cardiomyocytes (iPSC-CMs), aided with experimental and computational approaches.

**Objective:** We tested the potential of CRISPRi modulation in pre-differentiated iPSC-CMs combining all-optical cardiac electrophysiology and molecular analysis to characterize functional phenotypes of key ion channels in the human heart.

**Methods:** We designed single guide RNAs (sgRNAs) targeting KCNH2, KCNJ2, and GJA1. The sgRNAs were cloned into a lentiviral vector with eGFP reporter. A dox-inducible dCas9-KRAB was inserted into the AAVS1 safe harbor site of pre-differentiated hiPSC-CMs and transduced with lentivirus carrying the sgRNAs. To deploy all-optical electrophysiology, cells were transduced with an adenoviral vector containing ChR2-eYFP (for optical stimulation) and co-labeled with spectrally-compatible voltage and calcium sensors to obtain functional measurements upon 5 days of dox induction.

**Results:** A sgRNA targeting KCNH2 resulted in about 40% knockdown of KCNH2 mRNA and significant but mildly prolonged action potential duration, e.g., spontaneous APD90 (+12%, p<0.05). A sgRNA targeting KCNJ2 caused about 60% downregulation of mRNA levels and exhibited minimal changes in spontaneous beat frequency in high density cell preparations (+7%, n.s.) but more pronounced effects in reduced density cell preparations (+30%, p<0.01). Additionally, sgRNA targeting GJA1 exhibited about 40% knockdown of mRNA and resulted in significant slowing of conduction velocity (-19%, p<0.01) at 1 Hz pacing conditions.

**Conclusion:** Knockdown of key cardiac ion channels in our system yielded mild but specific functional changes. Using this platform for CRISPRi mediated knockdown of disease-associated genes in pre-differentiated cardiomyocytes can improve the assessment of gene function in cellular cardiac electrophysiology. In combination with sgRNA libraries and/or with CRISPRa mediated gene activation, it can allow a more comprehensive evaluation of the mechanisms controlling cardiac electromechanics.

**BS-514-04**

**ADENOVIRUS INCREASES ARRHYTHMIA SUSCEPTIBILITY DURING ACUTE CARDIAC INFECTION**

Rachel Lee Padget; Grace A. Blair; Michael D. North; David R. King; Michael Zeitz PhD; Gregory S. Hoeker PhD; Sharon Swanger PhD; Steven Poelzing PhD and James W. Smyth PhD

**Background:** Myocarditis underlies 42% of sudden cardiac death in young adults, yet viral arrhythmogenic mechanisms remain elusive. Adenovirus is a leading cause but species-specificity has limited disease modeling in mice. Gap junctions are comprised primarily of connexin43 (Cx43), enable electrical impulse propagation in the heart. Changes in Cx43 expression, localization, and/or function cause arrhythmias. Gap junctions propagate innate and adaptive antiviral responses, and our prior work has demonstrated that Cx43 expression and function are reduced during human adenovirus infection.

**Objective:** Utilize cardiotropic Mouse Adenovirus Type-3 (MadV-3) to investigate how acute viral infection generates an arrhythmogenic substrate.

**Methods:** Adult mice were inoculated with MadV-3 and viral genomes quantified by qPCR for organ tropism along with histopathology after 7 days. Cardiac function was measured by echo- and electrocardiography, and optical mapping. Changes to ion channel expression and localization were quantified using RT-qPCR, western blotting, confocal, and super resolution microscopy of infected tissue and isolated adult ventricular cardiomyocytes (ACMs), which also underwent patch clamping.

**Results:** Viral genomes were specifically enriched in heart tissue and no cardiomyopathy was detected, just as in human acute myocarditis. Reductions in cardiac ion channel and connexin mRNA levels occurred in infected hearts. Cx43 was phosphorylated at residues known to reduce function and decreased conduction velocity was found in infected hearts by optical mapping. Prolonged action potential duration was detected with impaired K+ currents in infected ACMs by patch clamping. Microscopy of infected cardiac tissue and ACMs revealed alterations in gap junction/scaffolding protein complexing at the cell-cell junction. In human iPSC-derived cardiomyocytes, Cx43 phosphorylation was increased and cellular uncoupling detected by optical mapping during human adenovirus infection.

**Conclusion:** Reduced cellular coupling and ion channel function during adenovirus infection generates an arrhythmogenic substrate prior to an appreciable immune response or cardiomyopathy development, highlighting contributions of active infection to electrical disturbances prior to inflammation.

**ABSTRACT CA-531:**

**Insights and Innovations in Ventricular Arrhythmia Ablation**

Saturday, April 30, 2022

10:30 AM - 11:30 AM

**CA-531-01**

**FAT INFECTION CONFERS PROPENSITY FOR VENTRICULAR TACHYCARDIA IN THE POST-INFARCT SUBSTRATE**

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**Background:** Infiltrating adipose tissue (inFAT) is present in the post-infarct substrate, but its role in ventricular tachycardia (VT) arrhythmogenesis is not well-established.

