Correction of CPVT-Related Electrophysiological Abnormalities by CASQ2 Overexpression

Meghan Soustek-Kramer, PhD[1]; David Wolfson, PhD[1]; Elaina Breznau, PhD[1]; Justin DeFrancisco, PhD[1]; Jamie L. Marshall, PhD[1]; Deni Kukavica, MD, PhD[2]; Andrea Mazzanti, MD[2]; Carlo Napolitano, MD[2]; Silvia G. Priori, MD[2]; Jessie Hanrahan, PhD, MS, MPH[1]; Nicolas Christoforou, PhD[1]

[1]Solid Biosciences Inc., Charlestown, MA, USA; [2]ICS Maugeri, Pavia, Province of Pavia, Italy

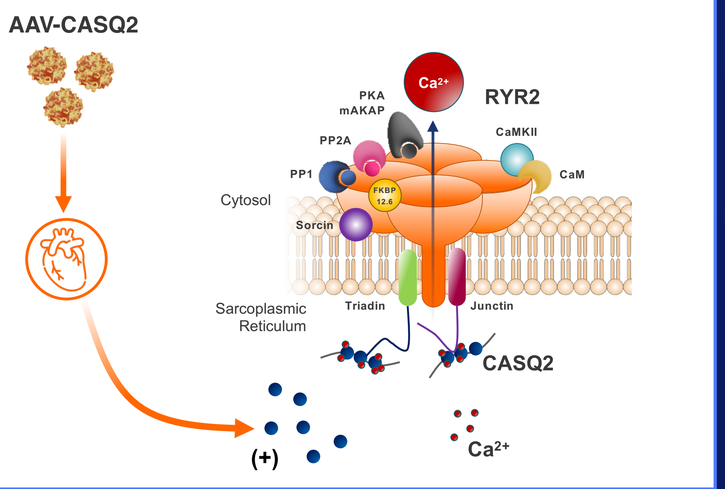
INTRODUCTION

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare life-threatening disease that is primarily linked to mutations in either the ryanodine receptor 2 (RYR2) or calsequestrin 2 (CASQ2) genes. CPVT patients typically present with physically or emotionally triggered syncope, cardiac arrest, sudden cardiac death, or seizure-like events. Current treatment options offer limited cardiac protection, leaving patients vulnerable to breakthrough cardiac events and in need of a novel gene therapy approach. We hypothesize that increasing CASQ2 levels will stabilize RYR2 in a closed state and serve as a dynamic luminal SR calcium storage therefore preventing Ca+ release during diastole. Here, we investigated the potential of CASQ2 overexpression in cardiomyocytes to correct beta-adrenergic stimulated-induction of ventricular tachycardia (VT) in two model systems harboring CPVT patient-derived RYR2 mutations. We assessed efficacy in hiPSC-derived cardiomyocytes carrying a disease relevant pathogenic mutation ($RYR2^{E2311D}$). Mutant and isogenic control cardiomyocytes were plated on electrode-embedded culture plates and divided into untreated (UNT), AAV-Luc (CTRL), or AAV-CASQ2 cohorts. Four days following transduction, impedance and electrode recordings showed $RYR2^{E2311D}$ mutant cardiomyocytes have significantly lower spontaneous beating rates compared to isogenic control (15.4 +/- 2.2 vs. 32.5 +/- 1.8 bpm). Treatment of $RYR2^{E2311D}$ cardiomyocytes with AAV-CASQ2 resulted in an improvement in the spontaneous beating rate compared to CTRL (20.1 +/- 3.3 vs. 16.0 +/- 1.6 bpm). Addition of beta-adrenergic agonist, isoproterenol (1uM), exemplified $RYR2^{E2311D}$ cardiomyocytes' arrhythmogenicity with higher beating irregularity (0.242 +/- 0.218 vs. 0.015 +/- 0.004, CoV seconds) and incidence of VT with pacing (7/12 vs. 0/6 wells) compared to isogenic control. Importantly, treatment of $RYR2^{E2311D}$ cardiomyocytes with CASQ2 rescued beat regularity compared to CTRL (0.012 +/- 0.005 vs. 0.159 +/- 0.138, CoV seconds) and incidence of VT (0/12 wells) with pacing back to isogenic control levels. To demonstrate *in vivo* efficacy, wildtype and RYR2 mutant mice ($RYR2^{R4496C/+}$) underwent surgery to implant a radio telemetry device for ECG assessment. At 16-17 weeks of age, $RYR2^{R4496C/+}$ mice were administered a single systemic injection of rAAV8-DES-CASQ2 at a low, mid, or high dose. All doses were well-tolerated with no clinical observations. Three-months post-dosing, ECG readings were recorded at baseline and post-administration of beta-adrenergic challenge (2mg/kg epinephrine and 120mg/kg caffeine). Following challenge, the incidence of VT was 13/25 (52%) in vehicle-treated $RYR2^{R4496C/+}$ mice, while a dose-dependent anti-arrhythmic effect was observed with treatment. A significant decrease in VT incidence was measured at the mid dose (4/25, 16%) and high dose (1/28, 4%) groups. Similarly, bioanalytical analysis demonstrated a clear dose-responsive effect on vector genome, *CASQ2* mRNA, and CASQ2 protein levels in the heart. Specifically compared to the low dose, CASQ2 protein in mouse hearts was 1.6-fold higher at the mid dose and 6-fold higher at the high dose. In conclusion, we established that increased expression of CASQ2 rescues electrophysiological abnormalities observed in RYR2 mutant human cardiomyocytes as well as a mouse model of disease, demonstrating the therapeutic potential of implementing a gene therapy approach in the clinic for CPVT.

