

# EVALUATION OF HYPERTROPHIC CARDIOMYOPATHY USING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES REVEALS ABNORMAL EXCITATION CONTRACTION COUPLING

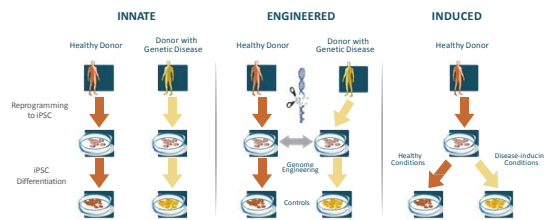


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## Abstract

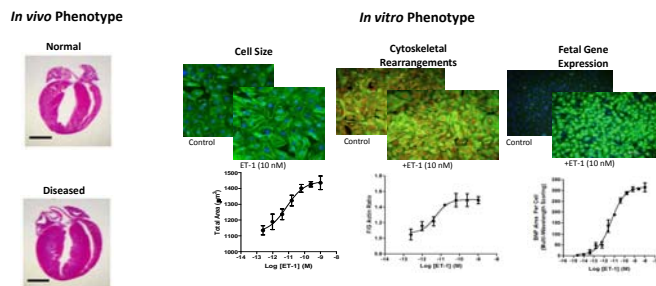
Sarcomeric cardiomyopathies, including hypertrophic cardiomyopathy (HCM), are an important cause of morbidity and mortality. Clinically, HCM is characterized by ventricular wall thickening as a result of enlarged cardiomyocytes, preserved ejection fraction concurrent with diastolic dysfunction, and arrhythmias. One of the most common forms of HCM arises from a missense mutation in the gene encoding the beta myosin heavy chain protein (MYH7), resulting in a change of amino acid 403 from arginine-to-glycine (R403Q). A major hindrance to detailed study of sarcomeric cardiomyopathies in humans has been lack of an appropriate in vitro cardiac tissue model. Here, we use human induced pluripotent stem cell derived-cardiomyocytes (hiPSC-CMs) to study the functional consequence of the HCM MYH7 R403Q mutation, specifically electrophysiology, calcium handling, and contraction. HiPSC-CMs were generated through reprogramming of somatic cells from a patient carrying the HCM MYH7 R403Q mutation. In addition, we use genome engineering strategies to correct the mutation, creating an isogenic control. Moreover, we developed an induced hypertrophy model by exposing control hiPSC-CMs to endothelin-1 (ET-1). Both inherited and induced models display classic hallmarks of hypertrophy, including up-regulation of fetal genes, cytoskeletal rearrangements, and increased hiPSC-CM size. In addition, the HCM MYH7 R403Q hiPSC-CMs display abnormal electrophysiological properties and calcium handling properties including significantly slower calcium decay rates and prolonged calcium handling kinetics (i.e., time to peak and time to baseline) concurrent with contractile dysfunction. These data illustrate the advantages of disease modeling using hiPSC technology. We conclude that patient-specific hiPSC-CMs exhibit classic clinical phenotypes relative to control. We show that the induced and inherited HCM phenotype hiPSC-CMs have common structural and functional features. In total, hiPSC technology enables a reliable and reproducible disease model not previously attainable and provides new solutions, tools, and opportunities for sarcomeric cardiomyopathy mechanistic elucidation and novel therapeutic research.

## Disease Modeling with iPSC – “Disease-in-a-Dish”



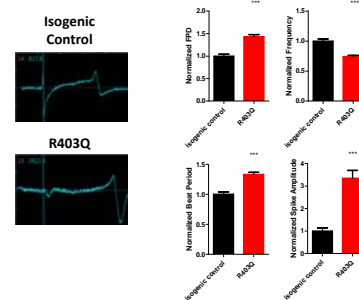
▲ **Figure 1. Disease modeling approaches.** Graphic depicting three primary disease modeling approaches. Innate model where a sample is taken from a diseased donor. Engineered model where genome engineering strategies are used to introduce or correct a mutation. An induced model where cells are exposed to disease causing conditions (e.g., ET-1).

## Induced Disease Models – Cardiac Hypertrophy



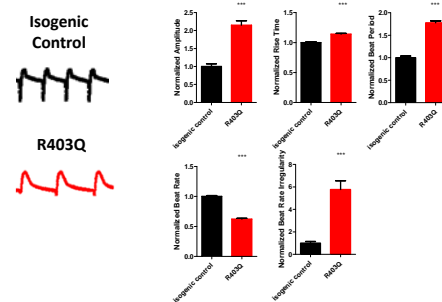
▲ **Figure 2.** Representative structure of diseased hearts (Left panel). Induced cardiac hypertrophy model (Right panel) where apparently healthy normal iCell® Cardiomyocytes were exposed to Endothelin-1 (ET-1) to induce a hypertrophy phenotype. Data depict a concentration-dependent increase in cell size, cytoskeletal rearrangements (F/G Actin), and fetal gene expression (BNP) evaluated using high content imaging.

## HCM MYH7 R403Q CMs Exhibit Abnormal Electrophysiological Properties



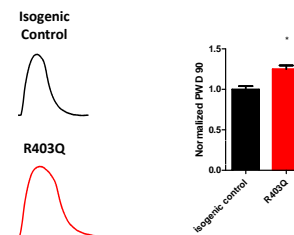
▲ **Figure 3. HCM (MYH7 R403Q mutation) hiPSC-CMs electrophysiological characterization.** Representative field potential recordings of isogenic control (upper panel) and HCM (lower panel) hiPSC-CMs. Data are mean ± SEM. Data show HCM MYH7 R403Q hiPSC-CMs have altered electrophysiological properties, including prolonged field potential duration of spontaneously beating cultures.

## HCM MYH7 R403Q CMs Display Increased Contraction Amplitude and Arrhythmias



▲ **Figure 4. HCM (MYH7 R403Q mutation) hiPSC-CMs contractile (impedance) assessment.** Representative impedance trace of isogenic control (Black) and HCM (Red) hiPSC-CMs. Data are mean ± SEM. Data show HCM MYH7 R403Q hiPSC-CMs have significantly increased impedance amplitude (Positive Peak Amplitude) and arrhythmic events.

## HCM MYH7 R403Q CMs Exhibit Altered Ca Handling Properties



▲ **Figure 5. HCM (MYH7 R403Q mutation) hiPSC-CMs Ca handling.** Representative twitch calcium transients from HCM (Red) and isogenic control (Black) hiPSC-CMs. Data are mean ± SEM. Data show significant Ca handling abnormalities in the HCM MYH7 R403Q hiPSC-CMs.

## Conclusion

- Patient-derived iPSC-CMs recapitulate innate disease pathophysiology.
- Control iCell Cardiomyocytes are responsive to ET-1 induced cardiac hypertrophy.
- HCM MYH7 R403Q CMs display a hypertrophic cardiomyopathy phenotype.
- Genome engineering strategies in iPSCs enable the correction of the MYH7 R403Q mutation, thus creating an isogenic control.
- Induced and inherited HCM phenotype hiPSC-CMs have common structural and functional features.