Neuromuscular Disorders 18 (2008) 633–640

www.elsevier.com/locate/nmd

Novel chloride channel mutations leading to mild myotonia among Chinese

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Received 18 October 2007; received in revised form 10 May 2008; accepted 15 May 2008

Abstract

We describe two Chinese families with a mild form of the myotonia congenita due to novel chloride channel (*ClCN1*) mutations. In one case, heterozygous I553F and H555N mutations were found. The patient shared the I553F mutation with his healthy father, and his mother had a history of mild myotonia when she was younger. In another family, autosomal dominant myotonia congenita was due to a L844F change. The physiological effects of the mutations were examined by using the two-electrode voltage-clamp technique after expression of the channels in *Xenopus* oocytes. All mutations drastically shifted the voltage required for half-maximal activation, more under conditions mimicking the homozygous situation, than under conditions mimicking the heterozygous situation. The larger effect was seen in the compound heterozygous situation combining the I553F and the H555N mutations. Our data suggest that myotonia congenita caused by *CLCN1* mutations in Chinese have similar variable features to those found in the West.

Keywords: Myotonia congenita; Mutation; Chinese; Chloride channel; Physiology

1. Introduction

Patients with myotonia congenita present with muscle stiffness, manifested after a voluntary contraction, which typically decreases with repetitive movement. The disorder is inherited in an autosomal dominant (Thomsen's disease) or in a recessive way (Becker's disease) [1]. Patients with the recessive disorder often have a more severe presentation, with weakness, in addition, when muscles are activated after a period of rest. A number of different mutations have been found in the chloride channel gene, ClC-1, and cases from families with autosomal dominant myotonia congenita are usually heterozygous for a mutated and a normal allele, while those in families with recessive myotonia congenita are homozygous or compound heterozygous. However, some mutations have been found to lead to autosomal dominant myotonia congenita in some families and to a homozygous recessive form in others. For example, two mutations leading to a similar molecular defect, a fs793X truncated protein, have been found to result in different types of inheritance. A 2330delG mutation has been found in a family with autosomal dominant myotonia congenita [2] and a 2264delC in another family with recessive inheritance [3]. A clear understanding of the genotype–phenotype correlation is still lacking [4]. In general, investigations of channel properties have established a diminished chloride conductance at the muscle cell membrane, which leads to a decrease in the threshold to

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doi:10.1016/j.nmd.2008.05.007

generate action potentials and, consequently, to repetitive firing. This can be assessed by characteristic EMG activity, usually described as ''mytonic runs'' that occur immediately following electrode insertion or following stimulated or voluntary muscle activity. Most of the patients reported so far are from Caucasian origin, and myotonia congenita has only recently and rarely been reported among Chinese [5,2], with a recent review of the Chinese literature describing 196 cases in 29 reports [6]. By contrast, thyrotoxic periodic paralysis, a disorder related to periodic paralysis due to mutations in the muscle sodium channel gene, *SNC4A*, is much more frequent among Chinese as compared to Caucasians [7]. The reasons for this variability remain completely unknown, and further data are needed to understand ethnic differences related to genotype and phenotype correlations.

In the present report, we provide additional evidence for the role of *CLCN1* mutations in myotonia congenita by demonstrating novel mutations in two unrelated families of Chinese origin. Furthermore, physiological analysis of these mutated channels expressed in *Xenopus* oocytes has allowed us to examine the effect of the mutations on chloride conductance.

2. Materials and methods

2.1. Mutation search

Blood was taken from the affected persons and other members of their families after informed consent. The study was approved by the local Ethics Committees. DNA isolation and PCR amplification for all exons of *CLCN1* was performed as previously described[8]. Published primers were used for the *CLCN1* gene [9,8]. PCR products were sequenced in an ABI automated DNA Sequencing System (PE Applied Biosystems, Foster City, CA, USA).

2.2. Site directed mutagenesis

Single- and double-point mutations were introduced into the cDNA of the human chloride channel purchased from IMAGE Consortium (No. 8322547) by a polymerase chain reaction (PCR)-based method. Comparison with the human genome indicated that this IMAGE clone contained an amino acid substitution (W118G). This mutation was corrected by PCR and this wild type (WT) was used for further analysis. WT or mutated chloride channels were epitope-tagged (FLAG) at their N-terminus by a similar strategy. All constructs have been subcloned into the pSD5 vectors [10].

2.3. Expression of WT and mutant ClC-1 in Xenopus laevis oocytes

Plasmids were linearized with NsbI (Fermentas). Capped complementary RNA (cRNA) was synthesized from the linearized plasmid DNA using the mMessage mMachine SP6 Kit (Ambion Europe, Leighton Buzzard, United Kingdom). *Xenopus* oocytes were injected with a solution containing the transcripts coding for either the WT or the mutant ClC-1 at a concentration of 150 nM. When a 1:1 mixture containing two transcripts was injected the concentration was 75 nM each. The rat γ-aminobutyric acid A ($GABA_A$) receptor subunits α1 and β2 were introduced into the oocytes at a concentration of 75 nM as a control to check for functional oocyte expression.

2.4. Functional analysis in X. laevis oocytes

Xenopus oocytes were prepared, injected, and defolliculated, and currents were recorded as previously described [11,12]. Electrophysiological experiments were performed by the two-electrode voltage-clamp method using a custom built amplifier with documented linearity up to current amplitudes of 20 μA. Voltage protocols to elicit opening of the chloride channels were produced, and data recordings were performed using the computer programes MacLab and Scope (ADInstruments GmbH, Spechbach, Germany). Instantaneous currents and steady state currents were determined 3 ms and 95 ms after the second voltage step. Overall relative open probabilities (Po) were calculated from the amplitude of the normalized tail currents 3 ms after voltage steps to −100 mV after 300 ms pre-pulses ranging from −140 mV to +120 mV. Individual curves were fitted using Kaleidagraph with a Boltzmann function:

$$I_v = I_o + (I_{max} - I_o)/(1 + \exp((V_{1/2} - V)/dx))$$

In which I_{max} is the maximal current, I_o is a constant offset, $V_{1/2}$ is the half-maximal activation voltage and dx is the slope factor. The relative P_o was obtained by the normalization $P_o = I_v / I_{max}$.

All data are shown as means ± SD from at least five different experiments. Currents elicited by the application of one mM GABA were measured at a holding potential of −80 mV.

3. Results

3.1. Case presentation

3.1.1. Family 1

A 19-year-old Chinese man was referred with complaints of lower-limb pain of 7 years' duration. Initially mild, the pain progressed to impair sport activities. He had bilateral calf and thigh cramping with tightness occurring when the patient ran, ascended stairs, or participated in sports such as distance running and basketball. These episodes sometimes caused him to fall. He noticed that sudden bursts of movements triggered the attacks, which lessened with activity and were not followed by muscle weakness. Symptoms were not exacerbated in the cold. At examination, muscle hypertrophy was found, and myotonia could be elicited on percussion and at EMG