INVESTIGATING MOLECULAR MECHANISMS OF TYPE 2 LONG QT SYNDROME WITH iPSC-DERIVED CARDIOMYOCYTES

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Patient-specific cardiomyocytes obtained from induced pluripotent stem cells (CM-iPSC) offer unprecedented mechanistic insights in the study of inherited cardiac diseases. The aim of this work was to model type 2 long QT syndrome (LQTS2) in CM-iPSC. Mononuclear peripheral blood cells obtained from two patients with a mutation in the voltage sensing region of KCNH2 (c.1600C>T) were isolated and enriched for erythroblasts. iPSC were generated and colonies were selected, expanded, karyotyped. iPSC were characterized by RT-PCR, immunofluorescence and spontaneous differentiation. Our results demonstrated an efficient enrichment of the erythroid population, with coexpression of CD36 and CD71 over 90%. iPSC emerged during days 15-20 and were selected manually for expansion. The selected cell lines expressed pluripotency markers and exhibited spontaneous differentiation. In addition, we inserted the same mutation in a normal iPSC line by CRISPR/Cas9 genome editing. Electrophysiology demonstrated that the action potential duration (APD) of cardiomyocytes differentiated from patient-derived LQTS2-iPSC as well as from CRISPR-LQTS2-iPSC was significantly longer than that of control iPSC. Moreover, triangulation of action potentials, implying a longer duration of phase 3, was also increase in cells carrying the mutation. Treatment with E4031 caused APD prolongation only in control iPSC, indicating that I_{Kr} channel does not contribute to repolarization mutated cardiomyocytes. Patch-clamp demonstrated a reduction of I_{Kr} current and immunofluorescence for Kv11.1 indicated a perinuclear staining of this channel in LQTS2 cell lines. We generated cardiomyocytes that recapitulate LQTS2 phenotype and our findings reinforce the hypothesis that iPSC reprogramed from LQTS2 patients constitute a promising platform to describe pathophysiological mechanisms and drug sensitivity in LQTS2.

	Control (n=31)		LQTS2-Patient 1 (n=28)		LQTS2-Patient 2 (n=30)		LQTS2-CRISPR (n=22)	
APD_{90}	231.2±69.3		343.6±136.7		384.3±75.4		420.9±136.9	
APD_{90}-APD_{40}	44.3±20.6		92.1±32.6		95.6±33.6		206±110.9	
	Before	After	Before	After	Before	After	Before	After
E4031 (n=4)	287.4±113	532.5±143.4	408±165	468.3±163	404.5±45	502±51	499.3±132	545±161.5