Basic Science – Basic Science, Cardiac Diseases, Arrhythmias

Inositol 1,4,5-triphosphate receptor (IP3R)-mediated calcium release: a compensatory mechanism in calcium release channel deficiency syndrome

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Background: Calcium release channel deficiency syndrome (CRCDS) arises from loss-of-function variants in the RYR2-encoded type 2 ryanodine receptor (RyR2), predisposing patients to sudden cardiac arrest/death (SCA/SCD) despite normal exercise testing. Previously, a novel homozygous RYR2 duplication causing ~ 80% RyR2 reduction was associated with exertion-related SCD. However, how Ca2+ homeostasis is maintained despite RyR2-loss remains unknown, as RYR2-/- animals are non-viable.

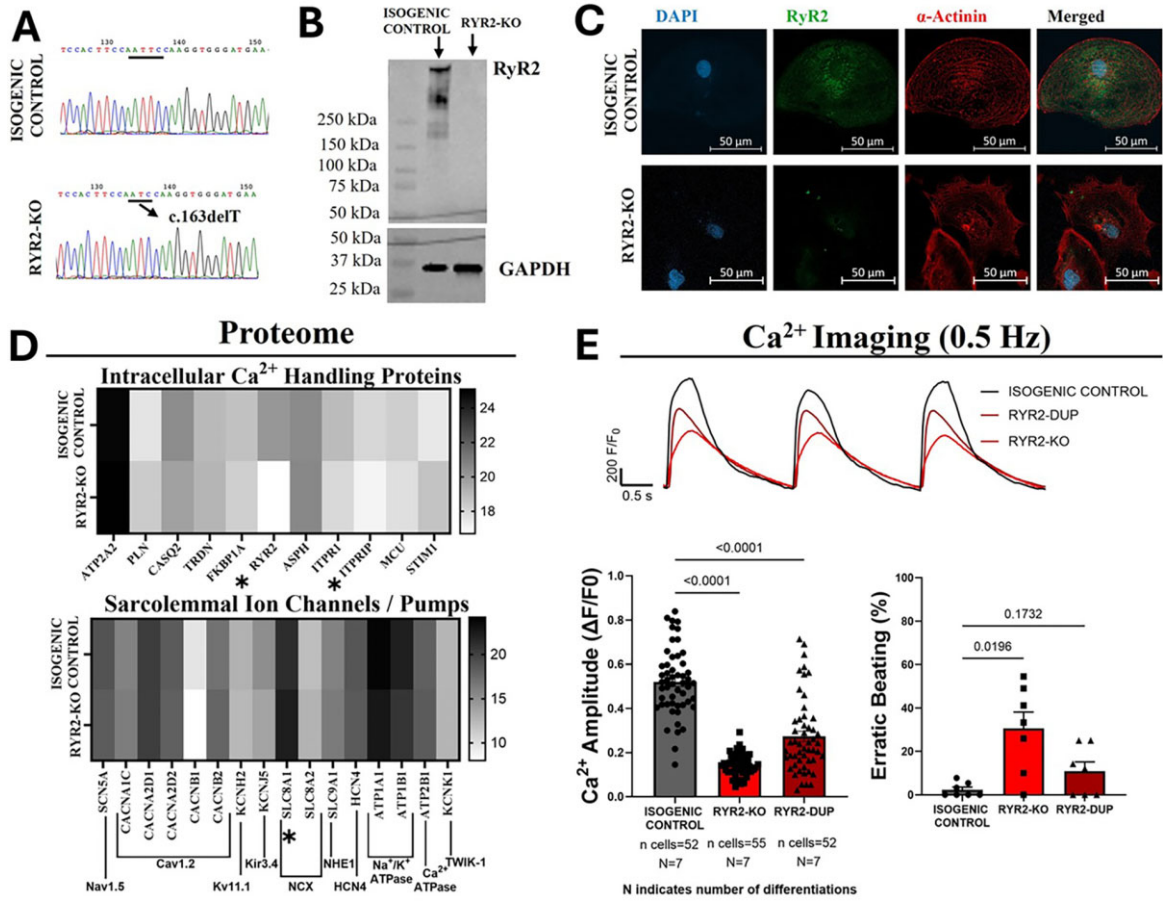
Purpose: To generate a RYR2-knockout (RYR2-KO-/-) induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) model to dissect compensatory mechanisms sustaining Ca2+ homeostasis and identify arrhythmogenic pathways predisposing to SCD.

Methods: CRISPR/Cas9 editing was used to introduce a homozygous c.163delT variant into a wild-type iPSC line (control), generating a RYR2-KO-/- model. A patient-derived RYR2-duplication (RYR2-DUP+/+) iPSC line was used for comparison. RyR2 expression was assessed by western blot (WB) and immunofluorescence (IF). Proteome and phospho-proteome were assessed using omics approaches. Intracellular Ca2+ handling was evaluated by Fluo-4 AM imaging (0.5 Hz and long-burst–long-pause [LBLP] protocol). Electrophysiological properties (action potential [AP], and sarcolemmal currents [ICaL, INa and INCX]) were assessed by patch-clamp.

Results: Despite complete RyR2-loss in RYR2-KO-/- iPSC-CMs as confirmed by WB and IF, contractility and contractile machinery expression was preserved. Ca2+ transient amplitude ($\Delta F/F0$) was reduced markedly in RYR2-KO (0.14 ± 0.01, $p<0.01$) and RYR2-DUP (0.27 ± 0.2, $p<0.01$) compared to control (0.52 ± 0.02). Proteomics revealed a significant NCX1 up-regulation and IP3R-interacting protein (ITPRIP) down-regulation in RYR2-KO compared to control, with pathway-analyses showing significant up-regulation of IP3-related pathways. Pharmacological IP3Rs block (2-APB, 10μM) abolished Ca2+ transient generation in RYR2-KO iPSC-CMs (Amplitude: baseline: 0.15 ± 0.01, 2-APB: 0.06 ± 0.01, $p<0.01$). RyR2-ablation induced electrical remodeling (pA/pF), including ICaL reduction, INCX up-regulation and hyperpolarization of the INa window current in RYR2-KO (ICaL [at 0mV]: -8.5 ± 1.6; INCX [at -110mV]: -4.1 ± 1.1) as compared to control (ICaL:-7.5 ± 3.7, $p<0.01$; INCX:-1.5 ± 0.5, $p=0.02$). RYR2-KO CMs demonstrated increased delayed after-depolarizations and beat-to-beat variability as compared to control. Despite Ca2+ sparks abolishment, RYR2-KO CMs displayed higher erratic beating (at 0.5Hz) and arrhythmogenic propensity than control, showing ventricular tachycardia-like events during LBLP stimulation.

Conclusions: Genetic RYR2-ablation disrupts physiological Ca2+ cycling and triggers electrical remodeling, including INCX up-regulation, overall promoting arrhythmogenesis. Compensatory IP3Rs activation sustains Ca2+ transient generation, revealing a mechanism preserving excitation-contraction coupling that may paradoxically promote arrhythmogenesis in CRCDS.

RYR2-KO: Generation and Characterization



Electrophysiological Assessment