MECHANISM OF ACTION

The MoA of AAV-CASQ2 proposed by Dr. Silvia Priori et al., is based on increasing levels of CASQ2 in the SR

- Increased CASQ2 levels enhance buffering of free Ca^{2+} in the SR resulting in stabilization of RYR2 in its closed conformation making diastolic Ca^{2+} leak through the RYR2 into the cytosol less likely in context of *RYR2* variants
- Stabilization of RYR2 in its closed conformation supports maintenance of normal cardiac rhythm and protects against triggered activity and arrhythmias



RESULTS

Experimental design of *in vitro* studies

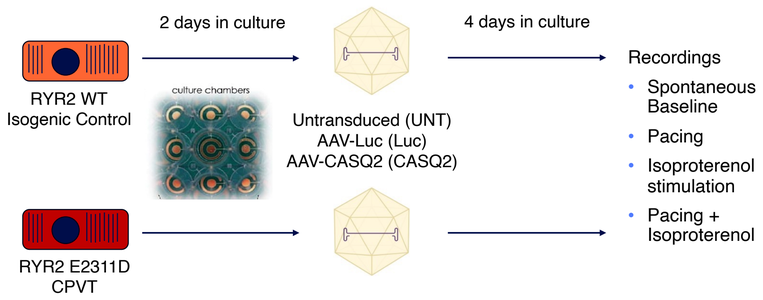


Figure 1. *In vitro* experimental design. Isogenic controls and RYR2 E2311D mutant (Fujifilm iCell® 01434) hiPSC-cardiomyocytes were seeded on Nanion CardioExcyte plates at a density of 50k cells/well. Cells were cultured for 2 days prior to transduction. Cells were then treated with formulation buffer or transduced with either AAV-luciferase (Luc) or AAV-CASQ2 (CASQ2) at an MOI of 1e5 vg/cell. Cells were cultured for an additional 4 days prior to recording beat rate: at baseline, with pacing, with isoproterenol stimulation, or with pacing and isoproterenol stimulation using the Nanion CardioExcyte 96.

