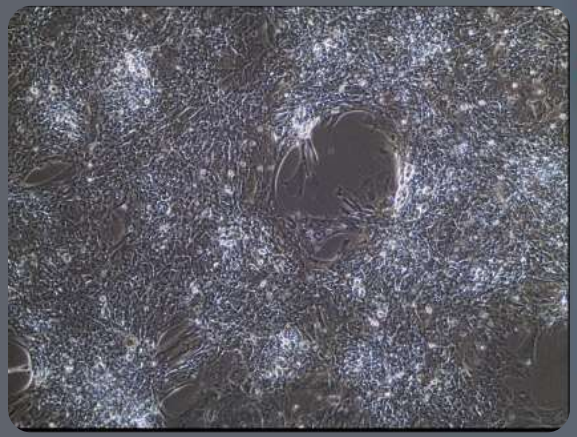
REGENERON
SCIENCE TO MEDICINE®

GENETICS GUIDED DRUG DISCOVERY USING HUMAN INDUCIBLE PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES AND HUMAN ENGINEERED CARDIAC TISSUES

SCOTT MACDONNELL
ASSOCIATE DIRECTOR – CARDIOVASCULAR AND RENAL THERAPEUTIC AREA

Contracting iPS-Derived Human Cardiomyocytes

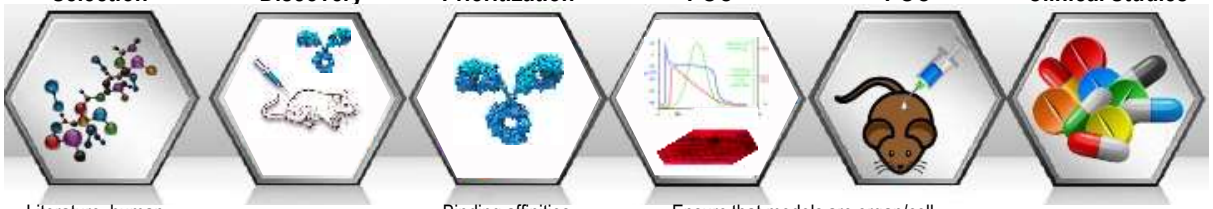


AGENDA:

- GENETIC GUIDED DRUG DISCOVERY
- IPSC DERIVED HUMAN CARDIOMYOCYTES
- USING IPSC CARDIOMYOCYTES TO EVALUATE FUNCTION OF R403Q CELLS AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY
  - 2D vs. 3D Models

GENETICS GUIDED DRUG DISCOVERY

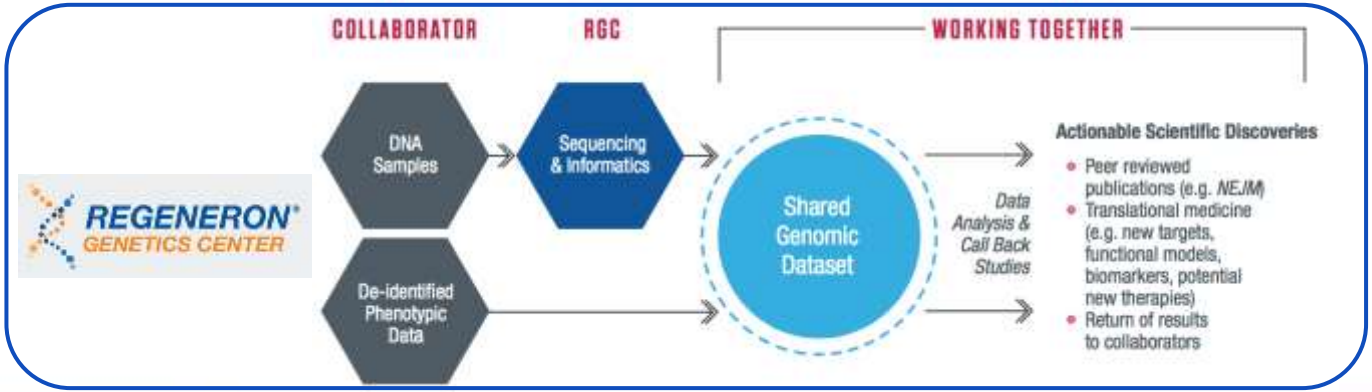
Drug Development

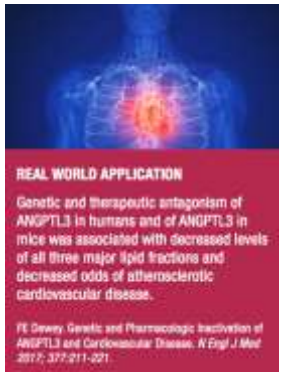


Literature, human genetics, animal studies, clinical data

Binding affinities, selectivity, biophysical characterization

Ensure that models are organ/cell + species specific. Also are relevant to disease.





MICE ARE NOT HUMANS….

Editorial | Published: 04 April 2013

Of men, not mice

Nature Medicine **19,** 379 (2013) | Download Citation

OF MICE NOT HUMANS…

FUNDAMENTAL PHYSIOLOGIC DIFFERENCES BETWEEN HUMAN AND MOUSE CARDIOMYOCYTES

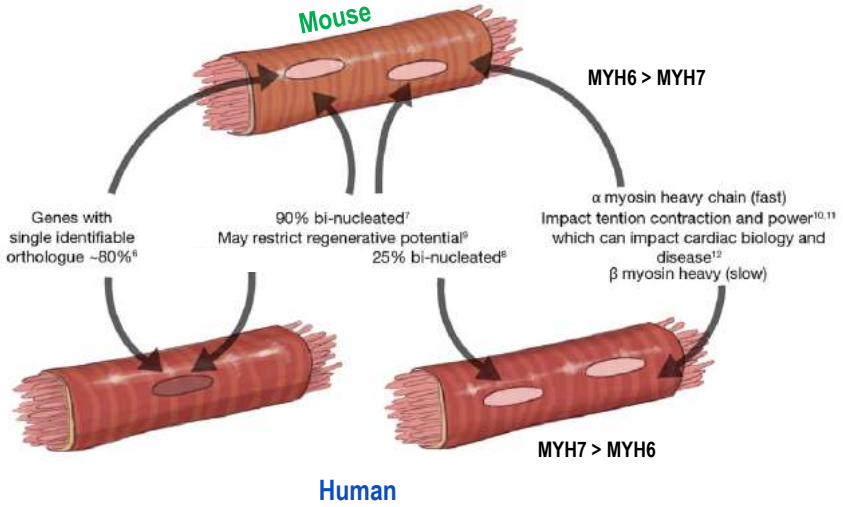
General Characterization

Mouse Cardiomyocytes

Properties
Mass 0.15 g
600 beats per minute
Energy Production 1350 kcal/kg/day
Sarcomeres : mitochondria 2:1

