THOMSEN OR BECKER MYOTONIA? A NOVEL AUTOSOMAL RECESSIVE NONSENSE MUTATION IN THE CLCN1 GENE ASSOCIATED WITH A MILD PHENOTYPE

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ABSTRACT: We describe a large Brazilian consanguineous kindred with 3 clinically affected patients with a Thomsen myotonia phenotype. They carry a novel homozygous nonsense mutation in the CLCN1 gene (K248X). None of the 6 heterozygote carriers show any sign of myotonia on clinical evaluation or electromyography. These findings confirm the autosomal recessive inheritance of the novel mutation in this family, as well as the occurrence of phenotypic variability in the autosomal recessive forms of myotonia.}


Myotonic diseases are classically divided into dystrophic and non-dystrophic myotonias. The non-dystrophic myotonias share a common etiology, dysfunction of the skeletal muscle ion channel. Non-dystrophic myotonias may be divided into two different types of channel dysfunction: those with chloride channel dysfunction (the congenital myotonias) and those with sodium channel dysfunction (congenital paramyotonia, potassium-aggravated myotonia, and hyperkalemic periodic paralysis with myotonia).

The congenital myotonias are inherited disorders caused by mutations in the CLCN1 gene that encodes the major skeletal muscle chloride channel. This channel is important for the normal repolarization of muscle action potentials and consequent relaxation of the muscle. Dysfunction of this channel is related to muscle membrane hyperexcitability and a delay in muscle fiber relaxation. Clinically, the patients show muscle stiffness after voluntary contraction, which is known as myotonia. The myotonia decreases after repetitive movement, which is often referred to as the warm-up phenomenon. Muscular hypertrophy is another important clinical sign, which confers an athletic phenotype on the patients. Muscle atrophy and transient or permanent weakness can be observed in some patients. The worldwide prevalence of congenital myotonia has been estimated to be approximately 1 in 100,000 population.

The phenotypic spectrum of congenital myotonias ranges from mild myotonia evident only on clinical examination to severe and disabling myotonia with transient weakness and myopathy. Based on the phenotype and inheritance, two allelic forms are described: Thomsen disease with milder symptoms and dominant inheritance, and Becker disorder with a severe phenotype inherited as a recessive trait. The diagnosis and genetic counseling of families has become a challenge in the molecular era because of the identification of a large number of mutations in the CLCN1 gene that can be found in either the homozygous or heterozygous state.

In this report, we investigate a large Brazilian family, displaying either autosomal dominant (AD) or autosomal recessive (AR) mild Thomsen myotonia. The high degree of consanguinity suggests an AR disease. Molecular analysis identified a novel homozygous stop codon mutation in the CLCN1 gene in 3 affected children, whereas their parents, all carriers, were clinically asymptomatic. Clinical and electrophysiological evaluation confirmed an AR trait.

METHODS

The index case (Fig. 1, V-13), his affected brother (Fig. 1, V-14), and their affected cousin (Fig. 1, V-18) were first seen in 2003, when they were 9, 4, and 6 years old, respectively. The parents were highly consanguineous: 2 sisters married 2 brothers, and all were first-degree cousins (Fig. 1). At that time, clinical manifestations of the 3 affected patients were mild. They only had mild muscle pain associated with global muscle hypertrophy.

The clinical history of the index case included an uneventful pregnancy and delivery. The patient

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had normal psychomotor development up to age 1 year. Symptoms started when he was 11 months old. He began to develop leg muscle stiffness while walking. At the age of 2–3 years, global muscle hypertrophy was evident. Afterwards, he began to experience repeated episodes of leg stiffness with difficulty walking and climbing steps. He also began to have delayed relaxation of hand grip after clenching. The myotonia was more intense on cold days.

A careful and extensive new evaluation was performed 7 years later, in 2010, when the index case was 16 years of age (Fig. 2). His neurological examination revealed global muscular hypertrophy with discrete proximal weakness in his limbs. His gait and coordination were normal. Deep tendon reflexes were absent. There was no grip myotonia, but there was evident calf myotonia during the effort of climbing steps. Percussion of the thenar eminence and the tongue elicited myotonia.

Cardiological evaluation, including electrocardiography and echocardiography, were normal.

Nerve conduction study was normal, and electromyography showed positive waves, fibrillation potentials, and short-duration polyphasic motor unit potentials associated with spontaneous bursts of potentials in rapid succession with waxing and gradual waning. These results were consistent with myotonia.

Serum creatine kinase (CK) analysis showed a fourfold increase above the upper limit of normal. The muscle biopsy showed discrete myopathic alterations including moderate variation in fiber size but no internally located nuclei or endomysial or perimysial connective tissue proliferation. ATPase stain showed type II predominance, with an absence of type IIb fibers (Fig. 3). There were neither vacuoles nor tubular aggregates in the muscle fibers.

At 4 years of age, the younger brother (V-14) showed first signs of mild muscle hypertrophy and calf stiffness during stair climbing. At age 11 years, his neurological examination was almost normal, except for the presence of muscle hypertrophy and percussion myotonia, which was milder than that seen in his older brother (V-13). He had no muscle weakness or grip myotonia. His serum CK level was almost normal (one or two times of the upper limit of the reference value). Electromyography also showed myotonic discharges.

Detailed clinical evaluation was extended to other members of the family. Neither the parents nor the young sister of the proband showed neurological abnormalities. The parents were submitted to electromyography to verify possible latent myotonia. Both had a normal pattern with no signs of myotonic discharges or myopathic alterations.

