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Topical Review

Clinical and Histologic Findings in *ACTA1*-Related Nemaline Myopathy: Case Series and Review of the Literature

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ABSTRACT

BACKGROUND: Nemaline myopathy is a rare congenital disease of skeletal muscle characterized by muscle weakness and hypotonia, as well as the diagnostic presence of nemaline rods in skeletal muscle fibers. Nemaline myopathy is genetically and phenotypically heterogeneous and, so far, mutations in 11 different genes have been associated with this disease. Dominant mutations in *ACTA1* are the second most frequent genetic cause of nemaline myopathy and can lead to a variety of clinical and histologic phenotypes. **PATIENTS AND METHODS:** We present a series of *ACTA1*-related cases from a Brazilian cohort of 23 patients with nemaline myopathy, diagnosed after Sanger sequencing the entire coding region of *ACTA1*, and review the literature on ACTA1-related nemaline myopathy. **RESULTS:** The study confirmed *ACTA1* mutations in four patients, including one with intranuclear rods, one with large intracytoplasmic aggregates, and two with nemaline intracytoplasmic rods. A repeat muscle biopsy in one patient did not show histological progression. **CONCLUSION:** Despite the recognized phenotypic variability in ACTA1-related nemaline myopathy, clinical and histological presentations appear to correlate with the position of the mutation, which confirms emerging genotype/phenotype correlations and better predict the prognosis of affected patients.

Keywords: Nemaline myopathy, *ACTA1*, congenital myopathy, muscle biopsy

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Introduction

Nemaline myopathy (NEM) is a rare congenital myopathy characterized by hypotonia and progressive muscle weakness affecting mainly axial and respiratory muscles. The typical histologic feature is the presence of nemaline rods in skeletal muscle fibers, which are Z-line-derived structures detectable on light and electron microscopy.[1]

NEM is genetically and phenotypically heterogeneous. The disease spectrum ranges from severe neonatal forms with akinesia or hypokinesia, respiratory failure, and swallowing difficulties at birth to mild muscular involvement noted in adult life.[2] According to the severity and age of onset of the disease, the European Neuromuscular Centre International Consortium on Nemaline Myopathy has divided this group of congenital myopathies into six different subtypes: severe, intermediate, typical, mild childhood onset, mild adult onset, and other forms, which include the Amish type. The typical form is the most common one and consists of neonatal hypotonia, delayed motor milestones, and symmetric, generalized weakness with a predilection for neck flexor and respiratory muscles.[1,3] Genetically, mutations in at least 11 different

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genes have been reported, which include nebulin (*NEB*), alpha-actin (*ACTA1*, MIM *102610), beta-tropomyosins (*TPM2*, MIM *190990, and *TPM3*, MIM *191030), troponin T type 1 (*TNNT1*, MIM *191041), and cofilin 2 (*CFL2*, MIM *601443), all related to thin filaments; genes encoding members of the BTB proteins superfamily (*KBTBD13*, MIM *613727; *KLHL40*, MIM *615340; and *KLHL41*, MIM *607701), involved in ubiquitination and protein degradation; leiomodin 3 (*LMOD3*, MIM *216112) and myosin 18B (MYO18B, MIM *616549).[4-9]

ACTA1 is the most frequent gene associated with dominant cases of NEM, which account for 15% to 25% of individuals with typical forms and around 50% with lethal forms.[2,10] Mutations in this gene cause mostly dominant diseases. Recessive disease is uncommon; it usually results in functionally null alpha-actin, and heterozygous carriers are unaffected. Besides causing NEM, mutation in this gene leads to a heterogeneous spectrum of muscle and histological phenotypes, including intranuclear rod myopathy,[11] cap disease,[12] actin myopathy,[13] congenital fiber-type disproportion,[14] zebra-body disease,[15] congenital myopathy with core-like areas,[16] and, more recently, progressive scapuloperoneal myopathy.[17] A combination of one or more histopathological features can be seen in the same patient, although there is usually a predominant pathologic picture.[18]

The clinical picture in NEM associated with *ACTA1* variants is heterogeneous, but is often severe. There are few detailed clinical descriptions of milder presentations, especially in older patients.[19] We present here four patients, three adolescents and one adult, with NEM due to mutations in *ACTA1* to illustrate the clinical and histological variability of ACTA1-related NEM. In addition, we reviewed the literature on clinical, histological, and molecular findings in patients with ACTA1-related NEM.

Patients and Methods

The patients were recruited after screening muscle biopsy banks from two large neuromuscular centers in the city of São Paulo, which draw referrals from the entire country. Forty-nine patients were identified meeting the histological criteria for NEM, of whom 23 could be contacted and consented to participate in the study. Patients were recruited for an initial consultation whereby they were submitted to a standard clinical protocol, including a detailed general medical evaluation, neurological examination, and collection of blood samples (parents and patient) for DNA extraction. All available ancillary examinations such as serum creatin kinase levels, electromyography, pulmonary function tests, and cardiac tests were noted. This study was approved by the local ethics committee.

All six coding exons of *ACTA1* were amplified via polymerase chain reaction and Sanger sequencing using primer pairs as described elsewhere.[6] Segregation was investigated by Sanger sequencing of the parents in all mutated cases. We also performed a systematic review of the literature for previous reports of actinopathies and NEM, using the terms "nemaline myopathy," "*ACTA1*," and "congenital myopathy."

Results

Heterozygous *de novo* mutations in *ACTA1* gene were identified in four of the 23 patients with NEM (17.4%). Clinical and histologic data for the patients are presented in Table.

Patient 1 is a 12-year-old boy with three unaffected siblings from nonconsanguineous parents. Pregnancy and delivery were uneventful. Hypotonia was noted in the first months of life and his motor milestones were delayed—head support was achieved at age 14 months and gait at age 24 months. At the evaluation, he was found to have difficulty gaining weight and reported to take more than 30 minutes to finish meals but denied swallowing difficulties. He eventually developed stable motor features; he could walk for more than 1000 meters and climb stairs without support but could never run or jump. The physical examination showed a myopathic face with facial weakness but normal ocular movements. He presented a mild proximal cervical weakness combined with a severe involvement of cervical flexors and moderate involvement of feet dorsiflexors. He had no cardiopathy but exhibited a mild pulmonary

TABLE.
Clinical and Histologic Features of the Patients With *ACTA1*-Related NEM

Patient	Age (y)	Muscle Biopsy	Head Support	Gait	Feeding Compl	Orthopedic Compl	Facial Weakness	Weakness	Restrictive Ventilatory Disturbance
1	12	Few NR + FTD	18 m	30 m	No	Mild scoliosis	Mild	Mild proximal, severe cervical flexors and feet dorsiflexors weakness	Mild
2	14	NR + AM	9 m	36 m	Neonatal	Scoliosis surgery at 12 y Toes, knees, hip, elbows, and wrists contractures	Mild	Severe proximal, cervical flexors and dorsiflexors	Severe
3	13	NR + INR Increased glycogen Type 1 atrophy	15 m	30 m	Neonatal	Thoracic deformity, mild scoliosis, rigid spine	Myopathic face	Moderate proximal, severe cervical flexors and dorsiflexors	Moderate
4	46	NR	12 m	5 y	Neonatal	Thoracic deformity, severe scoliosis	Myopathic face	Moderate proximal, cervical flexors and feet dorsiflexors	Moderate

Abbreviations:
A = Aggregates
AM = Actin myopathy
Compl = Complications
FTD = Fiber-type disproportion
INR = Intranuclear rods
m = Months
NEM = Nemaline myopathy
NR = Nemaline rods
y = Years