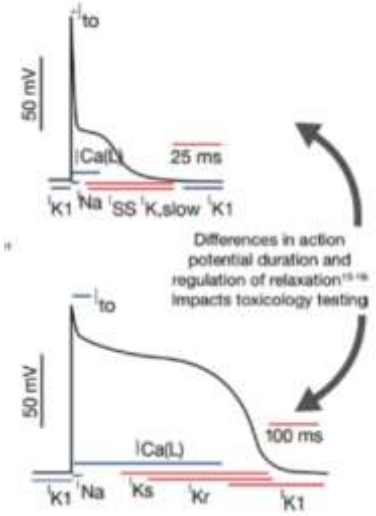
Human Cardiomyocytes

Properties
Mass 300 g
70 beats per minute
Energy Production 700 kcal/kg/day
Sarcomeres : mitochondria 2:1



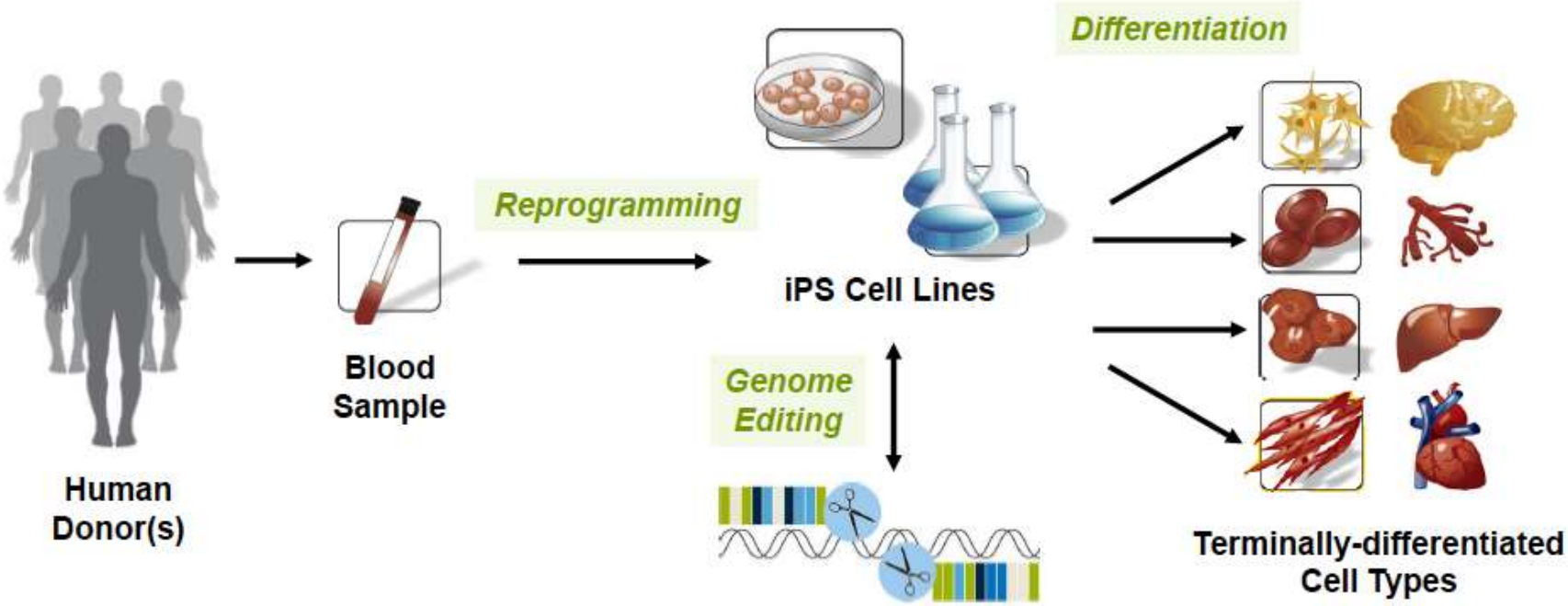
This impacts how tension and power are generated during contraction, affecting the mechanisms underpinning cardiac disease and function

Electrophysiologic Differences



Mice are less reliant on hERG channel. Challenges modeling arrhythmia in mice.

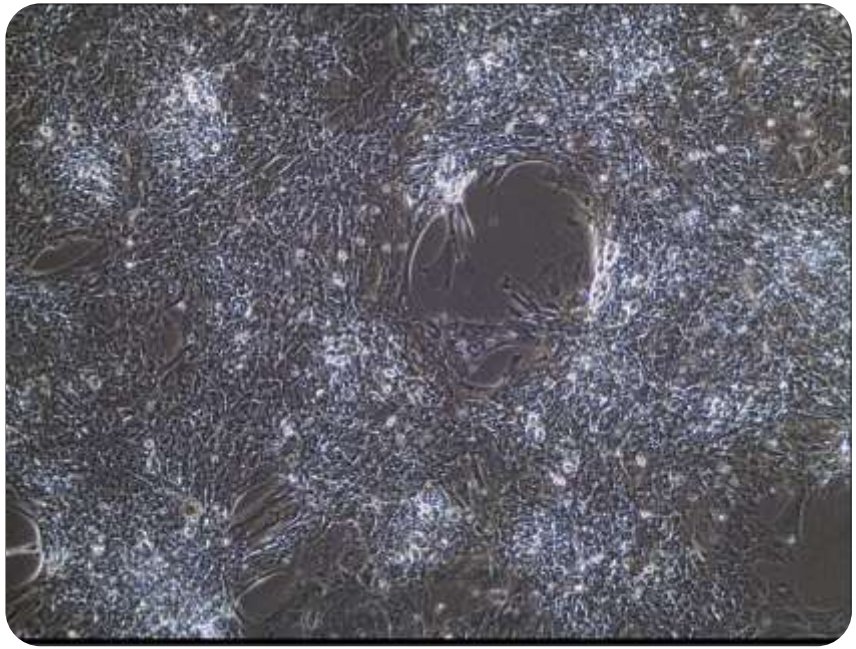
START WITH HUMANS - IPSC TECHNOLOGY



Circ Genom Precis Med. 2018;11:e000043.