RESULTS (cont'd)

RYR2 E2311D cardiomyocytes have slower and irregular beating rates compared to isogenic control, both marginally improved with CASQ2

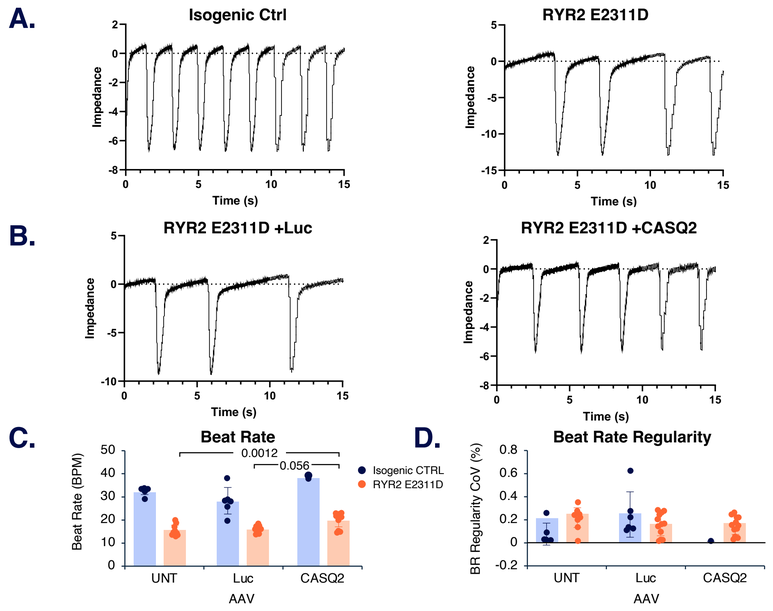


Figure 2. A. Representative impedance traces of spontaneous contractions from isogenic wild type control (left) and RYR2 E2311D mutant (right) hiPSC-cardiomyocytes. **B.** Representative impedance traces of spontaneous contractions from RYR2 E2311D mutant hiPSC-cardiomyocytes transduced with AAV-Luc (left) or AAV-CASQ2 (right). **C.** Average spontaneous beat rates of isogenic control (blue) and RYR2 E2311D mutant (orange) hiPSC-cardiomyocytes across the three treatment groups. Two-way ANOVA with Tukey's multiple comparisons, n=12 wells. **D.** Beat rate regularity measured by the coefficient of variation (CoV) for isogenic control (blue) and RYR2 E2311D mutant (orange) hiPSC-cardiomyocytes across the three treatment groups. Two-way ANOVA with Tukey's multiple comparisons, n=12 wells.

CASQ2 overexpression improves beating rate and regularity of RYR2 E2311D in response to isoproterenol

