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SCN4A mutation as modifying factor of Myotonic Dystrophy Type 2 phenotype

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Abstract

In myotonic dystrophy type 2 (DM2), an association has been reported between early and severe myotonia and recessive chloride channel (*CLCN1*) mutations. No DM2 cases have been described with sodium channel gene (*SCN4A*) mutations. The aim is to describe a DM2 patient with severe and early onset myotonia and co-occurrence of a novel missense mutation in *SNC4A*. A 26-year-old patient complaining of hand cramps and difficulty relaxing her hands after activity was evaluated at our department. Neurophysiology and genetic analysis for DM1, DM2, *CLCN1* and *SCN4A* mutations were performed. Genetic testing was positive for DM2 (2650 CCTG repeat) and for a variant c.215C>T (p.Pro72Leu) in the *SCN4A* gene. The variation affects the cytoplasmic N terminus domain of Nav1.4, where mutations have never been reported. The biophysical properties of the mutant Nav1.4 channels were evaluated by whole-cell voltage-clamp analysis of heterologously expressed mutant channel in tsA201 cells. Electrophysiological studies of the P72L variant showed a hyperpolarizing shift (−5 mV) of the voltage dependence of activation that may increase cell excitability. This case suggests that *SCN4A* mutations may enhance the myotonic phenotype of DM2 patients and should be screened for atypical cases with severe myotonia.

Keywords: Myotonic dystrophy type 2; Myotonia; SCN4A; N-terminus

1. Introduction

Myotonic dystrophy type 2 (DM2, PROMM, OMIM # 602668) is an adult onset muscular dystrophy caused by a CCTG repeat expansion in *CNBP* gene on chromosome 3q21 [1]. The expanded RNA transcripts modify the activity of specific RNA binding proteins involved in regulating alternative splicing. The resulting missplicing of several genes is thought to account for the multisystem nature of the disease including cardiac, cerebral and endocrine involvement, along with proximal weakness and myotonia [2]. Missplicing of the skeletal muscle chloride channel, CLC-1, leads to a transcript coding for a non-functional channel. Consequent reduced resting chloride conductance increases the electrical excitability of the muscle and causes myotonia [3].

Myotonia in DM2 is usually mild and sometimes may be difficult to elicit even with electromyographic evaluation [4,5]. Recent studies have found an association between DM2 patients with prominent myotonia and heterozygous recessive *CLCN1* mutations on chromosome 7q35 [6,7]. Mutations of *CLCN1* which encode the skeletal muscle voltage-gated chloride channel (CLC-1) are responsible for myotonia congenita (recessive Becker disease, OMIM # 255700; dominant Thomsen disease, OMIM # 160800). Mutations are "loss of function" causing reduced sarcolemmal chloride conductance and enhanced excitability [8]. The additive effects of *CLCN1* missplicing caused by DM2 expansion and *CLCN1* mutation may cause an atypical DM2 phenotype characterized by severe and early myotonia [7].

Another gene implicated in myotonic disorders is *SCN4A* on chromosome 17q23.3 (Myotonia, Potassium-aggravated,

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OMIM # 608390). *SCN4A* codes for Nav1.4, the voltage gated sodium channel (VGSC) expressed in skeletal muscle [9]. *SCN4A* mutations usually produce a "gain of function" effect causing impaired inactivation or enhanced activation of the Nav1.4 channel resulting in muscle excitability [8]. To date, a possible additive influence of *SCN4A* mutation to the DM2 phenotype has never been evaluated.

In our study we investigated a DM2 patient with severe and early onset myotonia without mutation in *CLCN1* gene. We identified a novel missense mutation c.215C>T (p.Pro72Leu) in *SCN4A* gene that represents the first mutation ever reported affecting the cytoplasmic N terminus domain of Nav1.4. To investigate the biophysical alteration of P72L substitution we performed whole-cell voltage clamping in a heterologous expression system showing the Nav1.4 mutant gain of function effect.

2. Materials and methods

2.1. Patient

The patient, a Caucasian 26-year-old woman, was evaluated in the Department of Neurology at the Penn State Hershey Medical Center. The patient gave written informed consent for genetic analysis in agreement with Helsinki convention. Clinical information on other family members was obtained during the interview.