IPSC-DERIVED CARDIOMYOCYTES – OPPORTUNITY AND LIMITATIONS

iPSC-Derived Cardiomyocyte Monolayer



Opportunity

- Human Cells (patient derived or engineered)
- Near limitless source of material – well powered studies
- Exhibit functional properties (contractile and electrical) of human cardiomyocytes
- Evaluate the impact of variants across multiple genetic backgrounds in parallel
- Unable to acquire and culture human primary cardiomyocytes
- In-vitro – mechanistic evaluation in the absence of sympathetic tone

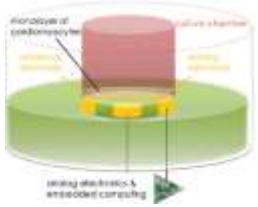
Limitations

- Disorganized sarcomere structure
- Immature phenotype
- Variation - Require multiple lines to define phenotype
- Isolated system

MODELING CARDIAC CONTRACTILITY AND ELECTRICAL ACTIVITY

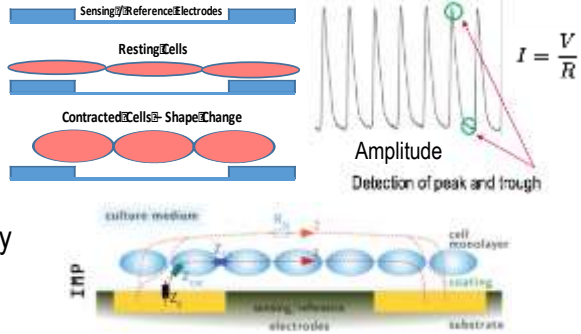
NANION CARDIOEXCYTE 96

Nanion CardioExcyte96

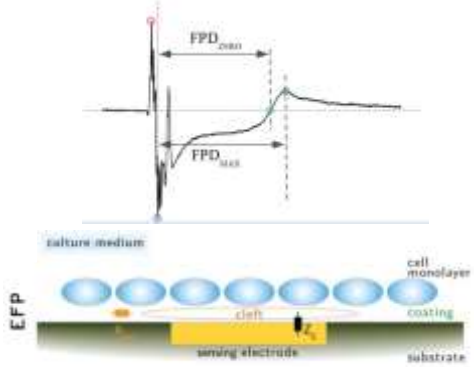


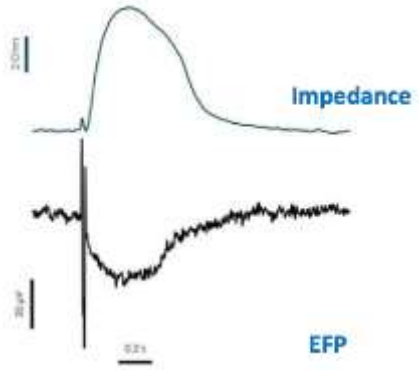
Pacing

Contractility



Electrical Activity

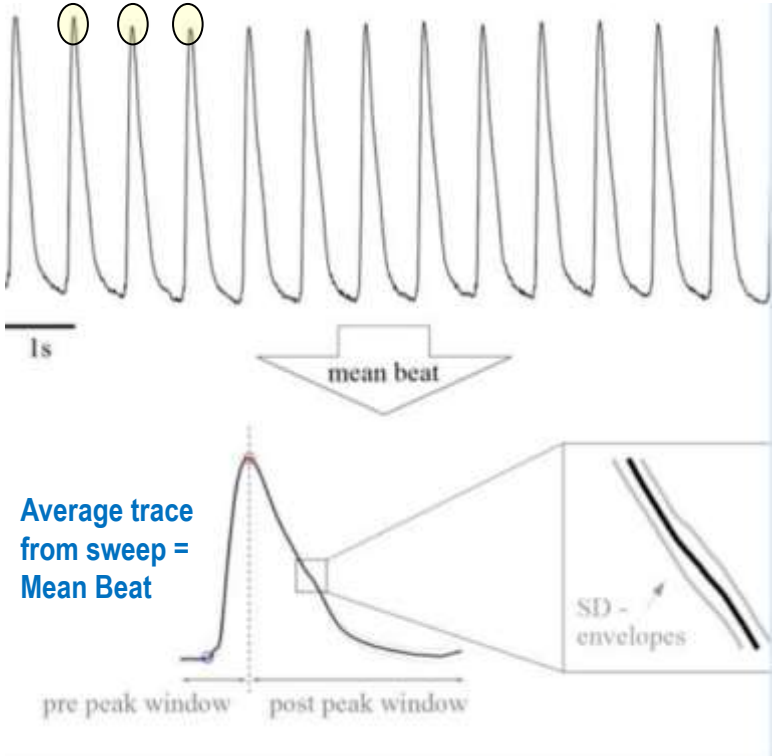




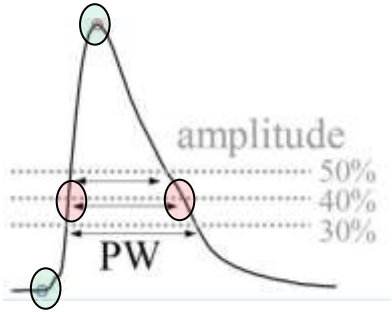
Impedance measurements to assess contractile function and extracellular field potential (EFP) to evaluate action potential duration are collected in parallel.

