ABSTRACT: Rippling muscle disease (RMD) is a benign myopathy with symptoms and signs of muscular hyperirritability. We report a 17-year-old patient who presented with muscular hypertrophy, local mounding on percussion, and a rippling phenomenon. Needle electromyography showed electrical silence during the rippling phenomenon. Muscle protein immuno-histochemical analysis showed a partial deficiency of caveolin-3. Molecular analysis revealed a novel heterozygous A>C transition at nucleotide position 140 in exon 2 of the caveolin-3 gene. We associated this novel mutation with RMD.

A NOVEL MISSENSE MUTATION IN THE CAVEOLIN-3 GENE IN RIPPLING MUSCLE DISEASE

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Rippling muscle disease (RMD) is a benign myopathy with symptoms and signs of muscular hyperirritability.13 In 1975, Torbergsen described the main clinical manifestations of this disease in a family with autosomal-dominant inheritance and pointed to the presence of a persistent muscle contraction resembling myotonia, but with an involuntarily rolling contraction of the muscles (“rippling”) provoked by mechanical stimuli such as tapping and stretching of the muscle.12 The term “rippling muscle disease” was introduced by Ricker et al., referring to the most characteristic symptom in this disease when it was demonstrated that the rippling phenomenon was associated with complete electric silence during needle electromyography.11

RMD is genetically heterogeneous, and both autosomal-dominant and autosomal-recessive inheritance have been described as well as sporadic cases.13–15 Most reports, however, are of RMD families with autosomal-dominant inheritance and only a few cases with recessive inheritance have been found.5,13

The diagnosis is based on clinical findings, increased serum creatine kinase (CK) level, and electromyography. Since the first description of RMD, few families with this disease have been identified and only one previous case, without molecular analysis, has been reported in Brazil.7,10–12,16

CASE REPORT

A 17-year-old girl was referred because of progressive walking difficulty with frequent falls, painful muscle stiffness with muscle cramps, and slowness of movements after rest since birth. She was born at term and was the first child of consanguineous parents (second cousins). At 9 years of age, her gait became more impaired and the stiffness more pronounced in the lower-limb muscles during physical activity.

On neurological examination she had mild exophthalmos after effort; severe diffuse muscle hypertrophy and hypertonia; mild weakness in proximal lower-limb muscles (grade 4 on the Medical Research Council scale); normal muscle strength in the upper limbs; preserved deep tendon reflexes; a tip-toe pattern during walking; and a myopathic gait. Tapping on a skeletal muscle with a reflex hammer produced a prolonged muscle contraction and local mounding, as well as peculiar rolling muscle contractions characteristic of the rippling phenomenon.

Routine hematological and biochemical screening were normal. Serum CK levels were 1640 U/L.
(normal, <140 U/L). Electrocardiogram and echocardiography were normal.

Both parents were asymptomatic with normal neurological examination performed by two neurologists, and both had normal serum CK levels.

Needle electromyography revealed spontaneous activity (fibrillation potentials) in the first dorsal interosseous and tibialis anterior muscles, with no myotonic potentials or myokymic discharges. Voluntary motor unit potentials were myopathic in nature because of low amplitude and short duration in the first dorsal interosseous, biceps brachialis, tibialis anterior, and quadriceps femoris muscles. In addition, during the provoked rippling phenomenon after muscle percussion, needle electromyography showed electrical silence.

Histological analysis of muscle biopsy showed myopathic alterations with mild proliferation of the endomysial connective tissue, marked variation in fiber size, rare fibers with internal nuclei, and foci of degeneration with necrosis and phagocytosis. Immunohistochemical analysis of muscle proteins showed normal immunolabeling for dystrophin and sarcoglycans and partial dysferlin deficiency. Caveolin-3 (CAV-3) was almost absent from the sarcolemma, but scattered fibers showed partial labeling (Fig. 1).

Genomic DNA was isolated from peripheral blood lymphocytes using standard procedures. The two coding exons of CAV-3 were amplified by polymerase chain reaction and directly sequenced in both directions as previously described. Sequencing of the father’s samples was normal, but a novel heterozygous A>C transition at nucleotide position 140 in the index patient and her mother was identified in exon 2, which predicts amino acid exchange from glutamate (E) to alanine (A) at position 47 (E47A) (Fig. 2). This sequence variation was not found in 100 Brazilian control chromosomes. All studies were done after informed consent was provided.

DISCUSSION

The CAV-3 mutation described here is most likely pathogenic because: (1) it was not found in 100 control chromosomes; (2) it was documented in the patient; (3) it causes the replacement of a negatively charged amino acid (E) by a neutral charged amino acid (A), a net charge alteration that can adversely affect the structure and function of proteins at critical positions of the amino acid sequence; (4) the affected residue at nucleotide position 140 of the protein is highly conserved in all vertebrate caveolins; and (5) the mutation resulted in a significant deficiency of the encoded protein, caveolin-3, based on immunohistochemistry. Moreover, the mutated amino acid residue is directly adjacent to the alanine at position 46 that was found to be mutated in several patients with RMD, emphasizing the important functional role of this part of the protein.

Mutations in the CAV-3 gene on chromosome 3p25 were first described in the autosomal-dominant form of limb-girdle muscular dystrophies type 1C.
LGMD-1C) and recently confirmed in RMD, and in patients with idiopathic hyperCKemia, demonstrating a large phenotypic variability.\(^1,8,13–15\) Nine missense mutations in CAV-3 have been reported in RMD; seven of these are heterozygous and two are homozygous.\(^2,4,6,13–16\) In our case, the presence of the one allele of the CAV-3 gene caused the RMD phenotype, confirming the association between RMD and CAV-3 mutation in heterozygous patients. Also, we found a novel mutation of the CAV-3 gene falling in the oligomerization domain that is critical for homo-oligomerization and for interaction with several caveolin-associated signaling molecules. The consequent deficiency of CAV-3 in the muscle could be due to the dominant negative effect of the mutation, as previously observed.\(^16\)

In the patient reported herein, typical myopathic alterations were observed. Additionally, muscle protein candidates for neuromuscular disorders were all normal, with the exception of a partial reduction in dysferlin and a drastic deficiency of CAV-3 from the sarcolemma, but with some scattered small positive fibers. CAV-3 is a skeletal and cardiac muscle–specific protein from the caveolin gene family involved in sarcolemmal trafficking, sorting, transport, and signal transduction.\(^1,9\) The secondary dysferlin deficiency in our patient supports the previously proposed hypothesis that dysferlin may play a role in the signaling functions of caveolae by interacting with CAV-3.\(^8,14\)

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