TUBULAR AGGREGATE MYOPATHY CAUSED BY ORAI1AND STIM1MUTATIONS: A FUNCTIONAL STUDY IN MYOBLAST AND MYOTUBES OF AFFECTED PATIENTS TOWARD THE IDENTIFICATION OF NEW THERAPEUTIC TARGETS

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Introduction: Skeletal muscle functionality directly depends on changes in cytosolic Ca^{2+} levels, and small disturbances in Ca^{2+} homeostasis can severely impact on muscle contraction, differentiation or gene transcription. A major mechanism controlling Ca^{2+} homeostasis is store-operated calcium entry (SOCE), which is triggered by Ca^{2+} depletion of sarcoplasmic reticulum (SR) and results in extracellular Ca^{2+} influx. SOCE is coordinated by stromal-interacting 1(STIM1), a SR Ca^{2+} sensor, and ORAI1, a plasma membranous calcium release-activated Ca^{2+} channel, following Ca^{2+} ion release from SR. When SR Ca^{2+} store is depleted, oligomerization and translocation of STIM1from SR to adjacent plasma membrane junctions are induced causing ORAI1activation. Dominant mutations in STIM1or ORAI1were shown to cause tubular aggregate myopathy (TAM). TAM is a rare progressive muscle disorder, clinically heterogeneous and involving either proximal muscle weakness, muscle cramps and pain, or myasthenic features. Biopsies from patients with TAM, typically show the presence of tubular aggregates (TA). TAs appear as accumulations of densely packed tubules, which are suggested to arise from SR as they contain SR proteins. It has been postulated that TAs formation is triggered by some functional consequences due to disruption in the SR-T-tubule junction, such as altered Ca^{2+} homeostasis (Lee et al, Int J Mol Sci 2016). TAM mutants are gain of function mutants, as shown for some mutated Orai1or STIM1proteins expressed in heterologous system. However, the mechanisms underlying muscle weakness and TA formation from abnormal Ca^{2+} influx in skeletal muscle fibers of affected individuals remain to be fully clarified. Thus, to explore how calcium homeostasis dysregulation could be associated with TAM in an experimental condition similar to the cellular *in vivo* environment, we here perform a functional study on muscle fibers of affected individuals.

Materials and methods: A plethora of techniques raging from fura-2cytofluorimetric and High Content Imaging technology to real-time PCR was used to perform the functional characterization of myotubes deriving from patients carrying Gly98Ser Orai1mutant or Leu96Val STIM1mutant, with a clinical and histological phenotype already defined (Bohm et al, Am J Hum Genet 2013; Hum Mutat 2017).

Results: We demonstrated a significantly higher resting calcium concentration ($[Ca^{2+}]_I$) in both TAM mutants myotubes compared with controls and an increased SOCE activity, with a particular extent for Orai1mutant. A gene expression analysis highlighted an altered expression of RNAs coding for proteins regulating calcium handling and muscle cell proliferation and differentiation. Interestingly, a drastic reduction in Pax7expression, together with a distinct pattern of multi-nucleated cells formation and the geometric features of mitochondrial network observed in STIM1mutant myotubes, led to hypothesize an altered myogenesis process associated to TAM. Accordingly, SOCE controls human myoblast differentiation in a hyperpolarization-linked manner (Darbellay et al, J Biol Chem 2009). To investigate a possible impact of the Ca^{2+} dysregulation observed in TAM mutants on myoblasts proliferation and myotubes formation, we are currently performing electrophysiological experiments to measure changes in plasma membrane potential during myoblast differentiation as well as a gene expression analysis in TAM mutants at the myoblasts stage.

Discussion and conclusion: Our study provide functional evidences of the Ca^{2+} homeostasis dysregulation associated with myotubes of TAM patients allowing to define a genotype/phenotype correlation that could open the way to a personalized medicine. SOCE-related proteins have been suggested to be druggable targets (Riva et al, J Med Chem 2018), thus preventing excessive extracellular Ca^{2+} influx through a selective inhibition of Orai1or STIM1activity could be a therapeutic approach for TAM.