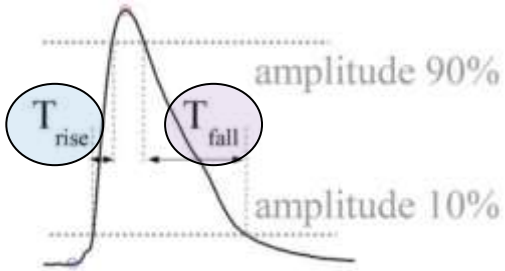
MODELING CARDIAC CONTRACTILITY

NANION CARDIOEXCYTE 96



Analysis of Impedance Traces



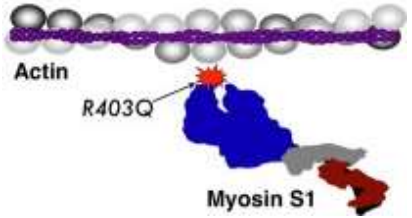


- **Contractility** – Trough-to-Peak
- **Arrhythmia** – Inter-spike interval variation
- Beat width – duration of contraction (**Calcium handling**)
- Time and Velocity of Upstroke – Calcium influx/SR release and contractile protein activation – **Systolic function**
- Time and Velocity of Relaxation – SR calcium uptake - **Diastolic function**

MODELING THE IMPACT OF GENETIC VARIANTS: MYH7-R403Q IPSC-CARDIOMYOCYTES AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY

Hypertrophic cardiomyopathy (HCM) affects **1 in 500 people** worldwide, with no discrimination for race or gender and is caused by the enlargement of cardiomyocytes, resulting in thickening of ventricular walls in the absence of increased external load. This thickening reduces left ventricle chamber size and impairs relaxation, eventually resulting in reduced cardiac output, fibrosis, arrhythmia, and failure.

The most prevalent mutations implicated in familial hypertrophic cardiomyopathy are in the MYH7 gene.



Decrease in myosin motor function with the MYH7-R403Q mutation leads to a compensatory asymmetric hypertrophic response.

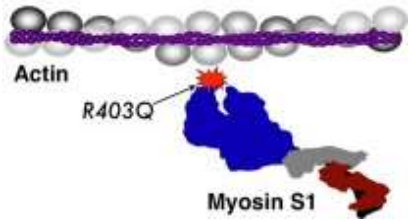
Patients carrying the MYH7-R403Q mutation are at increased risk for arrhythmia, heart failure, and sudden cardiac death. } **50% mortality by age 40**

Table 1. Sarcomere mutations in hypertrophic cardiomyopathy

Protein	Gene	Chromosome	Prevalence
Cardiac β-myosin heavy chain	*MYH7*	14q12	~40%
Cardiac myosin binding protein C	*MYBPC3*	11p11.2	~40%
Cardiac troponin T	*TNNT2*	1q32	~5%
Cardiac troponin I	*TNNI3*	19q13.4	~5%
α-Tropomyosin	*TPM1*	15q22.1	~2%
Myosin regulatory light chain 2	*MYL2*	12q24.11	~1%
Myosin, light chain 3	*MYL3*	3p21.3-p21.2	~1%
Actin	*ACTC1*	15q14	~1%
Titin	*TTN*	2q31	Rare
Myozenin	*MYOZ2*	4q26-q27	Rare
α-Myosin heavy chain	*MYH6*	14 28.01 cM	Rare

Sevcan Atay, Aslı Tetik, Vildan Bozok Çetintaş, Selcen Yakar Tülüce, Kamil Tülüce, Meral Kayıkçıoğlu, Zuhal Eroğlu. Beta myosin heavy chain mutations R403QLW, V606M, K615N and R663H in patients with hypertrophic cardiomyopathy. Anatol J Cardiol. 2014; 14(3): 244-250

MODELING THE IMPACT OF GENETIC VARIANTS: MYH7-R403Q IPSC-CARDIOMYOCYTES AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY



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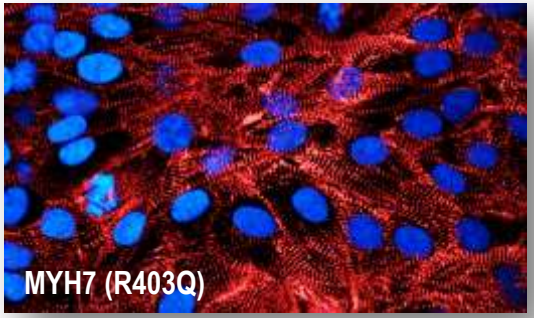
Patients carrying the MYH7-R403Q mutation are at increased risk for arrhythmia, heart failure, and sudden cardiac death.

50% mortality by age 40

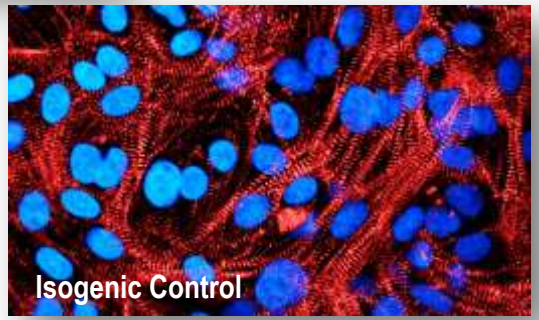
Determine if iPSC-derived cardiomyocytes from patients carrying the R403Q variant demonstrate a phenotype consistent with the clinical manifestations.

Can cells be used to model/explore the impact of genetic variants on cardiac function?

