BRIGHAM AND
WOMEN'S HOSPITAL

HARVARD
MEDICAL SCHOOL

Molecules, Mutations, and Mechanisms of Heart Failure

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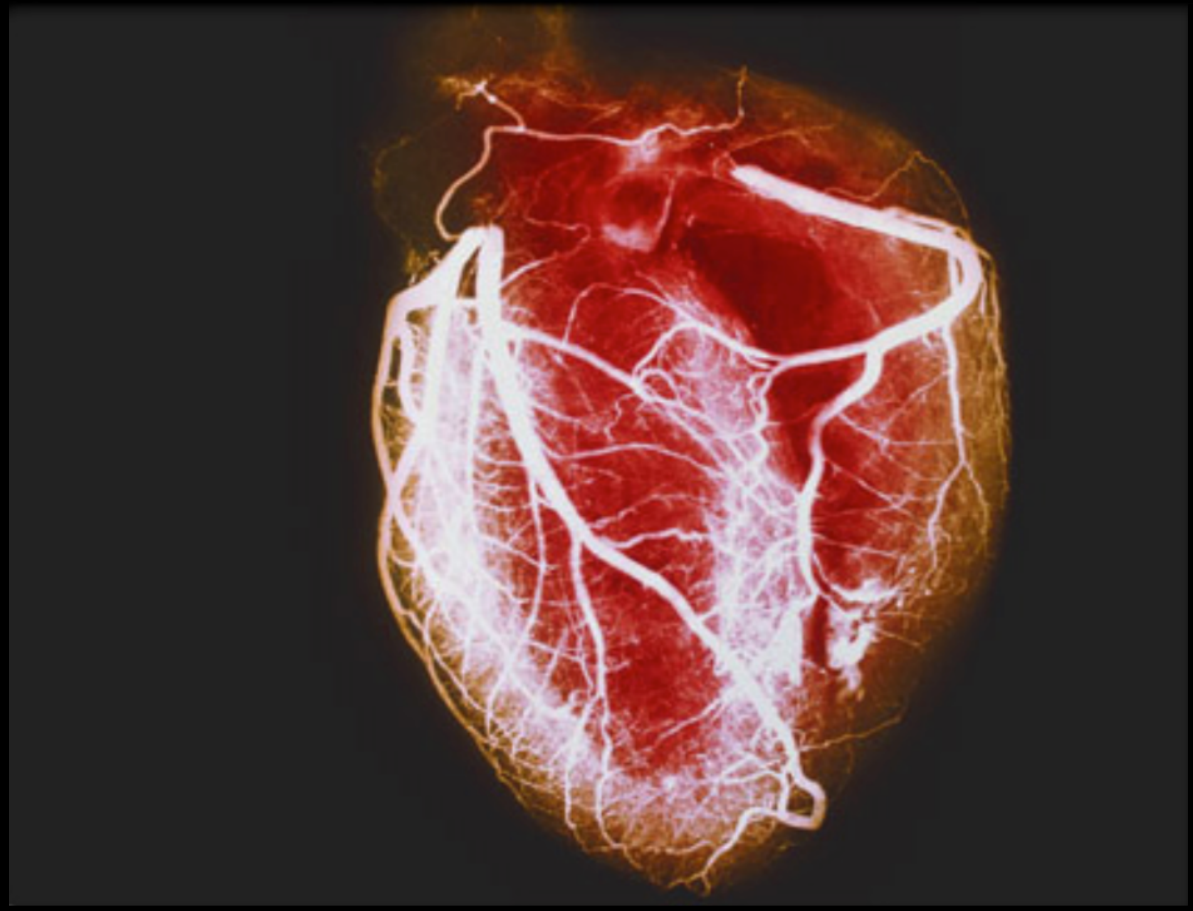
CES is a founder and owns shares in Myokardia Inc.,
a startup company that develops therapeutics that target the sarcomere



Heart Failure:

