Heterozygous Deletion of *KCNH2* Models Long QT Type 2 in Human Pluripotent Stem Cell-Derived Cardiomyocytes

Vincenzo Macri[1], Adam Hirst[1], Jessica Norberg[1], Melanie Kardel[1], Arthur Sampaio[1], Wing Chang[1], Terry Thomas[1], Allen Eaves[1,2], Stephen Szilvassy[1] and Sharon Louis[1]

[1] STEMCELL Technologies Inc., Vancouver BC, Canada [2] Terry Fox Laboratory, BC Cancer Agency, Vancouver BC, Canada

Introduction

Heterozygous variants are most frequently associated with cardiac arrhythmias, including long QT syndrome, in the general population and within families. Genome-editing of human induced pluripotent stem cells (hiPSC) can be used to model arrhythmias. *KCNH2* encodes for the hERG channel which is important for cardiomyocyte repolarization. It was previously reported that a heterozygous point mutation in the PAS domain of hERG was associated with familial long QT type 2 using an overexpression heterologous system.

Hypothesis

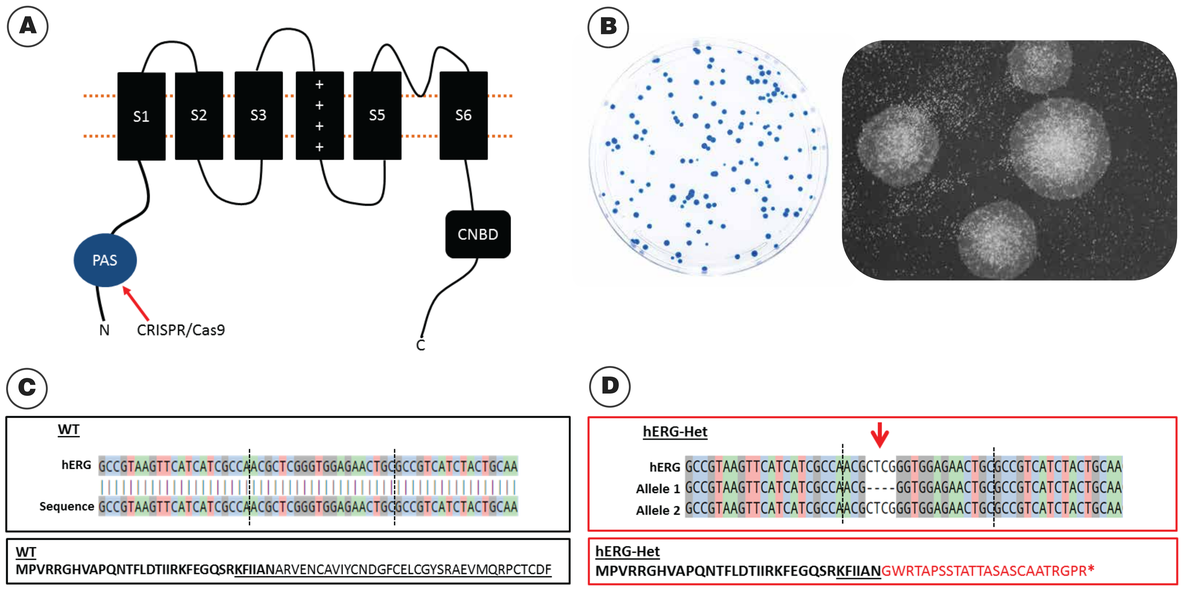
Targeted gene-editing to produce a heterozygous mutation in the PAS domain of hERG will elicit the long QT phenotype in hiPSC-derived cardiomyocytes (hiPSC-CMs).

Methods

CRISPR/Cas9 and a highly efficient cloning supplement were used to edit *KCNH2* and derive a clonal hiPSC line, respectively. DNA sequencing was used to identify heterozygous editing of the PAS domain of *KCNH2.* Control and hERG edited isogenic hiPSC lines were differentiated to cardiomyocytes using an optimized cardiomyocyte differentiation media and protocol. On day 18 of differentiation, the hiPSC-CMs were harvested and replated on glass coverslips for immumocytochemistry or Microelectrode Array (MEA) culture plates for electrophysiology. Ten days post replating hiPSC-CMs, spontaneous electrical measurements were acquired for five minutes and analyzed.