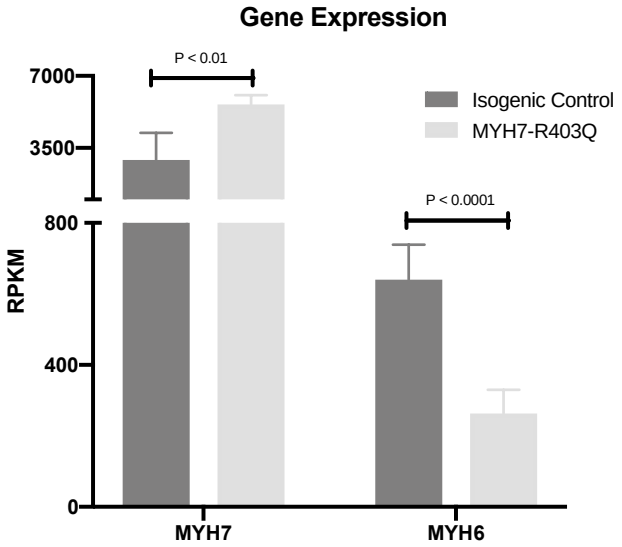
MODELING THE IMPACT OF GENETIC VARIANTS: MYH7-R403Q IPSC-CARDIOMYOCYTES AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY



Red = Sarcomeric Actin



Patient derived vs. isogenic control (corrected)

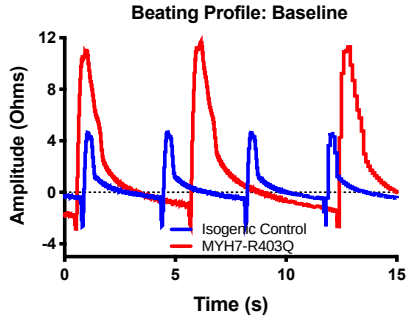


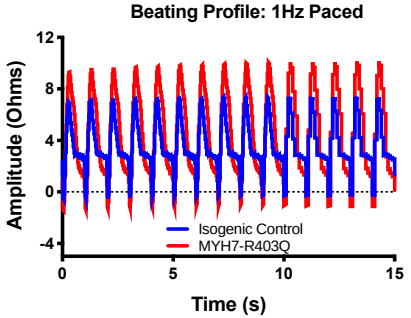
- Two myosin heavy-chain genes, **MYH6** and **MYH7** are expressed in mammalian hearts in opposing patterns during cardiac development and remodeling.
- Cardiac injury shifts this **ratio** toward **MYH7**.

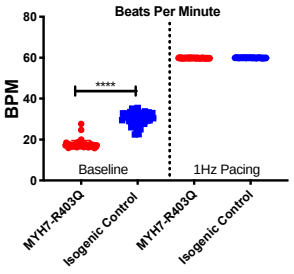
Link to HCM: Compensatory hypertrophic signaling

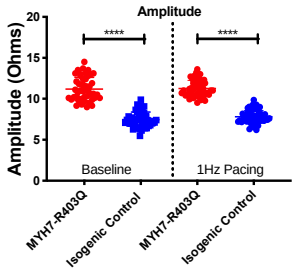
MODELING THE IMPACT OF GENETIC VARIANTS: MYH7-R403Q IPSC-CARDIOMYOCYTES AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY

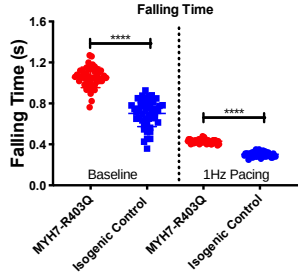
Contractility

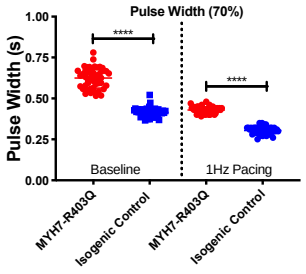










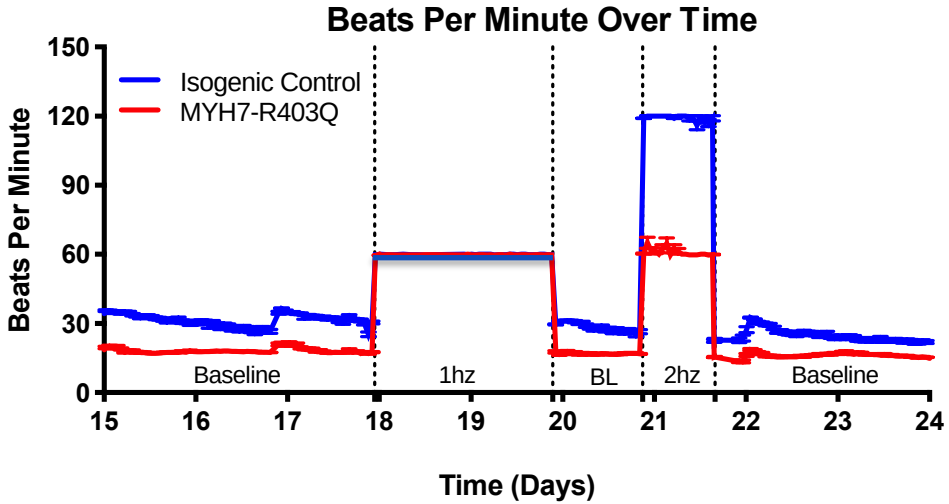


Conclusion: MYH7-R403Q cardiomyocytes demonstrate a slower intrinsic beat rate, slower relaxation times and a larger beat amplitude compared to isogenic control CMs. This phenotype persists when paced at 1Hz.

Link to HCM: Increased beat width, fall time, and reduced velocity suggests impaired diastolic performance in these R403Q derived cardiomyocytes.

MODELING THE IMPACT OF GENETIC VARIANTS: MYH7-R403Q IPSC-CARDIOMYOCYTES AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY

Question: Can the MYH7 (R403Q) cells respond to a pacing challenge?



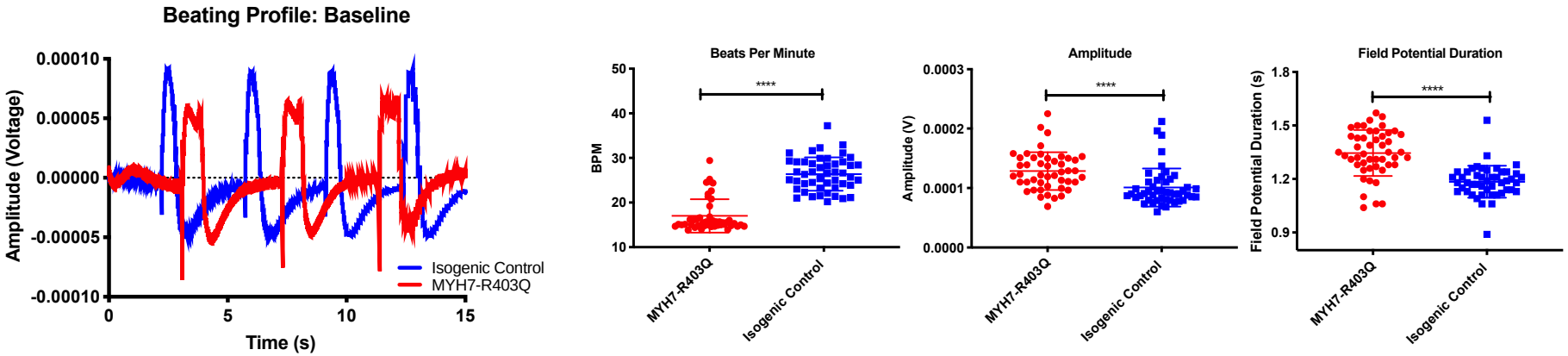
Conclusion: A lower intrinsic beat rate was observed in the MYHY-R402Q vs. control.