Affects ~5 million American; 500,000 new cases/year

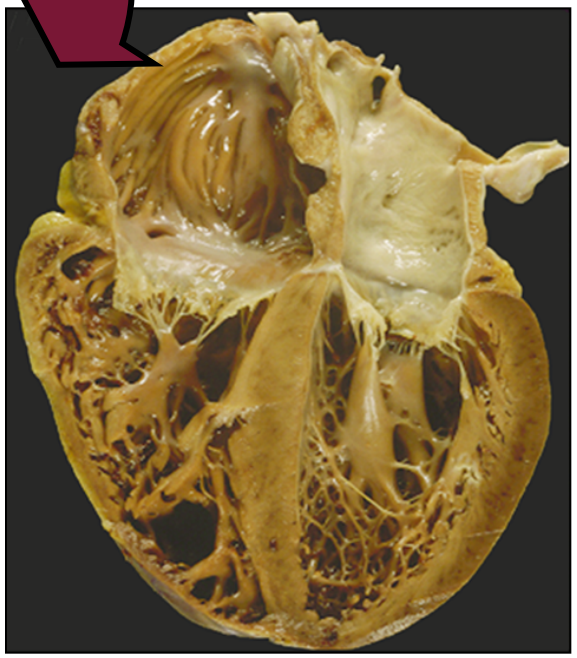
Expends $17.8 billion annually

Prognosis: 75% Die within 8 Years

Causes: Atherosclerosis, Hypertension, Valvular, Idiopathic

Sarcomere Proteins
Lysosome-associated Membrane Protein-2
Gamma-2 Subunit AMP-dependent Protein Kinase
Desmin
Trans-Thyretin
Alpha acid glucosidase
Alpha-D galactosidase
Myozenin-2
Actinin

Sarcomere Proteins
Lamin A/C
Z-disc Proteins
Intermediate Filaments
Dystrophin/Glycoproteins
RNA-binding motif protein 20
ATP-binding Cassette
Heat Shock Proteins
Presenilin
αB Crystallin

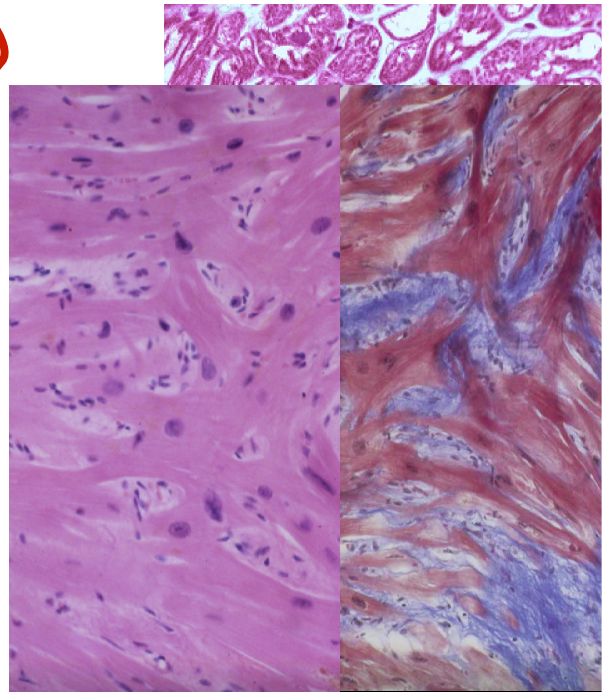


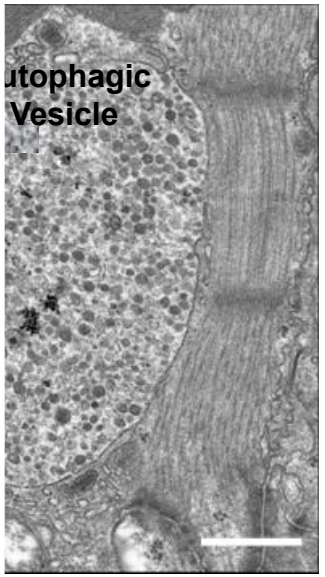
Distinct Clinical Outcomes in Genetic Hypertrophy

Gene	Gender	Expression & Outcomes
Sarcomere	M+F	Adolescent & Beyond Atrial Fibrillation in ~25% Variable SCD & HF Risk
PRKAG2	M+F	Adolescent Onset Progressive AVB/Pacemaker Uniform Need for Pacemaker
LAMP2	M>>F	Massive LVH, Arrhythmias Often has Systemic Disease Uniform Early Death
GLA	M>>F	Late Onset HF, Renal Disease Median Survival = 50 yrs Genetic Rx: α-Galactosidase