**Objective:** To investigate the role of post-infarct inFAT in VT propensity.

**Methods:** 24 post-infarct patients who underwent VT ablation were prospectively enrolled across two centers. For each patient, contrast-enhanced computed tomography (CE-CT), late gadolinium-enhanced magnetic resonance imaging (LGE-MRI), and substrate mapping during sinus rhythm were acquired. Voltage amplitude, deceleration zones (DZ) which represent regions of activation slowing, and substrate-based ablation locations were compared with the inFAT and scar distributions. To glean deeper insights into arrhythmogenic propensity, novel hybrid digital heart twins representing the patient-specific inFAT from CT and scar from MRI were reconstructed. VT circuits were then induced, assessed, and compared to mapping data (Fig.A).

**Results:** Combined inFAT and scar had lower voltage and exhibited stronger correlations with voltage amplitude than that of scar alone (0.76±0.56 vs. 1.19±0.47 mV, p<0.0005; r = -0.54 vs. -0.45). inFAT and scar exhibited greater isochronal crowding than scar alone (3(2) vs. 2(1) isochrones, p<0.0005). Most DZs consisted of combined inFAT and scar (71.1%) rather than scar alone. The amount of ablated inFAT was strongly correlated with ablated scar (r = 0.734, p<0.0005). In the digital hearts, 140 VTs comprised of both inFAT and scar (110/140 VTs) (Fig.B). In a
multivariate regression adjusting for age, infarct age, and VT circuit tissue volume, inFAT remodeling within the VT circuit, but not scar, was a predictor of the amount of DZs ($\beta = 0.355$, $p < 0.05$) and clinical ablations ($\beta = 0.323$, $p < 0.005$) (Fig.B).

**Conclusion:** Post-infarct inFAT remodeling creates a critical arrhythmogenic substrate for VT that needs to be prioritized during ablation.

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**CA-531-02**

**ABLASTION OF PREMATURE VENTRICULAR CONTRACTIONS: DISSOCIATION BETWEEN ACUTE AND LONG-TERM OUTCOMES**

Kenichi Tokutake MD; Kanae Hasegawa MD, PhD; Travis D. Richardson MD; Jay A. Montgomery MD; Sharon Shen MD; J.C. Estrada MD; Pablo J. Saavedra MD; Arvindh Kanagasundram MD, FHRs; Gregory F. Michaud MD, FHRs and William G. Stevenson MD, FHRs

**Background:** Acute and long-term outcomes after premature ventricular contraction (PVC) ablation may vary with structural heart disease (SHD) and PVC origin.

**Objective:** To compare acute and long-term outcomes after PVC ablation according to SHD and origin.

**Methods:** We reviewed 213 consecutive patients who underwent PVC ablation. Acute success was defined as abolition of the target PVC. Follow-up included 12 lead electrocardiographic, ambulatory monitoring, and symptoms. The origin of PVC was defined by mapping and elimination by ablation.

**Results:** Of 213 patients, 125 (59%) had structural heart disease (SHD) (coronary disease in 42, cardiomyopathy in 64, valve disease in 19). Acute ablation success was achieved in 93% of patients. During long-term follow-up (391 ± 253 days), 20% of patients recurred. After acute failure, late success occurred in 6 of 14 (43%). In patients with SHD, the long-term recurrence rate was higher compared to patients without SHD (26% vs 11%; $p < 0.05$), but the acute ablation success rate was similar (92% vs 95%; $p = 0.31$) (Figure 1). Outcome varied with PVC origin (Figure 2). Long-term success was greater in patients with outflow tract (RVOT and LVOT) PVCs than for other PVC origins (85% vs 67%; $p = 0.001$) despite similar acute ablation success rates (93% vs 91%; $p = 0.70$). Thirty seven (17%) patients had PVCs from more than 1 segment. Acute success (96% vs 84%; $p < 0.05$) and long-term outcome (82% vs 68%; $p < 0.05$) were better for patients with PVCs from only one versus multiple segments.

**Conclusion:** In patients with PVCs, the acute effect of ablation predicts the long-term outcome, but with limited accuracy, particularly in patients with structural heart disease and PVCs from multiple origins. Late success after acute failure occasionally occurs.

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**CA-531-03**

**FIRST-IN-HUMAN EXPERIENCE WITH ULTRA-LOW TEMPERATURE CRYOABLATION FOR MONOMORPHIC VENTRICULAR TACHYCARDIA**

Tom De Potter MD, PhD; Jippe C. Balt MD, PhD; Lucas V.A. Boersma MD, PhD; Frederic Sacher MD, PhD and Atul Verma MD, FHRs