Interestingly, while both MYH7-R403Q and control cells displayed rhythmic beating at 1Hz, the MYH7-R403Q cells were unable increase rate up to 2Hz.

Link to HCM: Increased arrythmia risk, limited reserve, reduced ability to adapt to stress.

MODELING THE IMPACT OF GENETIC VARIANTS: MYH7-R403Q IPSC-CARDIOMYOCYTES AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY

Extracellular Field Potential – Action Potential

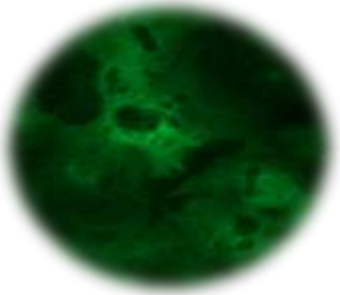


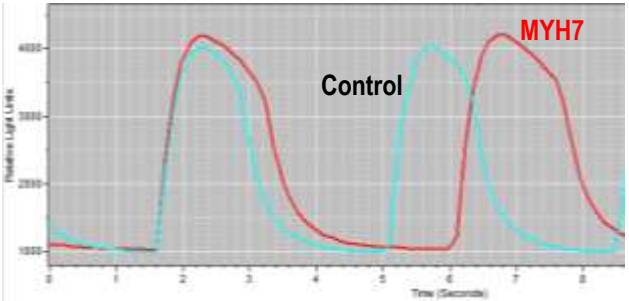
Conclusion: MYH7-R403Q cardiomyocytes demonstrate slower electrical beat rates, larger beat amplitude, and longer field potential durations compared to isogenic controls.

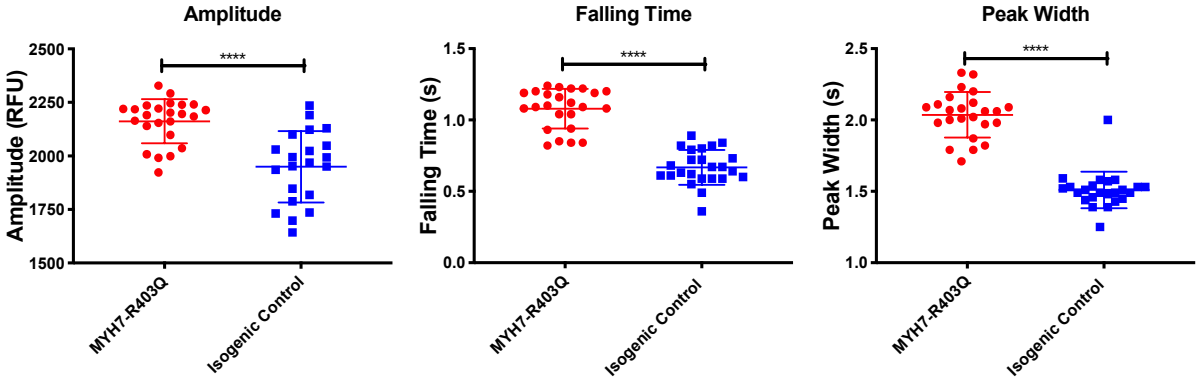
Link to HCM: Increased action potential duration can be a trigger for arrythmia.

MODELING THE IMPACT OF GENETIC VARIANTS: MYH7-R403Q IPSC-CARDIOMYOCYTES AS A MODEL OF HYPERTROPHIC CARDIOMYOPATHY

Calcium Flux using FLIPR (Ca^{2+} 5 Dye)





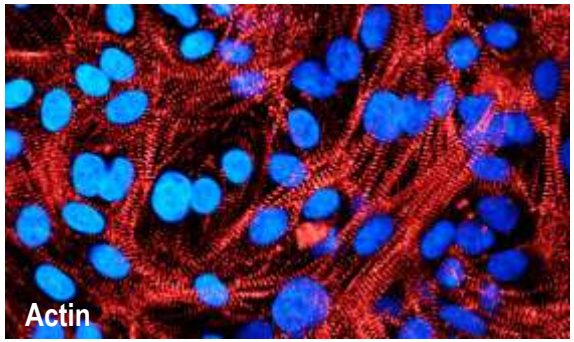


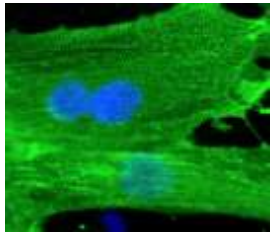
Conclusion: MYH7-R403Q demonstrate increased Ca^{2+} transient amplitude with reduced fall time suggesting impaired diastolic performance.