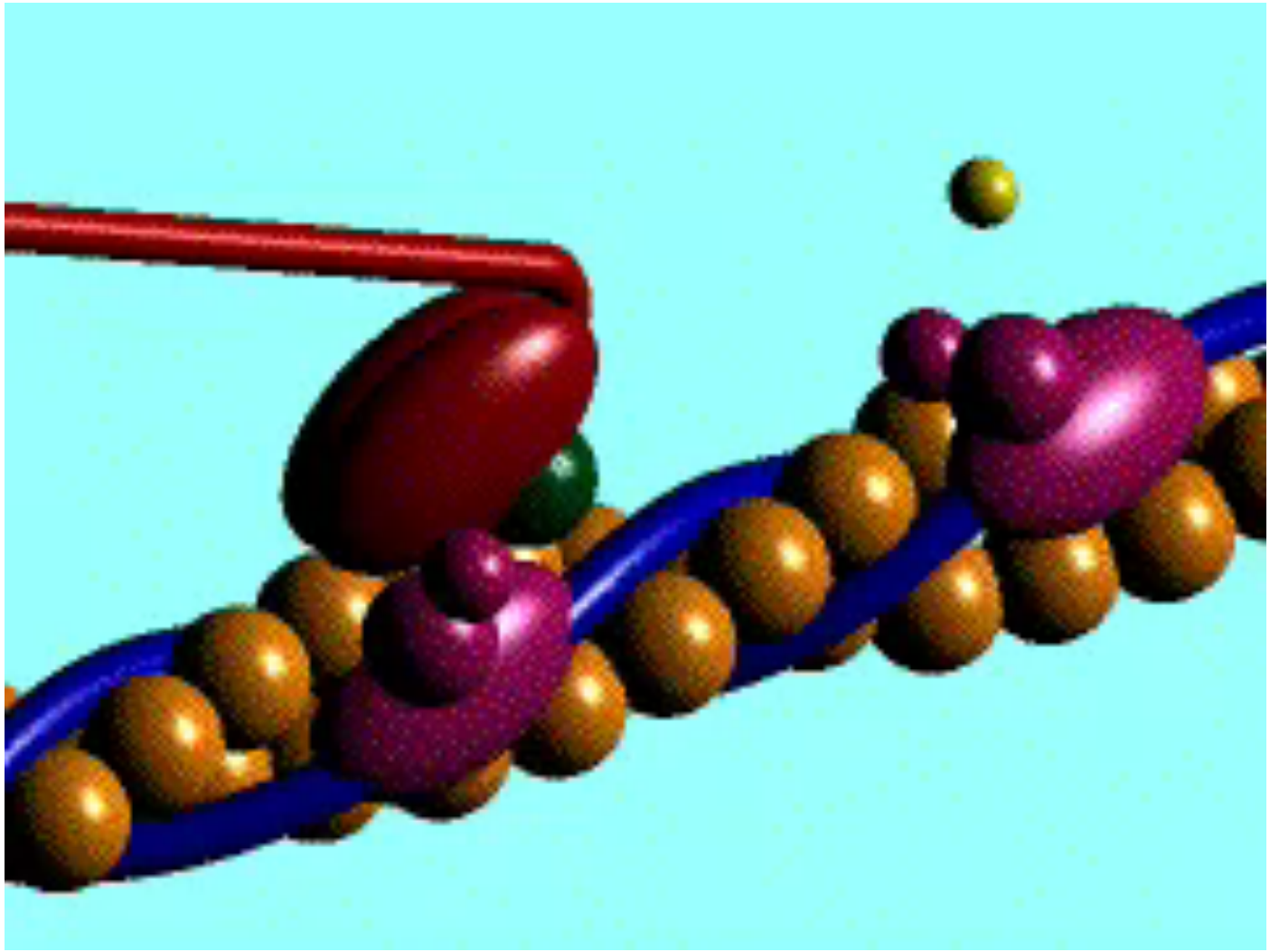
Genetic Causes of "Unexplained" Hypertrophy

