

A Novel Missense Mutation in *CLCN1* Gene in a Family with Autosomal Recessive Congenital Myotonia

Mohammad Miryounesi¹, MD, PhD;
Soudeh Ghafouri-Fard², MD, PhD;
Majid Fardaei³, PhD

¹Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran;

²Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran;

³Department of Medical Genetics, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence:

Majid Fardaei, PhD;
Department of Medical Genetics,
Fakhrabad Street, Shiraz, Iran
Tel/Fax: +98 71 32349610
Email: mfardaei@sums.ac.ir
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What's Known

- Congenital recessive myotonia is a rare genetic disorder.
- It is caused by mutations in *CLCN1*.

What's New

- We found a new missense mutation in the *CLCN1* gene (c.1886T>C).
- *In silico* analyses predicted that this nucleotide change would impair the protein function

Abstract

Congenital recessive myotonia is a rare genetic disorder caused by mutations in *CLCN1*, which codes for the main skeletal muscle chloride channel CIC-1. More than 120 mutations have been found in this gene. The main feature of this disorder is muscle membrane hyperexcitability. Here, we report a 59-year male patient suffering from congenital myotonia. He had transient generalized myotonia, which started in early childhood. We analyzed *CLCN1* sequence in this patient and other members of his family. We found a new missense mutation in *CLCN1* gene (c.1886T>C, p.Leu629Pro). Co-segregation of this mutation with the disease was demonstrated by direct sequencing of the fragment in affected as well as unaffected members of this family. In addition, *in silico* analyses predicted that this nucleotide change would impair the protein function. Thus, this new nucleotide variation can be used for prenatal diagnosis in this family.

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Keywords • Myotonia congenita • *CLCN1* protein • Mutation

Introduction

Congenital myotonia is a genetic channelopathy that affects skeletal muscles. The main feature of this disorder is muscle membrane hyperexcitability.¹ The worldwide prevalence of this disorder has been estimated at 1:100,000.² Hereditary pure myotonia is caused by mutations in two different genes coding for the voltage-dependent skeletal muscle specific Cl⁻ and Na⁺ channels. While Na⁺ channel myotonia is dominantly inherited, most of the Cl⁻ channel mutations are recessive traits. There are few dominant or semidominant Cl⁻ channel mutations in which dominant negative heterodimerization with normal subunits causes the disease phenotype.³ The Na⁺ channel causes the membrane to be more excitable and lets it fire action potentials, but the Cl⁻ channel reduces excitability and stabilizes the resting potential.³ *CLCN1* codes for the main skeletal muscle Cl⁻ channel. Mutations in this gene have been shown to reduce sarcolemmal chloride conductance and consequently cause membrane hyperexcitability.¹ Human CIC-1 is a 988 amino acid membrane protein encoded by the 23 exon *CLCN1* gene on chromosome 7q35.⁴ About 130 mutations have been found in this gene, which cause autosomal-dominant as well as autosomal-recessive forms of this disorder.¹ Myotonia as the main sign of this disorder is

described as unusual delay in muscle relaxation after voluntary forceful contraction. Muscular stiffness is present in movement initiation and becomes milder if the same movement is repeated. The phenotypes in affected individuals range from mild myotonia, revealed only by clinical examination, to severe and disabling myotonia with temporary weakness and myopathy. Patients with two mutated alleles suffer from the most severe phenotype. Heterozygotes are often asymptomatic, but heterozygosity has been shown to be sufficient to cause evident myotonia in some cases.⁵ Although the only culprit in this disorder is *CLCN1*, different mutations in this gene can cause autosomal-dominant (Thomsen disease, OMIM 160800) or recessive trait (recessive generalized myotonia or Becker myotonia, OMIM 255700).

Case Presentation

The patient was a 59-year man suffering from congenital myotonia (patient III-2). He had transient generalized myotonia, which started in early childhood. He reported amelioration of stiffness after repeated activity. No muscle weakness was seen in the physical exam. Electromyographic examination revealed myotonic discharges. His affected children suffered from the same symptoms since they were about 6 years old.

In order to find possible mutation in *CLCN1* gene, blood sample was collected from the

patient after informed consent was obtained according to the protocol approved by the local institutional review board. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. DNA was extracted from peripheral blood of the patient using salting out method.⁶ All exons and exon-intron boundaries of *CLCN1* genes were amplified and sequenced by Illumina's Genome Analyzer (BGI Clinical Laboratories, Shenzhen, China). A new missense mutation was found in this gene (c.1886T>C, p.Leu629Pro). The fragment corresponding to the observed nucleotide change was sequenced in other members of the family using the ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Co-segregation of this mutation with the disease was demonstrated by direct sequencing of the fragment in affected as well as unaffected members of this family. The pedigree of the family is shown in figure 1. Individuals known to be heterozygous for this nucleotide change do not have any movement disorder.