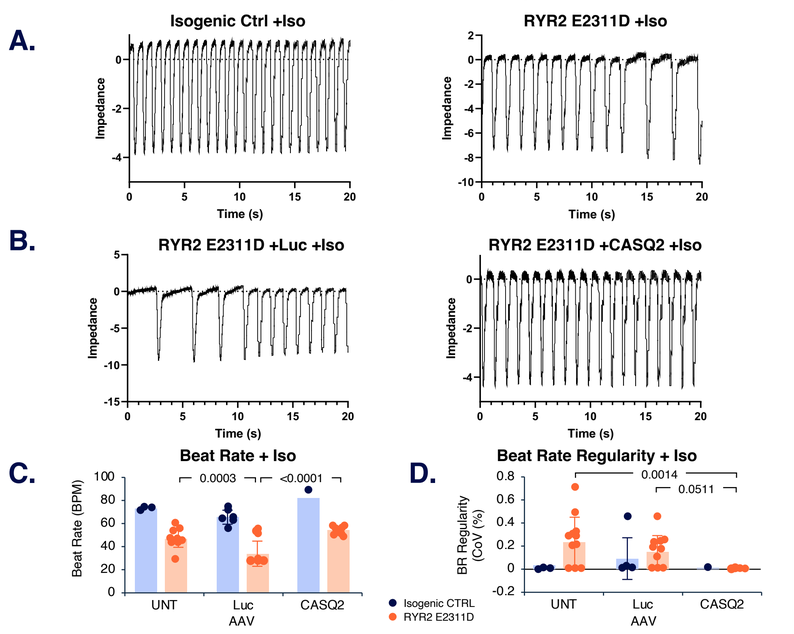


Figure 3. A. Representative impedance traces of spontaneous contractions with isoproterenol (1uM) treatment from isogenic wild type control (left) and RYR2 E2311D mutant (right) hiPSC-cardiomyocytes. **B.** Representative impedance traces of spontaneous contractions with isoproterenol (1uM) treatment from RYR2 E2311D mutant hiPSC-cardiomyocytes transduced with AAV-Luc (left) or AAV-CASQ2 (right). **C.** Average spontaneous beat rates of isogenic control (blue) and RYR2 E2311D mutant (orange) hiPSC-cardiomyocytes after isoproterenol (1uM) treatment. Two-way ANOVA with Tukey's multiple comparisons, n=12 wells. **D.** Beat rate regularity measured by the coefficient of variation (CoV) for isogenic control (blue) and RYR2 E2311D mutant (orange) hiPSC-cardiomyocytes after isoproterenol (1uM) treatment. Two-way ANOVA with Tukey's multiple comparisons, n=12 wells. Iso, isoproterenol.

CASQ2 overexpression improves RYR2 E2311D cardiomyocyte's ability to adopt rapid pacing rates and protects from tachycardia induction with isoproterenol

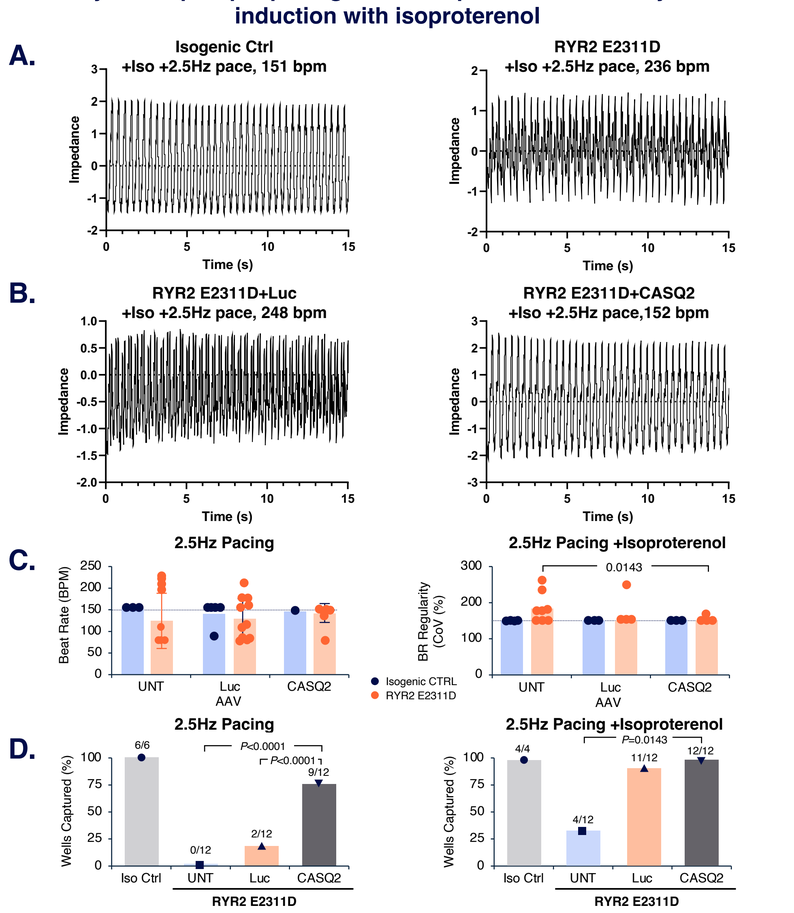
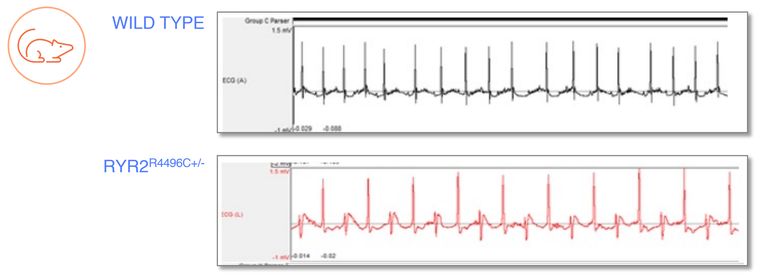