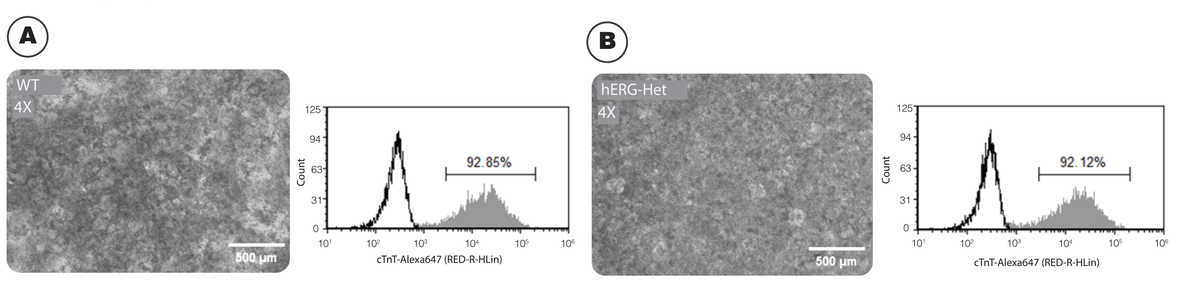
Results

Figure 1. Targeted CRISPR/Cas9 Editing of the PAS Domain of hERG Using an hiPSC Line Creates an Early Stop Codon in One Allele.



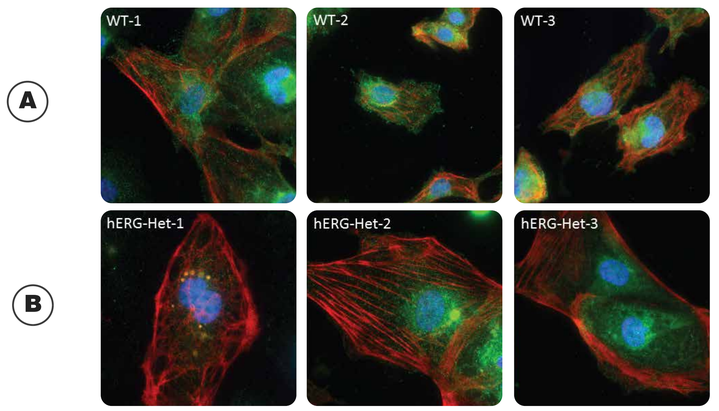
A) Single subunit of the hERG ion channel showing the CRISPR/Cas9 targeting the PAS domain (exon 2 of *KCNH2*) located in the N-terminus. **B)** Morphology of CRISPR/Cas9 edited clonal cell lines. **C, D)** DNA (above) and amino acid (below) sequences of the wild type (WT, black box) and Heterozygous PAS domain early stop codon (hERG-Het, red box) isogenic hiPSC lines. The red arrow highlights the four base pair deletion in a single allele of the CRISPR/Cas9 edited line. The underlined amino acids denotes the PAS domain in both the WT and hERG-Het isogenic hiPSC lines. The red star indicates a stop codon.

Figure 2. Both WT and hERG-Het isogenic hiPSC Lines Can be Differentiated to Produce Highly Pure Cardiomyocytes.



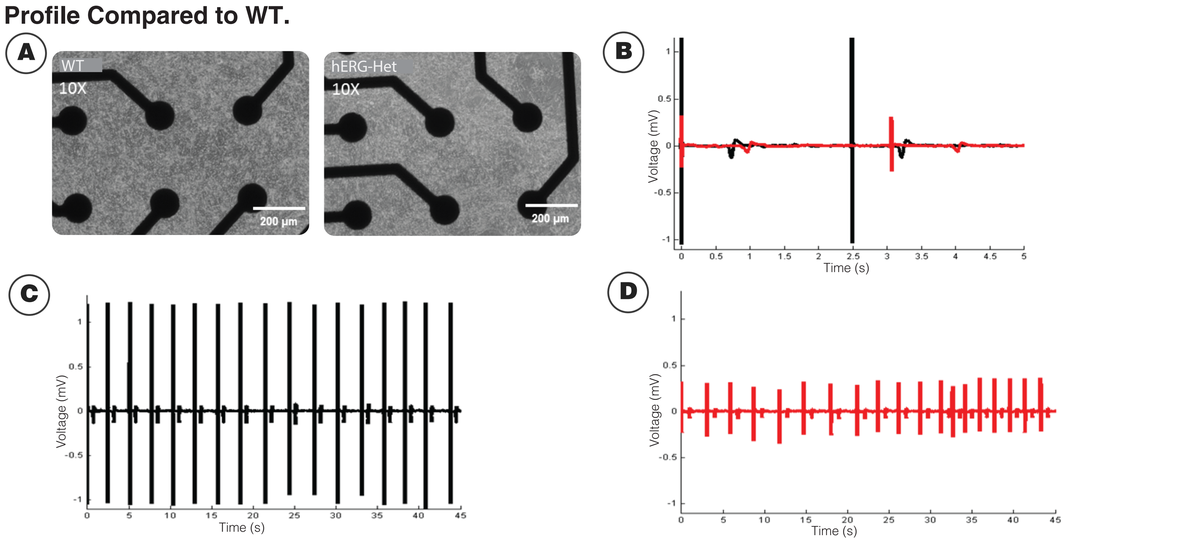
A, B) Bright field images at Day 15 showing confluent monolayers of WT and hERG-Het hiPSC-derived cardiomyocytes. Flow cytometry shows that both isogenic lines produce >90% cTnT-positive cells at Day 15.

