

## **CLASSIFICATION OF VARIANTS OF UNKNOWN/UNCERTAIN SIGNIFICANCE (VUS) FOR THE LONG QT SYNDROME BY USING PATIENT-SPECIFIC iPSCs-DERIVED CARDIOMYOCYTES (iPSC-CMs)**

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**Research Theme/Topic:** Translational Cardiology – Regenerative Medicine.

### **Main Abstract:**

Long QT syndrome (LQTS) is an arrhythmogenic disease characterized by prolonged ventricular repolarization that is associated with increased risk of ventricular tachycardia, syncope or sudden cardiac death. Inherited LQTS is due to mutations in genes encoding for ion channels, or related proteins, involved in ventricular action potential shaping.

Despite significant advances in the management of LQTS, the care of almost one third of LQTS patients is often plagued by inexact clinical genetic testing due to variants of uncertain significance (VUS). Even if a diagnosis is made, there is high heterogeneity in terms of risk of cardiac events, creating a management dilemma of committing patients to the more proper medical therapy. Thus, there is a strong need for better platforms, ideally of human origin, that can validate the functional significance of genetic variants associated with LQTS and allow responses to therapeutic agents to be assessed.

Human induced pluripotent stem cells-derived cardiomyocytes (iPSC-CMs) represent a physiologically relevant system characterized by biochemical, electrophysiological and genomic properties similar to those of primary human CMs, and faithfully recapitulating the conditions known as “patient in a dish” (individual level) and “clinical trials in a dish” (population level).

This PhD project, by using an approach based on LQTS patient-specific iPSC-CM, aims to unequivocally describe the contribution of specific genetic variants to LQTS pathogenesis.

The main objective is to validate a pipeline that allows a distinctive classification of VUS by using LQTS iPSC-CM. The project will be developed following consecutive milestones: 1) recruitment of LQTS patients carriers of VUS; 2) generation of iPSC lines from VUS carriers; 3) differentiation of iPSCs into iPSC-CMs; 4) CRISPR/Cas9 gene editing to generate isogenic iPSCs in which the VUS has been corrected; 5) CRISPR/Cas9 gene editing to generate isogenic iPSCs in which the VUS has been introduced; 6) molecular and electrophysiological characterization of iPSC-CMs in order to define the mechanisms of VUS pathogenicity and penetrance.

**Techniques:** hiPSC generation and culture, cardiomyocyte differentiation and culture, PCR, RT-PCR, immunofluorescence, western blot, genome editing.