Figure 4. A. Representative impedance traces of contractions with isoproterenol (1uM) and 2.5Hz pacing from isogenic wild type control (left) and RYR2 E2311D mutant (right) hiPSC-cardiomyocytes. Two-way ANOVA with Tukey's multiple comparisons. **B.** Representative impedance traces of contractions with isoproterenol (1uM) and 2.5Hz pacing from RYR2 E2311D mutant hiPSC-cardiomyocytes transduced with AAV-Luc (left) or AAV-CASQ2 (right). Two-way ANOVA with Tukey's multiple comparisons. **C.** Average beat rate of isogenic control (blue) and RYR2 E2311D mutant (orange) hiPSC-cardiomyocytes in response to 2.5Hz pacing before (left) and after (right) isoproterenol (1uM). Dashed line at 150 bpm indicates pacing frequency. Two-way ANOVA with Tukey's multiple comparisons, n=12 wells. **D.** Proportion of wells successfully captured with 2.5 pacing before (left) and after (right) isoproterenol (1uM). Wells captured out of total wells treated shown below x-axis. Chi-square statistical analysis.

MOUSE MODEL OF CPVT

- **Genetic Alteration:** The R4496C variant involves a single amino acid substitution in *RYR2*. This variant was discovered in CPVT patients.
- **Rationale for the Model:** Mice carrying *RYR2* R4496C exhibit many of the hallmark features of CPVT seen in humans: stress-induced arrhythmias, propensity for bidirectional VT, and diastolic Ca^{2+} leak[1]



The R4496C variant predisposes the heart to ventricular tachycardia in response to adrenergic stimulation.

STUDY SCHEMATIC

GROUPS	STRAIN	TEST ARTICLE	DOSE LEVEL (vg/kg)
1	WT	Vehicle	0
2			0
3	$RYR2^{R4496C+/-}$	AAV-CASQ2	Low
4			Mid
5			High

ECG, electrocardiogram; WT, wildtype.

A clear dose response was observed in all tissue types with the highest level of transgene expression measured in cardiac tissue