Force	Metabolism	Clearance
MYH7, MYBPC3	*PRKAG2:* Glycogen	*LAMP2:* Cellular Debris
TNNT2, TNNI3	*GAA* (Pompe): Glycogen	*DES*: Misfolded Protein
MYL3, MYL2	*GLA (Fabry):* Glycosphingolipids	*TTR:* Amyloid
TPMI, ACTC		
ACTN, MYOZ2		



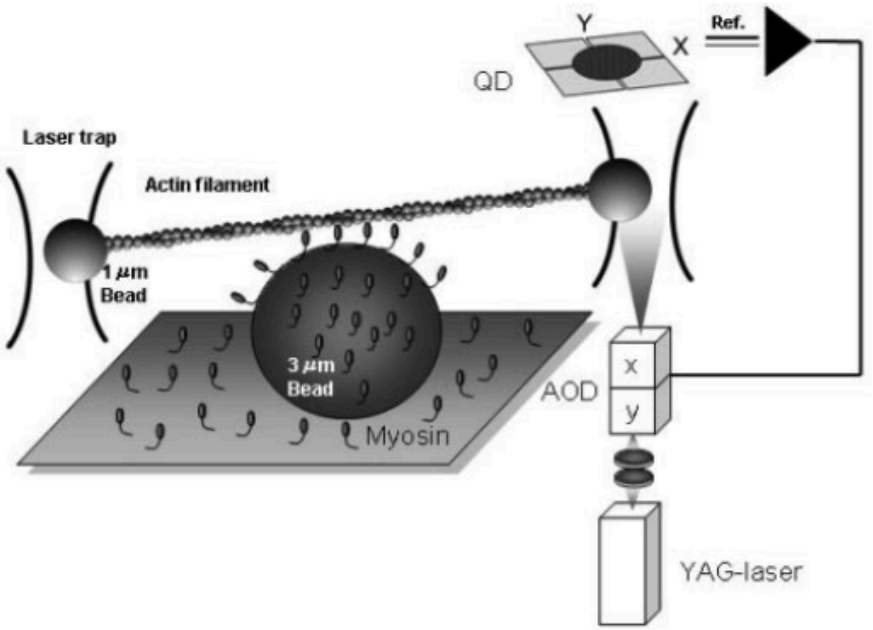


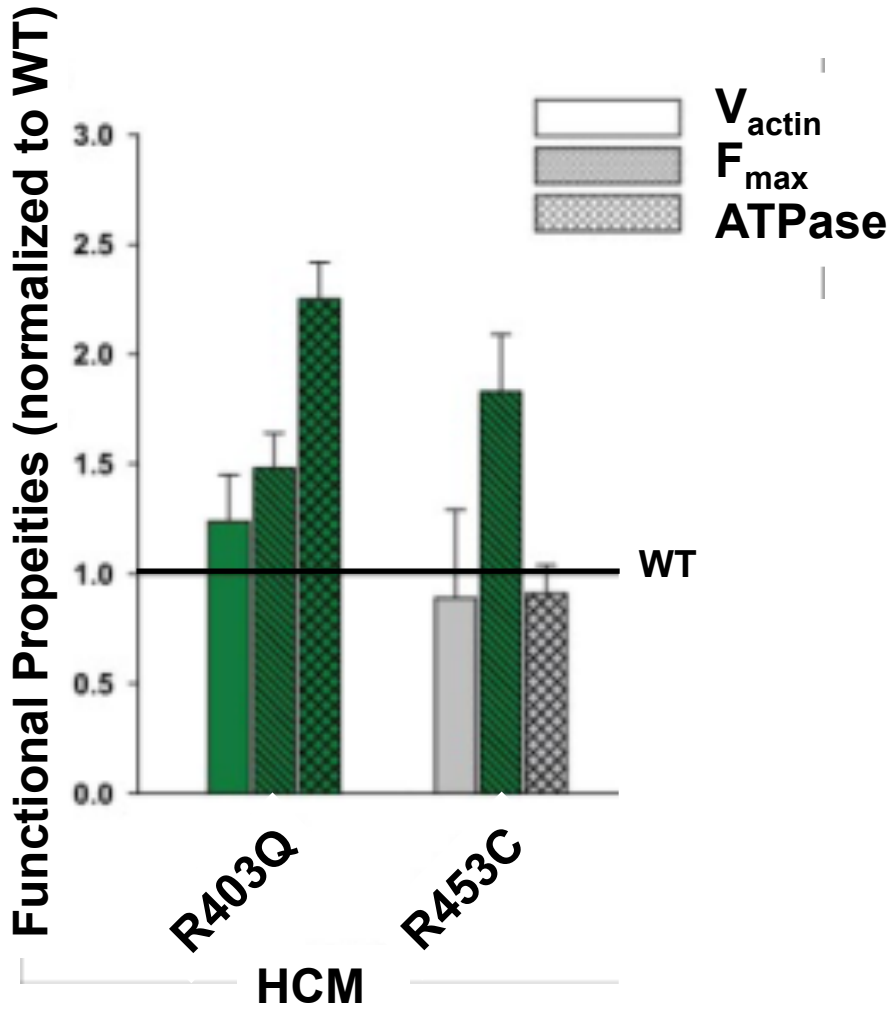
Sarcomere Gene Mutations:
Expected to Perturb Contractile Function



