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Neuromuscular Disorders 23 (2013) 437–440

www.elsevier.com/locate/nmd

Case report

A new *de novo* missense mutation in *MYH2* expands clinical and genetic findings in hereditary myosin myopathies

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Received 31 July 2012; received in revised form 6 February 2013; accepted 11 February 2013

Abstract

Congenital myopathy related to mutations in myosin MyHC IIa gene (*MYH2*) is a rare neuromuscular disease. A single dominant missense mutation has been reported so far in a family in which the affected members had congenital joint contractures at birth, external ophthalmoplegia and proximal muscle weakness. Afterward only additional 4 recessive mutations have been identified in 5 patients presenting a mild non-progressive early-onset myopathy associated with ophthalmoparesis. We report a new *de novo MYH2* missense mutation in a baby affected by a congenital myopathy characterized by severe dysphagia, respiratory distress at birth and external ophthalmoplegia. We describe clinical, histopathological and muscle imaging findings expanding the clinical and genetic spectrum of *MYH2*-related myopathy.

Keywords: MyHC IIa; *MYH2*; Congenital myopathy; Ophthalmoplegia; Joint contractures

1. Introduction

Hereditary myosin myopathies are a group of muscle diseases with variable age of onset and heterogeneous clinical features, caused by mutations in the skeletal muscle myosin heavy chain (MyHC) genes [1]. Three major isoforms are known in adult human limb muscle fibers: MyHC I, encoded by *MYH7* and expressed in slow type I muscle fibers and in heart ventricles; MyHC IIa, encoded by *MYH2* and expressed in fast type IIA muscle fibers, and MyHC IIx expressed in fast type IIB muscle fibers [1]. To date pathogenetic mutations associated to hereditary myopathies have been identified in both *MYH7* and *MYH2* genes [2]. The first pathogenetic *MYH2* mutation, reported by Martinsson and coauthors in 2000 [3], was a dominant missense mutation in exon 17 (c.2116, G>A), giving rise to an amino acid shift p.Glu706 Lys (E706K). All affected members showed multiple joint contractures at birth and external ophthalmoplegia. The disease was non-progressive in childhood but patients progressively developed proximal muscle weakness by the age of 30 years [4]. In 2005 two additional heterozygous missense mutations in *MYH2* have been identified in 2 unrelated patients affected by a mild proximal myopathy. However the pathogenetic role of these mutations was not completely proven [5]. Thus, with the exception of the original family harboring the dominant E706K, only 5 additional patients, from three unrelated families, with autosomal recessive mutations in *MYH2* have been reported [6]. All these patients had an early-onset myopathy characterized by mild generalized muscle weakness and pronounced ophthalmoparesis [6]. We report an unusual phenotype of neonatal-onset congenital

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http://dx.doi.org/10.1016/j.nmd.2013.02.011

myopathy related to a new *de novo* missense mutation in *MYH2*.

