CHARACTERIZATION OF A TUBULAR AGGREGATE MYOPATHY MOUSE MODEL

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Introduction: The Tubular agreggate myopathy (TAM) is one of a cluster of rare genetic diseases uncared for treatment with a prevalence of 1/250.000 newborns, characterized by mucle pain, cramps and progresive muscle stiffnes and blood disorders. These illness are related to gain-of-function mutations of two main protein (Orai1, a calcium cannel in the membrane; and STIM1, a calcium sensor in the reticulum, principal calcium store in the cell) involved in a ubiquitonious intracellular mecanism, named Store-Operated Calcium Entry (SOCE) When there is no calcium in the reticulum, STIM1interacts with Orai1, allowing the entrance of calcium at the cell. Gain-of function mutations heads to an increased activity of this mecanism and therefore, an enhanced calcium entry. Despite of its widespread presence, SOCE is crutial just in certain types of cells, as muscle cells and T-cells, reason why alterations in SOCE have no sistemic effects but at muscular and platelets levels. The aim of the proyect is to characterize a only-world STIM1-mutated knock-in mouse model, side-specific and not overexpressing, in order to follow as faithful as possible the human pathophysiology, affording the blowing up of the knowledge and understanding of these diseases, uncared for treatment. Simultaneously, this mouse model would consent a traslational sense in the researching of new modulators of SOCE, filling a current gap among in-vitro characterization and patients.

Material and methods: First of all, we have generated a knock-in animal model that bears one of the most frequent mutations in italian patients (STIM1^{I115F}). General characteristics have been demostrated, as life span, body weight, body lenght and heart and spleen weight. For evaluating distrophic-like phenotype, we used grip strengh, hanging test, rotarod and treadmil test. We measured KI and WT muscle weight, as well as the bleeding time and counted the number of platelets for determining the blood disorders. We use fluorescent probe to measure the calcium signalling in both WT and KI myotubes to demostrate the enhanced SOCE. The expression of genes related to calcium signalling pathways has been evaluated by Real Time PCR.

Results: We have demostrated that these mice respected the human physiopathology (i) impaired body and (ii) specific muscle growth, (iii) an enhanced SOCE in myotubes, (iv) muscle weakness, evaluated by the performance in rotarod and treadmil test and (v) platelets count alteration, showing a degradation with time up to 12months age in all of them. Also We demostrated that KI-STIM1^{I115F} survival is not affected by the mutation with a Kaplan-Meier curve in relation to WT. Moreover, we determined the presence of necrotic and fibrotic fibers and inflammatory cell infiltration in soleus and quadriceps muscles, as seen by Hematoxiline and Eosin, Masson Trichrome and Gomori's Trichrome staining. Futhermore, a deeper study in myeloid population has been done, demostrating a slightly increased number of LyC6middle monocytes.

Discussion and conclusions: In all this project we demostrated that KI-STIM1^{I115F} is a valid mouse model to reproduce the clinical phenotype saw in TAM patients. Our aim is to test new modulators of SOCE to fulfill this medical unneed and increasing the understanding of these mechanisms and its behaviour in physiological and patophysiological conditions. Futher characterization would be done, in order to define the alterated pathways by which the illness is ongoing.