CONGENITAL MYOPATHY WITH NEMALINE RODS AND CAP STRUCTURES CAUSED BY A MUTATION IN THE β-TROPOMYOSIN GENE (TPM2)

Homa Tajsharghi, PhD; Monica Ohlsson, MD; Christopher Lindberg, MD, PhD; Anders Oldfors, MD, PhD

Objective: To describe the clinical, morphologic, and genetic findings in a family in which one woman had nemaline myopathy, whereas her daughter showed features of cap disease.

Patients: A 66-year-old woman and her 35-year-old daughter had congenital, slowly progressive muscle weakness. They had weakness in both proximal and distal muscles and facial diplegia with bilateral ptosis, a long narrow face, a high arched palate, and micrognathia.

Results: Muscle biopsy specimens in the mother at age 57 years had shown nemaline myopathy, whereas a biopsy specimen at age 32 years had demonstrated no rods. Muscle biopsy specimens in the daughter at age 26 years had shown features of cap disease and no apparent nemaline rods. A missense mutation, Glu41Lys, in the β-tropomyosin gene TPM2 was identified in both patients but was absent in their healthy relatives.

Conclusions: The results indicate that mutations in TPM2 may cause nemaline myopathy as well as cap disease with a dominant mode of inheritance. These disorders may thus be phenotypic variants of the same genetic defect.

Arch Neurol. 2007;64(9):1334-1338

NEMALINE MYOPATHY IS A CONGENITAL MYOPATHY characterized by the presence of inclusions called nemaline rods in skeletal muscle fibers. The muscle weakness ranges in severity from neonatal life-threatening disease to mild muscle weakness in adulthood and is usually slowly progressive or nonprogressive. Nemaline myopathy has been associated with mutations in 5 different genes: skeletal α-actin (ACTA1), nebulin (NEB), α-tropomyosin (TM) slow (TPM3), β-TM (TPM2), and troponin T1 (TNNT1).

Cap disease is a congenital myopathy with abnormal accumulation of proteins forming caplike structures in the periphery of the muscle fibers under the sarcolemma, with reduced myosin adenosine triphosphatase activity but reactive for nicotinamide adenine dinucleotide, reduced-tetrazolium reductase. So far, 6 sporadic cases and 1 familial case of cap disease have been reported. The clinical features are similar to those of nemaline myopathy, with infantile onset of hypotonia and muscle weakness predominantly involving the proximal muscles, neck flexors, and facial muscles. A muscle biopsy specimen showed small type 1 fibers but no inclusions. Electromyography showed a myopathic pattern.

Clinical examination results at age 26 years had shown muscle weakness in both proximal and distal muscles. She had facial diplegia with bilateral ptosis, a long narrow face, a high arched palate, a tent-
shaped mouth, micrognathia, and scoliosis. A second muscle biopsy was performed at age 26 years (see the “Methods” section). An electrocardiogram and an echocardiogram were normal. Serum creatine kinase level was normal. A lung function study showed a vital capacity of 1.3 L (41% of predicted); because of nocturnal hypoventilation, she received ventilatory support at night. At age 27 years she had had surgery for scoliosis. At age 35 years she could walk only short distances and otherwise used a wheelchair.

CASE 2

A 66-year-old woman (Figure 1A, subject II:2) was examined. Her history showed that, in the neonatal period, she had had respiratory insufficiency. Motor milestones were delayed. Over the years she had experienced slowly progressive muscle weakness. Clinical examination results in 1972 at age 32 years had shown moderate muscle weakness predominantly in the proximal muscles, neck flexors, and facial muscles and in the foot extensors. She also had lumbar hyperlordosis. A muscle biopsy specimen (only paraffin sections) indicated a primary myopathy with fiber size variation. According to the report from 1972, no rods or other inclusions were identified. An electromyogram showed a myopathic pattern.

