muscle stiffness was experienced in his hands and legs, worsening during the last years. Upon clinical examination he had normal cognitive function and facial expression. He was generally thin and distal musculature was possibly mildly atrophic. However, his muscle strength was normal. Mild percussion and action myotonia were observed. EMG showed myotonic discharges.

Patient II:1 had myalgia as the only muscle symptom. His cognitive function and facial expression were normal. Clinical myotonias and muscle atrophy were absent. Shoulder abduction was possibly weakened. EMG demonstrated myotonic discharges.

Patient III:2 suffered from impaired balance from age 30. She complained of increasing stiffness of her fingers and weakness in her lower extremities. Myalgia was present. Patient III:5 experienced mild muscle stiffness, general muscle weakness, and myalgia. On clinical examination,
<table>
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<tr>
<th>Cases</th>
<th>CLCN1 p.Phe413Cys/WT</th>
<th>Onset of myotonia</th>
<th>Subjective symptom</th>
<th>Clinical myotonia</th>
<th>EMG myotonia</th>
<th>Subjective symptom</th>
<th>Clinical weakness</th>
<th>Muscle atrophy</th>
<th>EMG myopathy</th>
<th>Muscle pain</th>
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Table 1. Summary of subjective muscle symptoms and clinical features of affected family members

| aAll individuals in the table have an expansion in the CNBP gene. Some of them are also heterozygous for the CLCN1 mutation p.Phe413Cys. This table shows the variable clinical phenotypes present in this family. Both the patients’ subjective symptoms and clinical signs of the muscle system are summarized. As shown in the table, the result of clinical examination on muscle strength does not necessarily correspond to the subjective muscle weakness reported by the individual patient, and vice versa. Patients harboring the p.Phe413Cys mutation (II:5, III:7, III:9, III:10, IV:4, and IV:5) generally presented a more severe myotonia than those without. For subjective muscle stiffness and muscle pain, ++++, severely affected; ++, moderately affected; +, mildly affected; −, not present; EMG, electromyography. For clinical and EMG myotonia, as well as EMG myopathy, ++++, pronounced; ++, moderate; +, mild; +/−, suspected; −, not present. For muscle weakness and muscle atrophy, +, present; −, not present; +/−, suspected.

bPregnancy-induced myotonia.
both had normal mental status and facial expression. No muscle atrophy was observed. The muscle strength of the hip flexion of III:2 was mildly reduced. So was that of the proximal part of the upper extremities of III:5. No action or percussion myotonia was found. Both showed myotonic runs and mild myopathic features on EMG.

All five daughters of II:5 developed myotonia during their pregnancies (ages 20–26). After deliveries myotonia persisted. All of them had muscle stiffness in both the upper and lower limbs. Patient III:10 had the most severe myotonia with cold-induced stiffness in the perioral and periorbital muscles and occasional stiffness in the chest muscles, for example while coughing. Muscle weakness was experienced only by III:10 around her hips and in her thighs, starting during her first pregnancy. From the age of 35, she also experienced attacks of muscle weakness in her legs. Myalgia was reported by patients III:9, III:10, and III:11. Patients III:7, III:10, and III:11 also had symptoms from systems other than muscle (Table S1). Upon clinical examinations all had normal mental status and facial expressions. No muscle atrophy or hypertrophy was observed. They all showed normal muscle strength, including III:10 despite her subjective symptoms. Action and percussion myotonia were observed in all patients, to different extents (Table I). Patient III:10 was the most severely affected with stiff movements and pronounced clinical myotonia. Myotonic discharges were present on EMG.

Patient IV:1, investigated at age 26, had no symptoms from the muscle- or other systems. His mental status and facial expression were normal. However, EMG showed myotonic discharges and very mild myopathic changes.

Patient IV:4 had no muscle symptoms when evaluated at age 20. However, mild action and percussion myotonia were suspected on clinical examination. EMG revealed moderate myotonic discharges. Patient IV:5 noticed muscle stiffness from age 12 with no other symptoms. Results of clinical examination at age 28 were normal. However, EMG showed pronounced myotonic discharges, whereas EMG at age 18 was normal. Their sister, IV:3, suffered from muscle stiffness in her upper and lower limbs during the last year. She experienced difficulties climbing stairs and rising from sitting position. When attempting to open a jar lid, her fingers tended to get ‘locked’. She also experienced myalgia in the lower limbs and cold-induced weakness in her hands. Her neurological examination was normal. EMG at the age of 34 showed mild myopathic features without myotonic discharges.

Genetic analyses

Results from the genetic analyses are displayed in Fig. 1. None of the affected family members had (CTG)_n expansion in the DMPK gene (data not shown). Analysis of the CNBP gene revealed tetranucleotide expansions in all affected members.