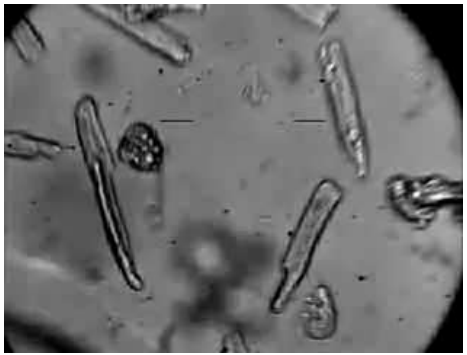
Link to HCM: Potential trigger for arrythmia, Ca^{2+} induced hypertrophic signaling

IPSC-DERIVED CARDIOMYOCYTES – OPPORTUNITY AND LIMITATIONS

iPSC-Derived Cardiomyocyte Monolayer







Opportunity

- Human Cells (patient derived or engineered)
- Near limitless source of material – well powered studies
- Exhibit functional properties (contractile and electrical) of human cardiomyocytes
- Evaluate the impact of variants across multiple genetic backgrounds in parallel
- Unable to acquire and culture human primary cardiomyocytes
- In-vitro – mechanistic evaluation in the absence of sympathetic tone

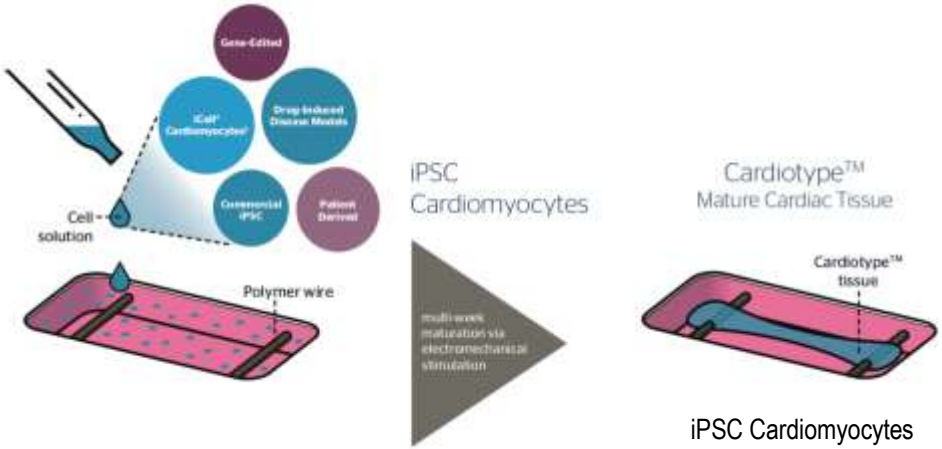
Limitations

- Disorganized sarcomere structure
- Immature phenotype
- Variation - Require multiple lines to define phenotype
- Isolated system (no fibroblasts)

EVALUATING THE IMPACT OF ACTIVIN A ON CARDIOTYPE TISSUES

3D CARDIAC TISSUES - TARA BIOSYSTEMS

Using human induced pluripotent stem cell derived cardiomyocytes, the patented BiowireTM II platform generates engineered adult human ventricular cardiac tissues, called CardiotypeTM tissues.



iPSC Cardiomyocytes and 10% Fibroblasts

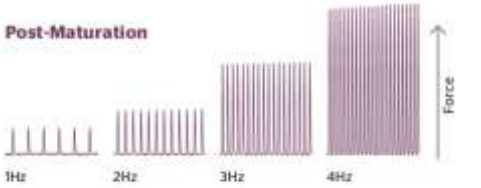
TARA Bioscience 3D Cardiac Tissue

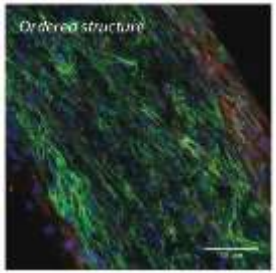
EVALUATING THE IMPACT OF ACTIVIN A ON CARDIOTYPE TISSUES

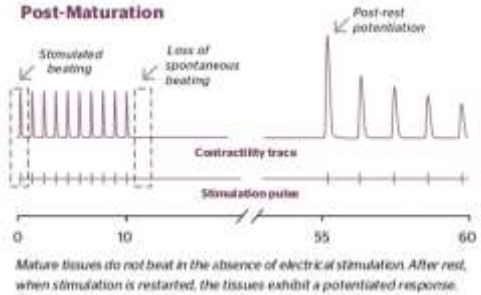
3D CARDIAC TISSUES - MIMIC ADULT MYOCARDIUM

The BiowireTM II platform biomimetically matures the tissues using electromechanical stimulation resulting in CardiotypeTM tissues that exhibit characteristic hallmarks of **adult human ventricular myocardium**.

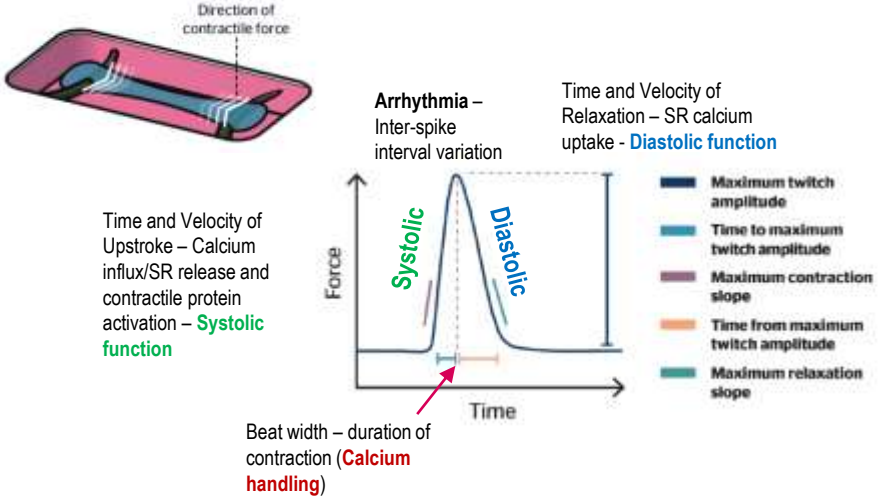
Properties of Adult Myocardium







- The positive force-frequency relationship is a property intrinsic to adult human myocardium.
- Post-rest potentiation and no spontaneous beating, properties intrinsic to adult human myocardium.