At age 57 years she had had moderate muscle weakness involving both proximal and distal muscles; she could walk without support. She had a long narrow face, a high arched palate, micrognathia, and dysphagia. An electrocardiogram and an echocardiogram showed no signs of cardiomyopathy. Her serum creatine kinase level was normal. A lung function study showed a vital capacity of 1.3 L (42% of predicted), and she received ventilatory support at night. A second muscle biopsy was performed at age 57 years (see the “Methods” section).

METHODS

MUSCLE MORPHOLOGY

Open muscle biopsy of the tibialis anterior muscle was performed in both patients. One specimen was frozen in liquid nitrogen and 1 specimen was fixed in 2.5% glutaraldehyde. Standard techniques were applied for enzyme histochemical staining of cryostat sections and for electron microscopy. Immunohistochemical analyses included TM, actin, α-actinin, myotilin, and desmin.
GENOTYPE ANALYSIS

Extraction of genomic DNA, polymerase chain reaction (PCR), and sequence analyses were performed as previously described.5 The entire coding sequences of ACTA1, TNNI1, TNNT1, TNNC1, TPM2, and TPM3 were determined in patient 1, using oligonucleotides that were designed from flanking intronic sequences for all exons of the genes. The sequence analysis of

Figure 2. Muscle morphology. Staining with Gomori trichrome demonstrates caplike structures in case 1 (A) and subsarcolemmal nemaline rods in case 2 (B). Electron microscopy demonstrates disorganized myofibrils, irregular and partly thickened Z bands, and scarce thick filaments in case 1 (C), whereas there are numerous nemaline rods in case 2 (D). Enzyme histochemical staining of nicotinamide adenine dinucleotide, reduced–tetrazolium reductase (E) demonstrates subsarcolemmal structures, which in both cases showed partly enhanced staining, presumably due to irregular distribution of mitochondria and sarcoplasmic reticulum. Enzyme histochemical staining of adenosine triphosphatase, pH 4.3 (F), showed weak activity in the subsarcolemmal structures in both cases, and immunohistochemical staining of tropomyosin (G) showed intense immunoreactivity in both cases.
ACTA1, TPM2, and TPM3 was also performed in patient 2. The removal of a BsuI restriction enzyme site was used to confirm the G1639A mutation in the patients by digesting a TPM2 exon 2 PCR product with BsuI. To identify the G1639A mutation in each family member (Figure 1A, subjects I:2, II:1, and III:2), a PCR fragment of exon 2 of TPM2 was sequenced and also digested with BsuI.

GenBank accession numbers were for genomic TPM2 sequence AF209746 and for complementary DNA TPM2 sequence NM-213674.

RESULTS

In both patients there was type 1 fiber uniformity and a considerable variability of fiber size. The majority of the small muscle fibers showed peripherally located caplike structures, sharply demarcated from the rest of the fiber (Figure 2A and B). In patient 1 the caplike structures appeared without any rods on modified Gomori trichrome staining. On electron microscopy, the caps were composed of disorganized myofibrils and thickened Z bands. Thick filaments appeared to be partly lacking. In patient 2 the caplike structures included numerous nemaline rods (Figure 2B and D). In staining for myofibrillar adenosine triphosphatase, the caps showed reduced enzyme activity in both patients. The caplike structures displayed strong immunoreactivity for all studied sarcomeric proteins as well as desmin in both patients. In patient 2 there were also numerous ragged red muscle fibers, some of which were negative for cytochrome c oxidase. These fibers showed intense accumulation of abnormal mitochondria and were more numerous than would be expected from the age of the patient. Electron microscopy did not demonstrate nemaline rods in these fibers.

Mutation analysis of TPM2 identified a heterozygous missense mutation in exon 2, G1639A (DNA), G360A (complementary DNA), changing the highly conserved and negatively charged glutamate at position 41 to the positively charged lysine (Figure 1B and C). The mutation was not identified in any of the 3 investigated healthy relatives or in any of 200 control chromosomes (Figure 1A).