The affected cousin (V-18, Fig. 1) had similar complaints to those of the index case. The first symptoms were noticed at age 1 year, with difficulty standing up associated with leg stiffness. When he was reevaluated at age 12 years, neurological examination showed global muscle hypertrophy (Fig. 2B), mild proximal weakness of the

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**FIGURE 1.** Pedigree of the family, highlighting the individuals tested for the K248X mutation. Black squares: clinical affected—homozygous for the K248X mutation; green-shaded squares and ovals: clinically asymptomatic—heterozygous for the K248X mutation; gold squares and oval: clinically asymptomatic—absence of the K248X mutation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
limbs, and grip myotonia, calf myotonia during the stair climbing, and percussion myotonia. His serum CK level was two- to fivefold above the upper limit of normal. His parents, who are double first cousins (brother and sister of the index case parents), also had a normal neurological examination.

Screening for Mutations in the CLCN1 Gene. DNA samples from 14 family members were extracted from whole blood by standard procedures and amplified by polymerase chain reaction (PCR) using primers for the 23 exons of the CLCN1 gene.

Mutations were screened through single-strand conformational polymorphism (SSCP) analysis, and denaturing high-performance liquid chromatography (DHPLC) screening amplicons from the exons showing abnormalities underwent sequencing analysis.

A c.742 A>T transition in exon 6 was found in the index case (V-13), resulting in a lysine to a stop-codon substitution at position 248 of the protein (K248X) (Fig. 4). The same homozygous mutation was also observed in affected patients V-14 and V-18. The 4 parents (IV-10 and V-11, V-12 and V13), 2 of the tested grandmothers (III-4 and III-10), and 2 sisters of V-18 (V-16 and V-17)
also had the mutation, but in a heterozygous state. This mutation was not found among other tested relatives from this family (III-11, III-12, and V-15).

**DISCUSSION**

Large consanguineous families, although rare, can be found at a higher frequency in some Brazilian regions. They provide important information regarding genetic diseases and help to clarify the mechanisms of new mutations.

In considering the two allelic forms of congenital myotonias, the family described here had a phenotype of Thomsen disease, with classical mild myotonia associated with early onset of symptoms. The mild progression was compatible with normal daily activities and did not require any medication to relieve the myotonia. Therefore, this family was studied at our center for many years looking for any possible clinical signs in the parents, because an autosomal dominant pattern of inheritance was expected.

 Signs of latent myotonia, that is, electrophysiological myotonia, without clinical manifestations, have been described in heterozygous carriers of congenital recessive myotonia. There is no consensus regarding the diagnostic value of this test, but in this family even careful electrophysiological study did not identify any myotonic discharges in the tested heterozygotes.

With the improvement of molecular biology techniques and further molecular analysis of the candidate gene CLCN1, we could determine the genetic defect and the pattern of inheritance of the disease in this family. A novel K248X stop codon mutation was identified. It segregated in homozygosity in the 3 symptomatic patients and was present in one allele in the 4 affected patients’ parents, 2 grandmothers, and 2 sisters, all clinically asymptomatic.

More than 100 different mutations have already been described in the CLCN1 gene, including missense and nonsense mutations. Most mutations produce recessive forms of myotonia, whereas only a few cause the dominant form. Point mutations include more than 20 identified missense mutations, located at the N terminus to transmembrane domain XII of the protein. Other mutations are deletions, splice-site mutations, and nonsense mutations. There are also approximately 10 mutations that have been associated with both autosomal recessive and autosomal dominant congenital myotonia, including the common Arg894X mutation. In these cases, it becomes difficult to clearly distinguish between the two modes of inheritance. Considering that the carriers of the novel K248X mutation described here are clinically

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**FIGURE 4.** Molecular analysis of the 23 exons of the CLCN1 gene. In exon 6, a c.742 A>T transition was found, resulting in a lysine to a stop-codon substitution at position 248 of the protein (K248X). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
and electrophysiologically asymptomatic, this mutation could fit the criterion of a recessive mutation.

The CLCN1 gene encodes the voltage-gated chloride channel 1 protein (skeletal muscle), which is mainly expressed in muscle. The channel is composed of two identical protein molecules. Each protein forms a separate ion-conducting pathway, called a protopore. Autosomal dominant Thomsen disease is related to one dominant-negative mutation that modifies either dimerization or ion selectivity of the channel. In autosomal recessive Becker disease, it is supposed that both subunits are affected by the mutations present in both alleles, causing loss of channel function. The consequence of recessive truncations has also been related to their location. According to Colding-Jørgensen, among the 18 recessive truncations described, the early truncations can safely be regarded as non-functional. Later truncations may be functional, especially if the membrane-spanning components of the subunit of the channel are intact.

The homozygous K248X mutation found in our family is expected to result in loss of function of the channel. In addition, this novel K248X mutation can be considered to be an early truncation, because it is located in exon 6 of the CLCN1 gene, close to the N-terminus region of the gene, and the residual 248 is located in the fourth transmembrane domain (http://www.ncbi.nlm.nih.gov/protein/119433677?report=graph). Therefore, severe clinical manifestations would be expected in affected patients. A reduction of chloride conductance to 50% apparently does not cause myotonia because heterozygous carriers of non-functional autosomal recessive mutations are asymptomatic. We speculate that this new mutation would be compatible with a more significant reduction of channel chloride conductance.

In conclusion, our findings support the presence of variability in the clinical course, pattern of inheritance, and molecular defects associated with mutations in the CLCN1 gene, and the diagnosis of either Thomsen or Becker myotonia. Further complementary functional studies are necessary to elucidate the effect of each mutation in the chloride channel affecting muscle function.

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