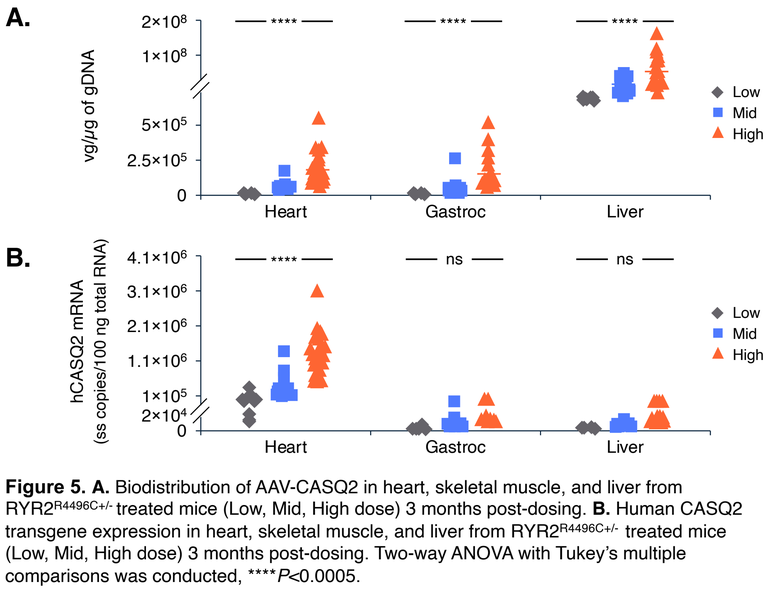


Figure 5. A. Biodistribution of AAV-CASQ2 in heart, skeletal muscle, and liver from $RYR2^{R4496C+/-}$ treated mice (Low, Mid, High dose) 3 months post-dosing. **B.** Human CASQ2 transgene expression in heart, skeletal muscle, and liver from $RYR2^{R4496C+/-}$ treated mice (Low, Mid, High dose) 3 months post-dosing. Two-way ANOVA with Tukey's multiple comparisons was conducted, ****P<0.0005.

A significant dose-responsive decrease in animals experiencing VT was observed in Mid and High dose groups compared to vehicle treated $RYR2^{R4496C+/-}$ mice

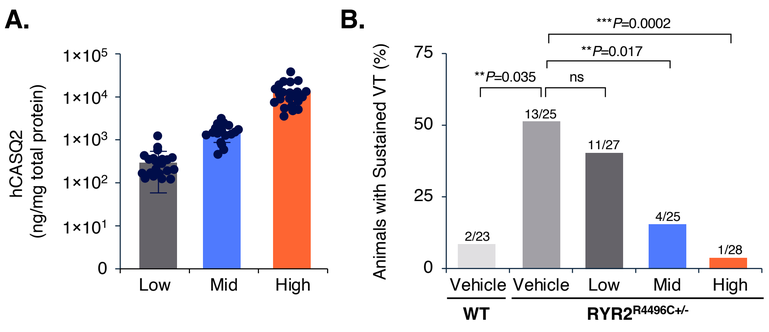


Figure 6. A. Human specific CASQ2 protein expression in $RYR2^{R4496C+/-}$ AAV-CASQ2 treated mice (Low, Mid, High dose) 3 months post-dosing measured by LC-MS. A 1.1x, 1.6x, or 6x fold increase in hCASQ2 protein levels was observed over endogenous baseline levels with the Low, Mid, and High dose, respectively. **B.** Quantification of the incidence of ventricular tachycardia (VT) determined by *in vivo* ECG recordings in WT, $RYR2^{R4496C+/-}$ vehicle, and $RYR2^{R4496C+/-}$ AAV-CASQ2 treated mice (Low, Mid, High dose) 3 months post-dosing following beta-adrenergic challenge (2mg/kg epinephrine and 120mg/kg caffeine). Chi-square statistical analysis is shown along with # of animals presenting with VT out of the total n/group.

CONCLUSIONS

- Here, we demonstrate the potential of AAV driven CASQ2 expression to correct beta-adrenergic stimulated-induction of ventricular tachycardia (VT) in two model systems of CPVT harboring different RYR2 mutations
- *In vitro*, AAV-CASQ2 treatment of RYR2 E2311D cells resulted in marked improvements in both beat rate and beat rate regularity under beta-adrenergic induced stress conditions. Additionally, treatment improved RYR2 mutant cardiomyocyte's ability to adapt to rapid pacing rates combined with isoproterenol treatment, conveying protection from stressed induced tachycardia.
- *In vivo*, AAV-CASQ2 treatment led to a dose-responsive increase in *CASQ2* mRNA and protein levels. Furthermore, this dose-responsive increase in CASQ2 expression significantly reduced the number of animals experiencing beta-adrenergic stress induced VT.

REFERENCES

1. Cerrone M, et al. *Circ Res.* 2005;96(10):e77-e82.

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DISCLOSURES

MSK: Nothing to disclose.

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