FIGURE 3. The WT and hERG-Het Isogenic hiPSC-Derived Cardiomyocytes Have a Different Expression Pattern of the hERG Ion Channel.



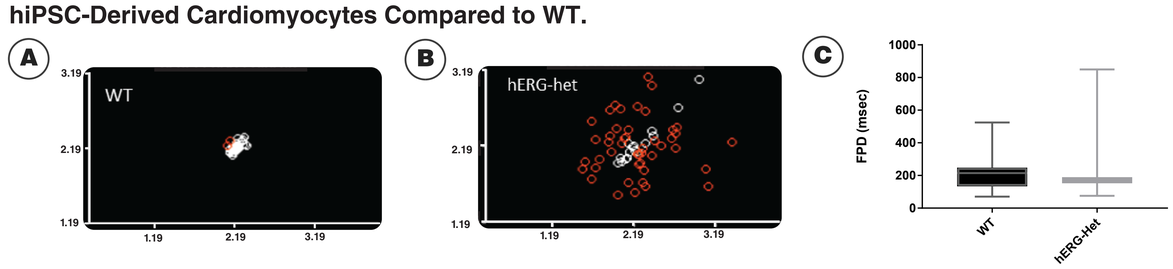
A, B) Three representative immunocytochemistry images of WT and hERG-Het hiPSC-derived cardiomyocytes stained for hERG (green), cTnT (red) and DAPI (blue), respectively. The hERG channel is distributed throughout the WT hiPSC-derived cardiomyocytes and is restricted near the nucleus in the hERG-Het hiPSC-derived cardiomyocytes.

FIGURE 4. The hERG-Het Isogenic hiPSC-Derived Cardiomyocytes Have an Unstable Excitability Profile Compared to WT.



A) Bright field images of replated WT and hERG-Het hiPSC-derived cardiomyocytes. **B)** Field potential recordings showing prolonged repolarization in the hERG-Het (red) compared to WT (black) hiPSC-derived cardiomyocytes. **C, D)** Forty-five second field potential recording showing stable electrical activity and arrhythmia in the WT (red) and hERG-Het (black) hiPSC-derived cardiomyocytes, respectively.

FIGURE 5. Greater Variability in Beat Period and Repolarization Observed in the hERG-Het Isogenic hiPSC-Derived Cardiomyocytes Compared to WT.



A, B) Beat Period Poincare plots showing greater beat period variability in the hERG-Het compared to WT hiPSC-derived cardiomyocytes. **C)** Box plox showing greater variability in field potential durations in the hERG-Het compared WT hiPSC-derived cardiomyocytes.

Conclusions

- A heterozygous four nucleotide deletion in exon 2 of KCNH2 was generated in a hiPSC line producing an early stop codon in the PAS domain of the hERG channel.
- Cardiomyocyte differentiation efficiency was similar for both the WT and hERG-Het isogenic hiPSC lines (>90% cTnT-positive).
- hERG channel expression was distributed throughout the WT hiPSC-derived cardiomyocytes but was restricted near the nucleus in hERG-Het hiPSC-derived cardiomyocytes.
- The hERG-Het hiPSC-derived cardiomyocytes had an irregular excitability profile with a variable beat period and prolonged field potential durations compared to the WT hiPSC-derived cardiomyocytes.
- Introduction of a heterozygous stop codon in the PAS domain of the hERG channel generates arrhythmias characteristic of long QT syndrome in isogenic hiPSC-derived cardiomyocytes.