Heterozygosity for the CLCN1 mutation c.1238C>G, causing p.Phe413Cys, was detected in family members II:5, III:7, III:9, III:10, IV:3, IV:4, and IV:5. Among them, IV:3 had no EMG myotonia.

Discussion

In this study, we describe a large family with apparently clinically different myotonic disorders in two brothers. One of them, II:2, had bilateral cataracts, myotonia, and heart manifestation that were compatible with DM1, supported by EMG and histological findings. However, he did not have the classical facial expression. Distal muscle wasting and weakness were minimal. At the age of 64, none of the serious complications that are typical for DM1 were present. The overall clinical picture was strikingly mild. His brother II:5, at the age of 74, was clinically diagnosed with myotonia congenita (MC). The presence of DM1 and MC in a single family has been reported previously by Höweler et al. (17). Within that family DM1 and MC segregated independently.

Preliminary molecular analysis of our family revealed a CLCN1 mutation c.1238C>G, causing p.Phe413Cys, previously shown to be associated with recessive MC (15). However, not all affected members carried this mutation. Haplotype analysis further excluded the CLCN1, the SCN4A, and the DMPK as the disease genes (data not shown). Subsequently, a CNBP (CCTG)_n expansion was detected in all affected members. This family demonstrates that it may be difficult to clinically differentiate dystrophic myotonias from non-dystrophic myotonias.

Recently, two separate research groups reported DM2 patients with a coexisting CLCN1 mutation (18, 19). Suominen et al. (19) reported that about 5% of the German and 3% of the Finnish DM2 patients carry the most frequent CLCN1 mutation p.Arg894X, whereas, the carrier frequencies in the normal German and Finnish population were 1% and 1.3%, respectively. In addition, 2% of the Finnish DM2 population carries another frequent CLCN1 mutation, p.Phe413Cys, compared
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to 0.7% in the normal population (19). The exact frequency of the common CLCN1 mutations in the DM2 population is unknown, and is not expected to be different from the normal population. Co-segregating CLCN1 mutations have been proposed to have an influence on the DM2 phenotype, correspondingly, the disproportional distribution of CLCN1 mutations among DM2 patients might reflect a selection bias towards diagnosing patients in the severe end of the phenotypic continuum (19). The family presented here might provide such an example as heterozygosity for p.Phe413Cys was detected in 6 of the 13 affected members. Generally, affected carriers experienced more severe muscle stiffness and showed more severe clinical and EMG myotonias than those having exclusively the CNBP expansion (Table 1). Hence, co-segregation of the CLCN1 mutation may modify the phenotypic effect of the CNBP expansion, contributing to a more severe phenotype.

Carriers of recessive CLCN1 mutations are usually asymptomatic but subclinical EMG myotonia has been reported (20). Family member IV:3, who is heterozygous for p.Phe413Cys but harboring normal CNBP alleles, experienced muscle stiffness, cold-induced muscle weakness, and myalgia. Neither clinical, nor EMG myotonia was found; however, mild myopathic features could be recorded by EMG. It remains unknown whether heterozygosity for p.Phe413Cys might be involved in her mild manifestations.

Cardiomyopathy is rare in DM (21), and is thought to be secondary to conduction failure and heart arrhythmias. In patient II:2 the pattern from echocardiography was suggestive for an abnormal muscle relaxation instead of a restriction filling. This indicates for the first time that the existence of myotonia in the myocardium may be the primary cause of heart manifestation in DM patients.

Both dystrophic and non-dystrophic myotonias may occur during pregnancy but usually resolve after delivery (22–24). Myotonia of the five daughters of II:5 started during pregnancy, but persisted after delivery. The role of p.Phe413Cys in this pregnancy-induced myotonia is less clear since this mutation did not segregate with this particular trait. The mechanism underlying the pregnancy-induced myotonia is currently unknown; however, the co-incidence with pregnancy might suggest an intriguing hormonal influence. In vitro studies done by Fialho and colleagues showed that both testosterone and progesterone have an inhibitory effect on CIC-1 channels via a rapid signaling pathway (25). Lacomis et al. proposed that increased progesterone during pregnancy may affect intra- and extracellular potassium level that may enhance myotonia (23). However, as most changes during pregnancy are thought to be temporary, the effect of pregnancy at the onset and persistence of myotonia in these five sisters remains a puzzle. Further research is required to identify pregnancy-specific factors that may modify the effect of CNBP tetranucleotide expansion.

Supporting Information

The following Supporting information is available for this article: Table S1. Summary of non-muscle system manifestations and the results of various analyses of affected family members

Additional Supporting information may be found in the online version of this article.

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References