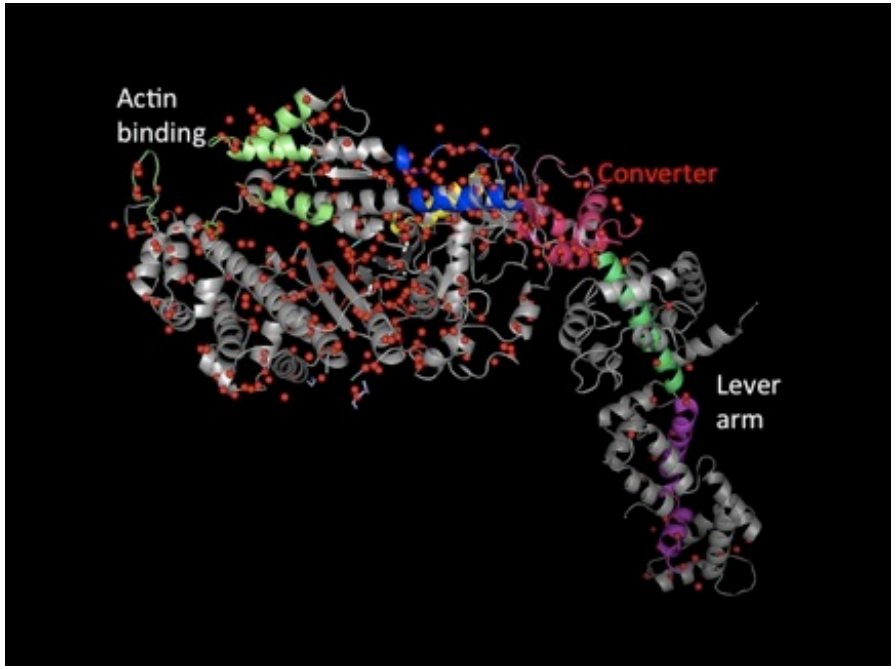
Biophysical Impact of HCM Myosin Missense Mutation