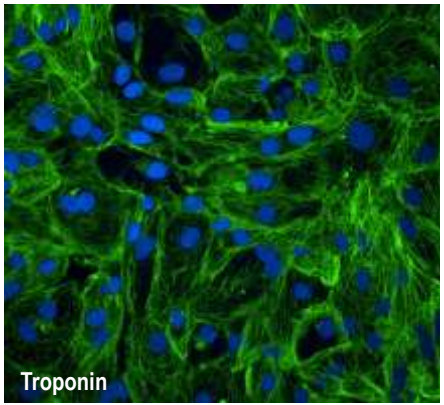


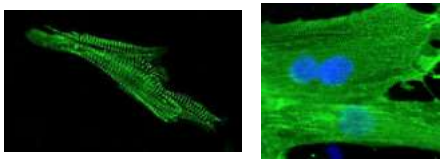
Functional Data Collected from CardioType Tissues

MOVING BEYOND 2D - 3D CARDIAC TISSUE AS AN IN-VITRO MODEL SYSTEM

TARA BIOSCIENCE – COLLABORATION TO EVALUATE MYH7 (R403Q) VARIANT

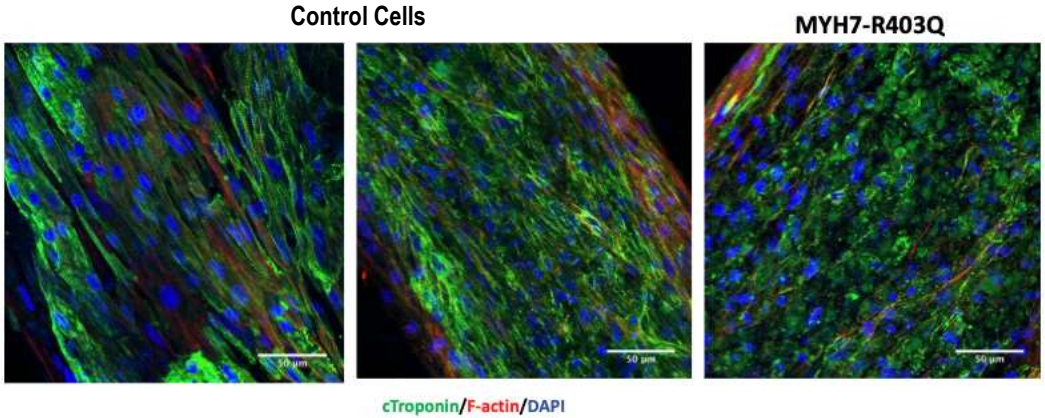
iPSC-Cardiomyocytes (2D)





Isolated human cardiomyocytes

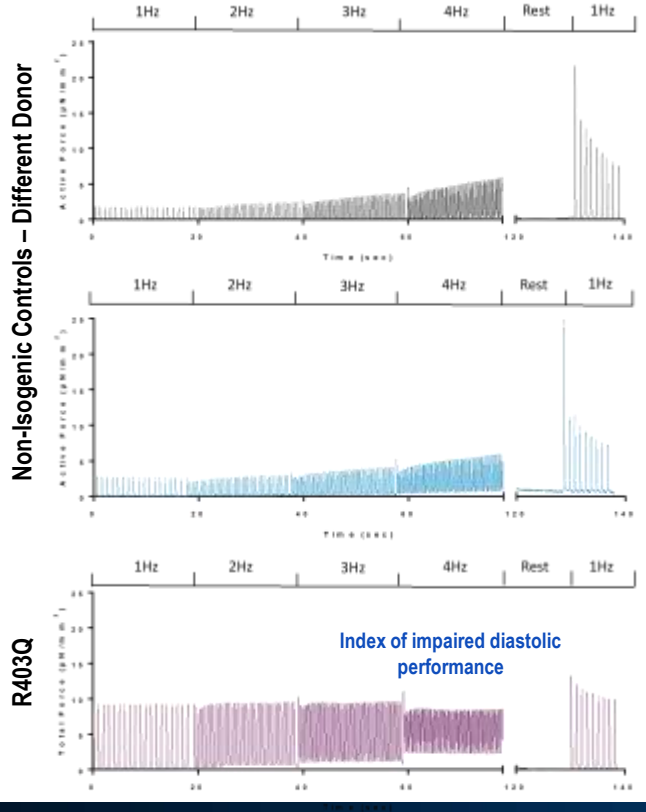
MYH7-R403Q in Biowire™ II 3D Tissue Platform

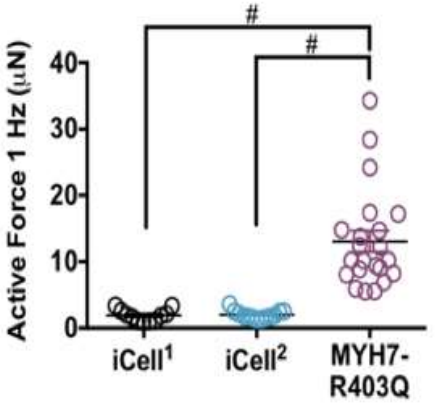


MYH7-R403Q 3D tissues have decreased structural alignment compared to control 3D tissues (cTroponin in green, F-actin in red, nuclei in blue).

MOVING BEYOND 2D - 3D CARDIAC TISSUE AS AN IN-VITRO MODEL SYSTEM

TARA BIOSCIENCE – COLLABORATION TO EVALUATE MYH7 (R403Q) VARIANT





Consistent with 2D impedance data

Conclusion: MYH7-R403Q 3D tissues display a negative force frequency relationship in response to external increasing electrical stimulation (1 to 4Hz).

MYH7-R403Q 3D tissues display a higher active force at 1Hz external stimulation.

Platform provides an opportunity to further profile targets of interest based on 2D findings

CONCLUSION: MYH7(R403Q) VS. ISOGENIC CONTROL

- Cardiomyocytes generated using iPS cells derived from a patient carrying the MYH7-R403Q variant maintain a hypertrophic phenotype in-vitro.
- Increased impedance amplitude suggest enhanced contractility frequently observed in hypertrophied cardiomyocytes as a compensatory mechanism.
- Increased contraction beat width and fall time suggest impaired diastolic performance and sarcoplasmic reticulum Ca^{2+} uptake.
- The diseased phenotype observed is maintained at both intrinsic and paired beat rates.
- Reduced FFR was observed in 3D Biowire tissues.

These data suggest that distinct differences in iPSC-CM from patients carrying diseased associated genetic variants can be observed *in-vitro.*

Functional studies using iPSC-CM may thus be used to define mechanisms involved in cardiovascular diseases and screen for novel therapeutics.