Discussion

Mutations in *CLCN1* gene have been shown to cause congenital myotonia. Here, we demonstrated a new mutation in this gene, which is segregated in a family with several members suffering from congenital myotonia. Protein sequence analysis demonstrated that this amino acid substitution occurs in the

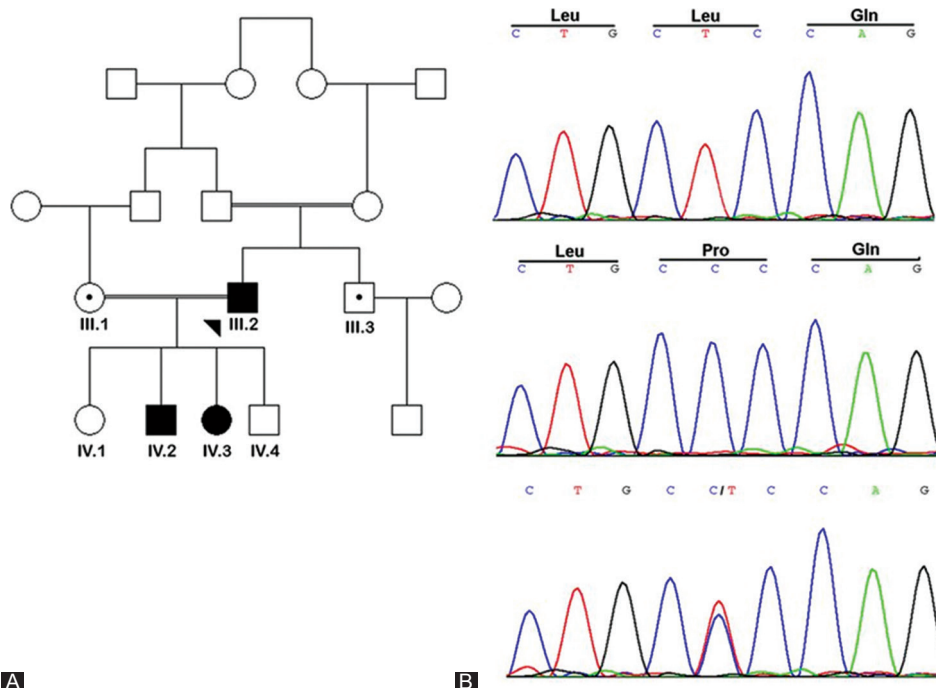


Figure 1: (A) The pedigree shows patients as well as carriers of the mentioned mutation. (B) Sequence analysis shows normal sequence (above), mutant homozygout (middle) and heterozygout (below).

cystathionine-beta-synthase (CBS) domain of the protein (amino acids 609-668). Point mutations in CBS domains have been previously shown to critically impair the specific protein function and are responsible for numerous hereditary disorders in humans.⁷ *In silico* analyses by MUpro (Prediction of Protein Stability Changes for Single Site Mutations from Sequences) revealed that this nucleotide change decreases the stability of protein structure (Confidence Score: -1).⁸ With MUpro, a negative score shows the mutation decreases protein stability, where lower scores imply higher confidence. In addition, SIFT software predicted that this mutation affects protein with a score of 0.00. This prediction is based on the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences. By this software, the amino acid substitution is predicted to be damaging if the score is ≤ 0.05 , and tolerated if the score is > 0.05 . PolyPhen-2 predicted that this mutation is probably damaging with a score of 1.000 and showed that this residue is conserved among all species. With this software, values nearer to 1 are more confidently predicted to be deleterious.

In brief, we demonstrated a new missense mutation in *CLCN1* gene in a patient with congenital myotonia. *In silico* analyses with multiple software as well as segregation analysis imply that this nucleotide change will impair the function of protein. In addition, we demonstrated that this mutation causes recessive form of the disorder.

However, detailed description of the effects of this nucleotide change on muscle-specific Cl⁻ channel is only possible by functional analysis. In a previous study, the functional consequences of different mutations on Cl⁻ channel has been tested by recording chloride currents from human embryonic kidney cells temporarily expressing mutant channels. This study has demonstrated that different *CLCN1* mutations cause distinctive clinical and electrophysiological effects. Such researches would help to understand the pathophysiology of this neurologic condition in addition to designing new therapeutic modalities for severe forms.⁹

Although there are many reports introducing novel *CLCN1* mutations in different populations, to our knowledge, this is the first report of molecular analysis of this gene in the Iranian population. Because of the low frequency of mutated alleles in most populations, genetic counseling has an important role in this kind of neurological disorder, to prevent its occurrence in subsequent generations.

Acknowledgment

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Conflict of Interest: None declared.

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