Dosage Effect of a Dominant CLCN1 Mutation: A Novel Syndrome

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Multiple mutations in the CLCN1 gene coding for the voltage-gated chloride channel have been documented to cause myotonia congenita. We report a kindred featuring an index patient who possesses 2 copies of a dominantly inherited mutated CLCN1 allele with a resulting novel phenotypic presentation. The index patient is a boy who presented initially for evaluation at the age of 5 years with a 2-year history of gait problems. Both parents and 3 male siblings were entirely well. Examination revealed a striking diffuse muscular hypertrophy, diffuse mild to moderate weakness, Gower sign, percussion, and grip myotonia. Electromyography confirmed myotonia, and molecular analysis revealed 2 copies of the T310M mutation on the CLCN1 gene. Testing of family members revealed a normal neurological examination without clinical myotonia in all and electromyographic evidence of myotonia and a single copy of the T310M mutation in both parents and 2 siblings. Our kindred is the initial demonstration of the dosage effect of a dominant mutated allele in the CLCN1 gene.

Keywords: myotonia congenita; CLCN1; channelopathy

Myotonia congenita is an inherited disease with both an autosomal recessive (Becker, OMIM 255700) and dominant (Thomsen, OMIM 160800) mode of inheritance. Both forms are rare and clinically similar.1 The few differences between the 2 forms are as follows: (1) Thomsen variant is characterized by early onset, mild to moderate myotonia and slight muscle hypertrophy, (2) Becker variant is characterized by later onset, moderate to severe myotonia with transient weakness, moderate muscular hypertrophy, and in some cases by permanent muscle weakness and wasting. The estimated worldwide prevalence of these 2 forms taken together is approximately 1:100 000.2 Both forms of the disease are caused by varied mutations in the CLCN1 gene on the long arm of chromosome 7. This gene codes for the voltage-gated chloride channel (ClC-1). More than 80 mutations have been thus far described. The majority of described mutations are responsible for a recessive form of the disease. Fifteen mutations (S132C, E193K, L198V, T268M, L283F, V286A, I290M, T310M, A313T, S471F, P480L, P480T, Q552R, I556N, fs872X) have been reported to result in the autosomal dominant form of the disease.3–6 Eight mutations (M128V, G230E, F307S, R317Q, R338Q, A531V, R894X, P932L) have been shown to result in either the recessive or the dominant variant of myotonia congenita.7–9

We report a family with 1 symptomatic family member having 2 copies of the T310M mutated allele, previously known to result in the autosomal dominant variant of myotonia congenita, with a novel clinical phenotype and 4 clinically unaffected family members with 1 copy only.

Methods

Detailed kindred, electrophysiologic, and molecular analyses of the affected patient and his family were performed, including complete history, physical examination, electromyographic studies, and genetic testing for mutations of the CLCN1 gene. Genetic testing was performed on 15 cc of whole blood collected in ethylenediaminetetraacetic acid tubes from each family member. Genomic DNA was extracted from blood samples according to previously described methods.10 The 23 exons with 100-bp

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flanking intronic regions of the CLCN1 gene were amplified by polymerase chain reaction and sequenced in each family member on an ABI prism automated DNA sequencer (Applied Biosystem International) at the Laboratory of Chemistry, Pitie-Salpetriere Hospital, Paris. Sequences were analyzed with SeqMan 4.03 (DNA Star, Madison, Wisconsin) and compared with reference genomic sequence (hg17 NM_000083, May 2005). Sequence variants were classified as polymorphism if documented as such in the literature or if they were found in both affected and nonaffected participants.

**Index Case**

Our patient presented at the age of 5 years with a 2-year history of gait disturbance. Both of his parents are from the Azores Islands (Portugal). Parental consanguinity was denied. There is no family history of apparent neuromuscular conditions, despite detailed questioning.

His perinatal history is unremarkable. His early development was normal. However, his parents had noticed some difficulties with gross motor tasks compared with his siblings and peers (eg, walking, running, and in sports). His past medical history is noncontributory.

The patient’s initial complaints were of leg weakness and gait disturbance. He also reported muscle cramps, (myotonia) initially in the lower extremities only but progressing over the next few years to involve also the upper extremities. These cramps are worse after a period of inactivity and improve with exercise (“warm-up phenomenon”). Over the subsequent years, he developed slowly progressive muscular weakness. At the age of 12 years, he can walk, run for short distances with some difficulties, go up and down the stairs, bicycle, swim, and play hockey, but with less competence then his peers.

