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Amphiphysins have been implicated in membrane remodeling in brain and skeletal muscle. Mutations in amphiphysin-2 were recently identified in autosomal recessive centronuclear myopathies. In order to understand the dynamics of amphiphysin-2 during its degeneration, we chronologically evaluate the expression of amphiphysin-2 and caveolin-3 in rat tibial muscles during a cycle of regeneration induced by cardiotoxin injection using immunohistochemistry and Western blot. Tibial muscles of male Wistar rats (7-8 weeks old) were injected with cardiotoxin. The cardiotoxin-injected muscles were removed on 1, 3, 5, 7, 14, and 28 days after the injection. Western blotting was performed as Laemmli’s methods. In immunohistochemical studies, amphiphysin-2 and caveolin-3 were weakly stained at T-tubules of some regenerating muscles. In the western blot analysis, amphiphysin-2 was first detected as a visible band on day 5, whereas caveolin-3 was first recognized as a visible band on day 3. During 3-5 days after cardiotoxin injection, satellite cells fuse and differentiate to mature muscle fibers. These results provide evidence that both amphiphysin-2 and caveolin-3 contribute muscle differentiation and membrane deformation.

Centronuclear myopathies (CNM) are a group of diseases with variable onset and severity sharing as a distinctive histological feature, a high frequency of muscle fibers with centralised nuclei. Myotubular myopathy (MIM#310400) the X-linked form of CNM is characterized by neonatal hypotonia and inability to maintain unassisted respiration. The MTM1 gene, responsible for this disorder, codes for a protein involved in myofiber differentiation and muscle cell architecture. In this work, eight patients were subjected to MTM1 MLPA analysis, selected according to the following criteria: (i) muscle biopsy compatible with CNM and (ii) exclusion of MTM1 point mutations by sequencing. We identified the first gross duplication spanning exons 1-5 (c.-76_342+76dup) in a 7 year old boy with progressive tetraparesis, ophthalmoplegia, facial diparesis and independent ambulation, the clinical course being milder than the classical myotubular myopathies. Analysis at the mRNA level revealed both normal transcripts and a mutated isoform lacking exon 6 (r.343_444del), suggesting somatic mosaicism. As suspected, this duplication was not detected in the patient’s mother. Considering the phenotypic expression in the patient, this mutational event most likely occurred de novo during early embryogenesis. We also describe the implementation of a locus-specific database (LSDB) for this gene using the Leiden Open Variation database (LOVD) software. The MTM1-LOVD (http://www.lov.d.nl/MTM1) contains 372 mutation entries identified in 370 patients (last accessed March 2011). A total of 223 unique MTM1 mutations are listed in this LSDB, including: 207 point mutations, 15 single or multi-exonic deletions and the large duplication described in the present work. Despite the significant advances in this field during the last decade about one third of the CNM cases remain genetically unresolved. Here we show that gross MTM1 gene duplications may account for a fraction of these cases.

The sarcoplasmic reticulum of muscle fibers is known to form tubular aggregates (TAs) in various diseases, in some constituting the most striking