COMMENT

We describe 2 patients, a woman and her daughter, with a congenital myopathy characterized by sharply demarcated, peripherally located structures in the muscle fibers. In the daughter these structures appeared very similar to what has been described in cap disease, whereas in the mother there was subsarcolemmal accumulation of nemaline rods. We also identified a novel heterozygous missense mutation in the β-TM gene (TPM2) that segregated with the disease in the family.

Tropomyosin is localized head-to-tail along the length of the thin filament, providing stability, and is essential for myosin-actin interaction. The binding of calcium through the troponin complex induces the movement of TM within the thin filament and thereby allows the binding of myosin to actin. Three major TM isoforms are present in striated muscle: α-TM (encoded from TPM1) is predominantly expressed in cardiac muscle and fast, type 2 muscle fibers; β-TM (encoded from TPM2) is mainly expressed in slow, type 1 muscle fibers; and γ-TM (encoded from TPM3) is predominantly expressed in slow, type 1 muscle fibers. Thus, the identification of a mutation in TPM2 in our family is compatible with the finding of structural alterations in slow, type 1 muscle fibers. Four different mutations in TPM2 have so far been described and associated with 4 different phenotypes: nemaline myopathy, distal arthrogryposis type 1, myopathy without rods combined with distal arthrogryposis type 2B, and a congenital myopathy without rods. This variation indicates that the functional and structural consequences differ between the mutations. This may be due to interactions with other proteins, which are important for the function of TM.

Nemaline rods were not found in the biopsy specimen of patient 1 at age 26 years or in the first biopsy specimen of patient 2 at 32 years of age. Therefore, the nemaline rods appear to occur at greater age in this disease, as demonstrated by the presence of numerous nemaline rods in patient 2 at age 57 years. There are additional examples of patients with nemaline myopathy in whom the first biopsy specimen did not demonstrate the diagnostic rods. It is possible that some of the myopathies with cap structures will turn out to be nemaline myopathy at a later stage because all the reported cases of cap disease were in patients younger than 32 years at the time of biopsy. The clinical expression along with the changes in the morphologic features suggest that cap disease may be a variant or early stage of nemaline myopathy. Additional histologic investigations and identification of the genetic cause in other patients with cap disease are necessary to test this hypothesis.

ADDENDUM

During the final processing of this manuscript for publication, another article describing a TPM2 mutation in cap disease was published, which further supports the association between cap disease and mutations in TPM2.

Accepted for Publication: January 11, 2007.
Correspondence: Homa Tajsharghi, PhD, Department of Pathology, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden (homa.tajsharghi@gu.se).
Author Contributions: Drs Tajsharghi and Ohlsson contributed equally to this work. Study concept and design: Tajsharghi, Ohlsson, Lindberg, and Oldfors. Acquisition of data: Tajsharghi, Ohlsson, Lindberg, and Oldfors. Analysis and interpretation of data: Tajsharghi, Ohlsson, Lindberg, and Oldfors. Drafting of the manuscript: Tajsharghi, Ohlsson, and Oldfors. Critical revision of the manuscript for important intellectual content: Tajsharghi, Ohlsson, Lindberg, and Oldfors. Obtained funding: Tajsharghi and Oldfors. Administrative, technical, and material support: Tajsharghi, Ohlsson, and Lindberg. Study supervision: Oldfors.

Financial Disclosure: None reported.
Funding/Support: This study was supported by the Swedish Research Council (project 7122) and the Association Francaise Contre les Myopathies.

(Reprinted) Arch Neurol/Vol 64 (No. 9), Sep 2007 www.archneurol.com

©2007 American Medical Association. All rights reserved.
REFERENCES


Call for Papers

Archives Express

The Archives launched a new ARCHIVES Express section in the September 2000 issue. This section will enable the editors to publish highly selected papers within approximately 2 months of acceptance. We will consider only the most significant research, the top 1% of accepted papers, on new important insights into the pathogenesis of disease, brain function, and therapy. We encourage authors to send their most exceptional clinical or basic research, designating in the cover letter a request for expedited Archives Express review. We look forward to publishing your important new research in this accelerated manner.

Roger N. Rosenberg, MD