Estrogen-induced phenotype in hiPSC-derived cardiomyocytes of a woman affected by both Long QT Syndrome Type 2 and Polycystic Ovary Syndrome

F. Bastaroli1, C. Maniezzi2, V. Dusi3, M. Mura1, F. Lodola2, A. Zaza2, M. Gnerchi1

1I.R.C.C.S. San Matteo Polyclinic, Department of Cardiothoracic and Vascular Sciences-Laboratory of Translational Cardiology, Pavia, Italy
2University of Milano-Bicocca, Department of Biotechnology and Biosciences, Milano, Italy
3Hospital Citta Della Salute e della Scienza di Torino, Cardiovascular and Thoracic Department, Turin, Italy

Funding Acknowledgements: Type of funding sources: Foundation. Main funding source(s): Leducq Foundation

Introduction: Long QT Syndrome (LQTS) is an arrhythmogenic disorder caused either by genetic alteration or by exogenous factors. Polycystic Ovary Syndrome (PCOS) is a disease associated with high levels of testosterone and low levels of estrogen (E2). It has been suggested that hormones can influence the occurrence of malignant cardiac events in LQTS type 2 (LQT2) women, carriers of mutations on the potassium channel (hERG). However, the precise mechanisms governing this process still need to be clarified.

Purpose: We studied a patient affected by both LQT2 and PCOS who started experiencing cardiac symptoms after initiation of hormonal replacement therapy. We observed a correlation with the occurrence of arrhythmic events and the irregular assumption of the hormonal replacement therapy, thus we aim to dissect the underlying mechanism in patient-in-a-dish cardiac cellular model.

Methods: Induced Pluripotent Stem Cells (hiPSC) were generated from a woman affected by both LQT2 and PCOS and from a healthy donor, and then differentiated into cardiomyocytes (hiPSC-CMs). hiPSC-CMs were stimulated with E2 10nM for five time points (1, 5, 10, 15 and 30 minutes). The expression of potassium channel hERG, and of the G-coupled estrogen receptor (GPER) regulating the fast non-genomic pathway, were quantified after E2 stimulation at different time points by In Cell Western (ICW). The Unstimulated sample were used to normalize GPER and hERG expression to 100%. Electrophysiological experiments were performed using the whole-cell patch-clamp technique in current-clamp mode to record spontaneous action potentials (APs). AP duration at 30%, 50% and 90% repolarization (APD30, APD50 and APD90) was quantified. During spontaneous activity, AP duration values were rate-corrected with Bazett’s formula, thus obtaining cAPD.

Results: LQT2 hiPSC-CMs express higher levels of GPER (p<0.05) and hERG (p<0.01) than healthy hiPSC-CMs. A significant decrease in GPER expression was detected during 10nM E2 stimulation in healthy cardiomyocytes at the five time points (p<0.0001), while any difference was detected in hERG expression levels. E2 stimulation at different time points did not affect neither GPER nor hERG expression in LQT2 hiPSC-CMs. In LQT2 hiPSC-CMs, APDc values were significantly increased (p<0.0001) compared to healthy cells; pre-incubation with E2 for 10 minutes significantly shortened cAPD50 and increased spontaneous firing rate. In healthy hiPSC-CMs, E2 had no effect.

Conclusions: The hiPSC-CM model reproduces the LQT2 clinical phenotype. cAPD50 shortening by E2 was unexpected based on the proarrhythmic effect in the patient. The mechanism of LQT2-specific effects of E2 are under investigation.