2. Case report and results

The patient is a 12-year-old Italian girl. She was the second child of healthy non-consanguineous parents. She was born at 36 weeks of gestational age by spontaneous vaginal delivery. Both amniotic fluid and fetal movements were reportedly normal. She developed severe respiratory distress during the first hour of life requiring assisted ventilation. At birth she had generalized hypotonia with preserved anti-gravity limb movements without joint contractures or limb deformities. The baby had a myopathic face with ptosis and ophthalmoparesis and she presented inability to swallow. Respiratory function progressively improved whereas severe dysphagia persisted requiring gastrostomy. CPK were normal and electromyography did not show any myotonic discharges. AChR antibodies and repetitive nerve stimulation were normal. Muscle biopsy, performed at the age of 9 months, showed marked variability in fiber size and moderate fibrosis without inflammation or necrosis. Immunochemistry for myosin isoforms showed predominance of type I fibers and few type II fibers (Fig. 1). This histological picture moved us towards a diagnosis of congenital myopathy that was over time supported by the observation of clinical gradual improvement. The baby acquired independent ambulation at the age of 2 years and swallowing problems gradually improved allowing the removal of gastrostomy at the age of 4 years. Genetic analysis for Ryanodine-Receptor 1 (RYR1), beta-tropomyosin (*TPM2*) and tropomyosin 3 (*TPM3*) genes were normal. At last clinical examination, at the age of 12 years, she still had a myopathic face with bilateral ptosis and ophthalmoplegia, arched palate and rhinolalia, waddling–steppage gait and mild dorsal scoliosis. Muscle MRI showed diffuse involvement of the thigh mainly involving vastus lateralis, rectus femoris and semitendinosus muscles. At calf level there were marked changes in the lateral head of the gastrocnemius (Fig. 2). The main clinical features of ophthalmoplegia and non-progressive myopathy prompted us to search for a *MYH2* defect. We performed direct sequencing analysis of all coding exons of *MYH2* (Ref. cDNA sequence NM_017534.5) disclosing a heterozygous c.5737T>C mutation in exon 39. This variant was not found in both parents and in 100 unrelated healthy controls. The prediction analysis by SIFT (sift.jcvi.org/) classified the mutation as damaging (score:0). The c.5737T>C mutation changed the strongly conserved hydrophobic leucine residue at position 1870 to proline (L1870P). L1870P is located in the highly conserved LMM domain within the myosin tail, whose staggered and tight intermolecular packing involving several myosin proteins leads to the formation of thick filaments [7]. The multiple sequence alignment of the region of MyHC IIa surrounding the Leu 1870 residue affected by mutation with a proline is shown in Fig. 3. Because proline is a potent α-helix and β-sheet structure breaker [8] it is likely that the L1870P mutation introduces a bending in the α-helical coiled coil structure in which the myosin tail is known to be organized. Distortions in this region are expected to affect the formation of myosin homodimers, because the latter require the intertwinement of the helical structures of myosins across their tail length. Such structural distortions are even amplified during the formation of thick filaments, which employ an ordered and almost parallel packing of several homodimer tails. Despite the L1870P change is located near the C-terminus of the protein, multiple sites of the L1870P mutation and related distortions are distributed at various positions within the thick filament [7] thus introducing alterations at different longitudinal points in the filaments. Moreover, despite the L1870P is heterozygous, the expression of the correctly folded MyHC IIa by the wild type allele cannot compensate the deleterious effects produced by the mutated allele. In fact, the formation of myosin filaments implies the assembly of multiple MyHC IIa proteins, which are contributed by both the wild type and mutant proteins, and thus cannot

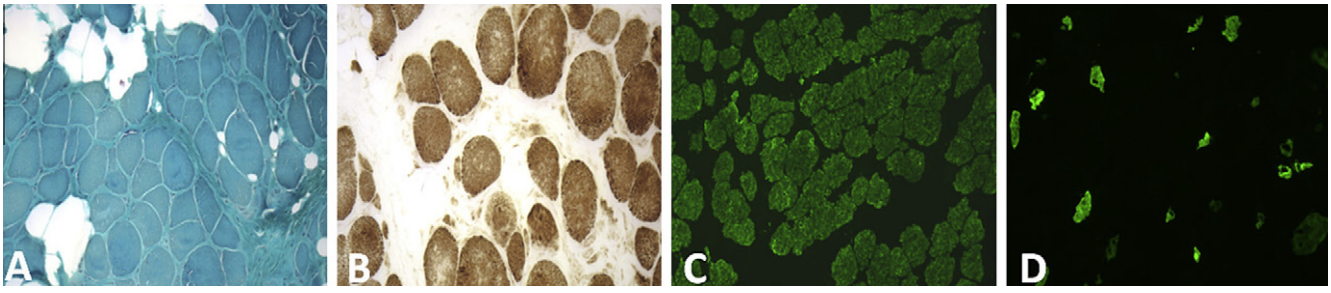

Fig. 1. Muscle biopsy. Quadriceps muscle biopsy shows marked variability in fiber size with proliferation of perimysial and endomysial connective tissue without inflammation and necrosis (A). Hystochemistry for cytocorme c-oxidase does not show disorganization of the intermyofibrillar network (B). Immunochemistry for Myosin isoforms shows type I fibers predominance (monoclonal antibodies against slow type I fibers, Leica Microsystems) (C). In (D) are shown the rare and hypotrophic type II fibers (monoclonal antibody against fast type fast IIa and IIx fibers, mAb A4.74, Hybridoma Bank, The University of Iowa, Department of Biology, Iowa City).