# Clinical, molecular and functional characterization of a novel *CLCN1* mutation associated to myotonia congenita

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Myotonia congenita (MC) is an inherited muscle disease characterized by impaired muscle relaxation after contraction, resulting in muscle stiffness. Both recessive or dominant MC are caused by mutations in the *CLCN1* gene encoding the voltage-dependent ClC-1 chloride channel. This channel is quite exclusively expressed in skeletal muscle where it normally stabilizes muscle fibre membrane potential. Over the years, the functional analysis of naturally occurring ClC-1 mutations in heterologous expression systems provided important insights into the pathomechanism of the disease (1,2,3). Indeed, the reduced chloride conductance, due to the loss-of-function mutations in ClC-1, predisposes the muscle membrane to the hyperexcitability that hampers muscle relaxation generating myotonia. However, various aspects of the disease remain to be clarified. In particular, the correlation between clinical symptoms, functional and genetic defects is not easily predictable, given the variability of symptoms reported among individuals carrying the same ClC-1 mutation (4). Interestingly, a recent study proposed at least two disease modifiers, among genes involved in muscle excitability, to explain the clinical phenotype, using patients' muscle biopsies in support of the functional data (2).

We recently identified a novel *CLCN1* heterozygous variant, the c.1004C>A, associated to a Thomsen MC phenotype in one Italian family. Clinical signs include lower limb muscle stiffness, sometimes painful and mainly occurring at the beginning of movement after prolonged rest and improving with exercise. The resulting T335N mutation is located in the I-J loop on the extracellular surface of the ClC-1 dimer.

Here we provide the functional characterization of T335N channels expressed in tsA201 cells by whole-cell patch clamp, with the aim to gain insight into the molecular mechanism underlying ClC-1 dysfunction in this family. Moreover, we decided to perform q-PCR analysis of selected ion channels transcripts in the available muscle biopsy of the affected patient, in order to better correlate the genetic and functional analysis to the clinical symptoms of the disease.

Electrophysiological recordings show that, in high intracellular chloride solution, T335N currents lack the fast deactivation at negative potentials typical of wild-type ClC-1 currents, instead showing slow activation at each test potential till reaching a steady-state. Instantaneous and steady-state current densities for T335N are at each voltage greatly reduced compared to WT. The voltage-dependence of T335N activation appears significantly shifted by about 70mV toward positive potentials compared to WT channels. Gene expression experiments using the available muscle biopsy of the affected carrier are in progress.

In conclusion, patch-clamp studies of ClC-1 mutants demonstrated the pathogenicity of T335N channels. We expect that the quantitative analysis of gene expression will confirm previous published data (2), thus supporting the idea that disease manifestations could involve additional genes than *CLCN1*. Thus, an in-depth characterization of newly identified MC mutations that includes clinical, functional and molecular analysis using heterologous expression systems and muscle biopsies would be highly advisable for a better understanding of the disease phenotype and for the identification of new drug targets and therapeutic options.Supported by Italian Health Ministry (grant GR-2009-1580433) and Telethon-Italy (grant GGP14096).

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4. Imbrici et al. (2015). Front Cell Neurosci 9:156