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Morphological and Molecular Abnormalities of the Skeletal Muscle Tissue from Pediatric Patient Affected by a Rare Genetic Chaperonopathy Associated with Motor Neuropathy

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Abstract : The neuromuscular system controls, directs, and allows movement of the body through the action of neural circuits, which include motor neurons, sensory neurons, and skeletal muscle fibers. Protein homeostasis of the involved cytotypes appears crucial to maintain the correct and prolonged functions of the neuromuscular system, and both neuronal cells and skeletal muscle fibers express significant quantities of protein chaperones, the molecular machinery responsible to maintain the protein turnover. Genetic mutations or defective post-translational modifications of molecular chaperones (i.e., genetic or acquired chaperonopathies) may lead to neuromuscular disorders called as neurochaperonopathies. The limited knowledge of the effects of the defective chaperones on skeletal muscle fibers and neurons impedes the progression of therapeutic approaches. A distinct genetic variation of CCT5 gene encoding for the subunit 5 of the chaperonin CCT (Chaperonin Containing TCP1; also known as TRiC, TCP1 Ring Complex) was recently described associated with severe distal motor neuropathy by our team. In this study, we investigated the histopathological abnormalities of the skeletal muscle biopsy of the pediatric patient affected by the mutation Leu224Val in the CCT5 subunit. We provide molecular and structural features of the diseased skeletal muscle tissue that we believe may be useful to identify undiagnosed cases of this rare genetic disorder. We investigated the histological abnormalities of the affected tissue via hematoxylin and eosin staining. Then we used immunofluorescence and qPCR techniques to explore the expression and distribution of CCT5 in diseased and healthy skeletal muscle tissue. Immunofluorescence and immunohistochemistry assays were performed to study the sarcomeric and structural proteins of skeletal muscle, including actin, myosin, tubulin, troponin-T, telethonin, and titin. We performed Western blot to examine the protein expression of CCT5 and some heat shock proteins, Hsp90, Hsp60, Hsp27, and α-B crystallin, along with the main client proteins of the CCT5, actin, and tubulin. Our findings revealed muscular atrophy, abnormal morphology, and different sizes of muscle fibers in affected tissue. The swollen nuclei and wide interfiber spaces were seen. Expression of CCT5 had been decreased and showed a different distribution pattern in the affected tissue. Altered expression, distribution, and bandage pattern were detected by confocal microscopy for the interested muscular proteins in tissue from the patient compared to the healthy control. Protein levels of the studied Hsps normally located at the Z-disk were reduced. Western blot results showed increased levels of the actin and tubulin proteins in the diseased skeletal muscle biopsy compared to healthy tissue. Chaperones must be expressed at high levels in skeletal muscle to counteract various stressors such as mechanical, oxidative, and thermal crises; therefore, it seems relevant that defects of molecular chaperones may result in damaged skeletal muscle fibers. So far, several chaperones or cochaperones involved in neuromuscular disorders have been defined. Our study shows that alteration of the CCT5 subunit is associated with the damaged structure of skeletal muscle fibers and alterations of chaperone system components and paves the way to explore possible alternative substrates of chaperonin CCT. However, further studies are underway to investigate the CCT mechanisms of action to design applicable therapeutic strategies.

Keywords : molecular chaperones, neurochaperonopathy, neuromuscular system, protein homeostasis
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