2.2. Molecular genetic analysis

Genetic analysis of *DMPK*, *CNBP*, *CLCN1* and *SCN4A* genes was performed by Athena Diagnostics, Inc. (Marlborough, MA) using the Complete Myotonia evaluation kit, which has been developed for the molecular diagnosis of myotonic dystrophy type 1 (DM1), myotonic dystrophy type 2 (DM2), myotonia congenita and sodium channel myotonia.

DM1 and DM2 repeat expansion mutations on *DMPK* gene (Chr19q13.3) and *CNBP* gene (Chr3q21.3) were evaluated utilizing polymerase chain reaction (PCR) amplification of genomic DNA followed by high resolution electrophoresis to determine the number and the size range of CTG and CCTG repeats.

DNA sequence analysis of *CLCN1* and *SCN4A* gene was performed by PCR amplification of highly purified genomic DNA, followed by automated bi-directional DNA sequencing of the entire coding region of *SCN4A* (24 exons, NM_000334) and *CLCN1* (23 exons, NM_000083). Sequencing also included the highly conserved flanking intronic sequence of the exon–intron splice junctions for all coding exons and 10 bases of intronic DNA surrounding each exon. Additional information is available by contacting Athena Diagnostic, Inc. *SCN4A* exon 1 has also been analyzed by direct sequencing in 100 control individuals.

2.3. Functional analysis of WT and p. P72L Nav 1.4 channels

The c.215C>T mutation was engineered into Wild-Type (WT) *SCN4A* complementary DNA (cDNA), received from Maria Essers Department of Clinical Research Ion Channels and Channelopathies University of Bern in the pRc/CMV2 plasmid by site directed mutagenesis using the QuikChange II site-directed mutagenesis kit (Stratagene). The construct was sequenced to confirm the correct introduction of mutation and ensure the validity of sequence. Beta 1 subunit (a kind gift from Prof. Hugues Abriel, University of Bern, Switzerland) was subcloned in a pIRES vector engineered with EGFP. WT and P72L SCN4A mutant channels were transiently expressed in tsA201 cells using Fugene. An equal amount (0.5 µg) of alpha and beta 1 subunits were transfected. To mimic the heterozygous condition, 0.25 µg of each alpha subunit were transfected. The expression of the channels was studied 48 h after transfection.

Membrane currents were measured using whole cell patch clamp with Axopatch Multiclamp 700B amplifier (Axon Instruments, Foster City, CA), as previously described [10]. Recordings were made at room temperature. Internal solution contained (mmol/L) 50 aspartic acid, 60 CsCl, 5 disodium ATP, 11 EGTA, 10 HEPES, 1 CaCl2, 1 MgCl2, pH 7.4 adjusted with CsOH. Extracellular solution contained (mmol/L) 130 NaCl, 2 CaCl2, 5 CsCl, 1.2 MgCl2, 10 HEPES, 5 Glucose, pH 7.4 adjusted with CsOH. In the experiments designed to measure the voltage dependence of activation, external sodium was reduced to 65 mM using n-methylglucamine as Na^+ substitute. Holding potential was −80 mV.

2.4. Statistical analysis

Pooled data are presented as mean ± SD; n denotes the number of cells. To minimize the effect of culture-to-culture variability, cells were prepared expressing each construct and data from at least three separate transfections were pooled for each comparison. An ANOVA was performed for multiple comparison, followed by a modified t test with Fisher correction (ORIGIN 10). Values of $p < 0.05$ were considered significant, in figures indicated with *.

3. Results

3.1. Patient

A 26-year-old female patient complained of hand cramps and difficulty relaxing her hands from the age of 20. She reported pain in her shoulders and soreness of forearms following exercise. There were no clear triggers and specifically neither cold nor diet exacerbated her symptoms. She had no episodic weakness. She tried mexiletine that improved her stiffness slightly but stopped it due to gastrointestinal side effects. Over the past 2 years, she had noted weakness in her hands. In terms of family history, her 54-year-old mother had cataracts removed and complained of hand cramping. Neurological examination of the patient showed grip and thenar percussion myotonia with warm up phenomenon and mild distal weakness. Needle EMG examination revealed abundant, overlapping, myotonic discharges in most of the muscles examined and myopathic motor units predominantly in her distal muscles. Short and prolonged exercise testing was normal.

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