On physical examination, a diffusely increased muscle bulk was noticed (“Popeye” appearance; Figure 1). Grip and percussion myotonia (tongue, abductor pollicis brevis, extensor digitorum communis) were present. Generalized weakness (4 to 4+ range) was noticed, with proximal muscle predominance. The Gowers maneuver was positive. The rest of the neurological examination was within normal limits.

When compliant, his muscle cramps, myotonia, and to some extent, his muscle weakness responded well (both subjectively and objectively) to carbamazepine.

Extensive investigations were performed. His serum creatinine kinase levels were always in the normal range. An echocardiogram was normal. He had normal skeletal survey, karyotype (46 XY, 450 bands), vitamin E, lipid profile, lactate, pyruvate, and urine organic acids. Because of the prevalence of the SCA3 in his ethnic group, Machado-Joseph disease gene testing was performed and was negative (ie, normal number of repeats on both alleles). An ophthalmology examination was normal. The electromyographic studies revealed normal nerve conduction studies and typical myotonic discharges (“dive-bombers”) on concentric needle examination. The muscle biopsy revealed non-specific changes (ie, marked variation in fiber size, preponderance of type II fibers, no deficiency of type IIb fibers, some internally located nuclei, and some fibers containing subsarcolemmal accumulation of dense material). This finding was believed to be nondiagnostic. Molecular testing revealed 2 copies of the CLCN1 T310M mutated allele.

Detailed testing of family members revealed a normal neurological examination without clinical myotonia in both parents and 3 siblings. Electromyographic studies revealed evidence of myotonia in both parents and 2 siblings. The muscles examined were the extensor digitorum communis in all and the deltoid in 1 sibling, and myotonia was present in both proximal and distal muscles.

Molecular studies on all family members showed a single copy of the index’s T310M CLCN1 mutation in both parents and in both siblings with myotonic discharges demonstrated on electromyography (Figures 2 and 3).

**Discussion**

The muscle chloride channel (ClC-1) is a voltage-gated ion channel. It is opened, closed, and inactivated according to fluctuations in the membrane potential to regulate the
electrical excitability of the skeletal muscle membrane. Skeletal muscle cells have a much greater resting chloride conductance compared with other cells in the body. Muscle chloride conductance is predominantly mediated by ClC-1 channel. A reduction of this conductance by malfunction of the ClC-1 channel leads to electrical instability, manifesting clinically as either myotonia or muscular cramps.

The ClC-1 protein is encoded by the CLCN1 gene located on the long arm of chromosome 7 (7q35). The coding sequence contains 23 exons. More than 80 mutations, representing potential missense, nonsense, insertions, deletions, frameshift, and splice mutations have thus far been identified.

In both forms of myotonia congenita, mutations of the CLCN1 gene are thought to affect ClC-1 channel gating, resulting in decreased chloride conductance. This finding causes an electrical instability of the sarcolemma, leading to repetitive sustained electric discharges that result in the electrical and clinical phenomena of observed myotonia. In the Becker phenotype, both subunits carry a disease-causing mutation. This is thought to cause a non-functional channel. In the Thomsen phenotype, the pathophysiology is thought to be one of a dominant-negative mutation. A mutation causing an alteration of 1 of the 2 protopore coding alleles could alter the gating or selectivity of the channel to chloride ion flux.

To our knowledge, only 3 cases of Thomsen’s disease with T310M mutation have been described. Wu et al reported 1 asymptomatic patient who developed proximal more than distal weakness and a symptomatic patient who developed proximal more than distal weakness, as well as symptomatic myotonia during her first pregnancy. The symptoms resolved after her delivery. The authors do not mention any genetic testing of other family members. It would be interesting to perform EMG and genetic testing on her family members, because our kindred shows that heterozygous carriers can be completely asymptomatic clinically.

Our kindred is the first report of a dosage effect of dominantly inherited alleles modulating ClC-1. Possession of a single copy can be compensated and asymptomatic or only mildly symptomatic whereas when 2 copies are present, this finding can lead to clinically apparent muscle dysfunction with functional neuromuscular limitations.

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References