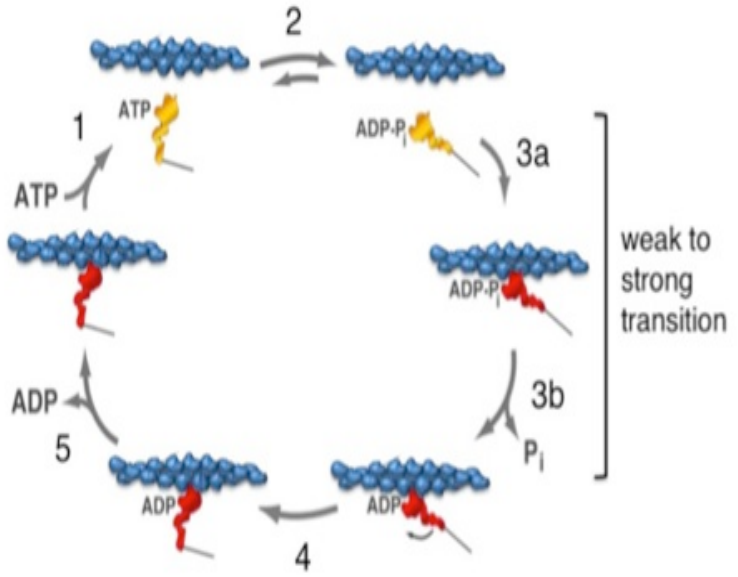
De Bold et al, Am J Physiol 2007

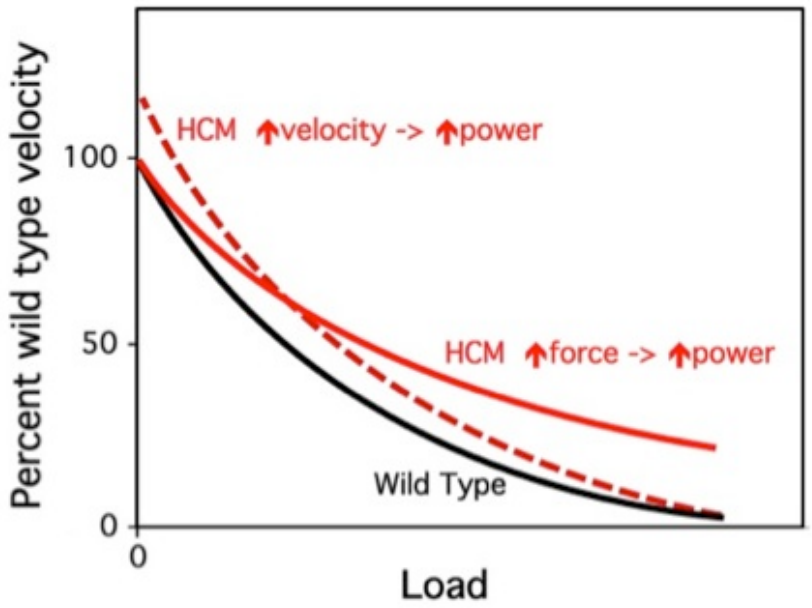


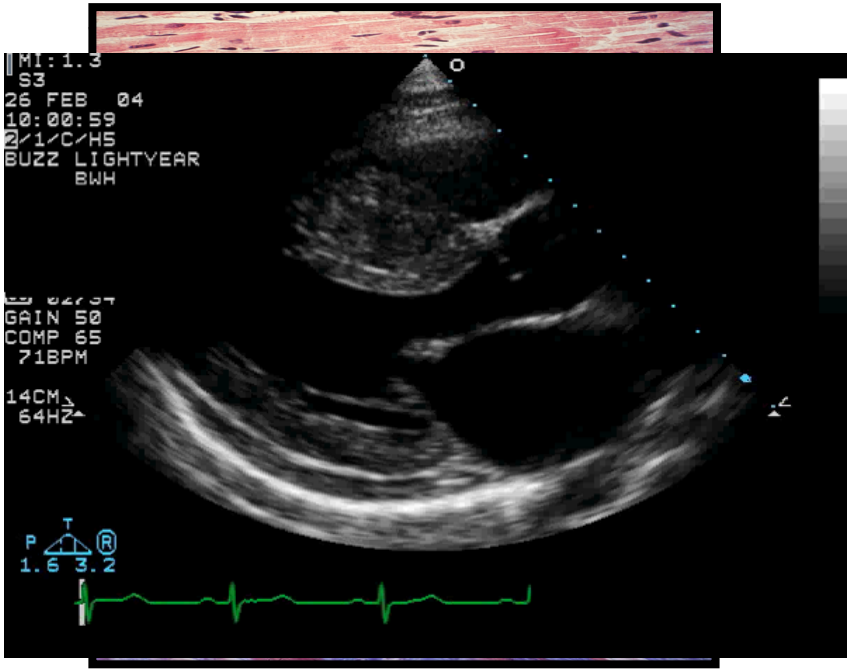


HCM Myosin Mutations Increase Sarcomere Power

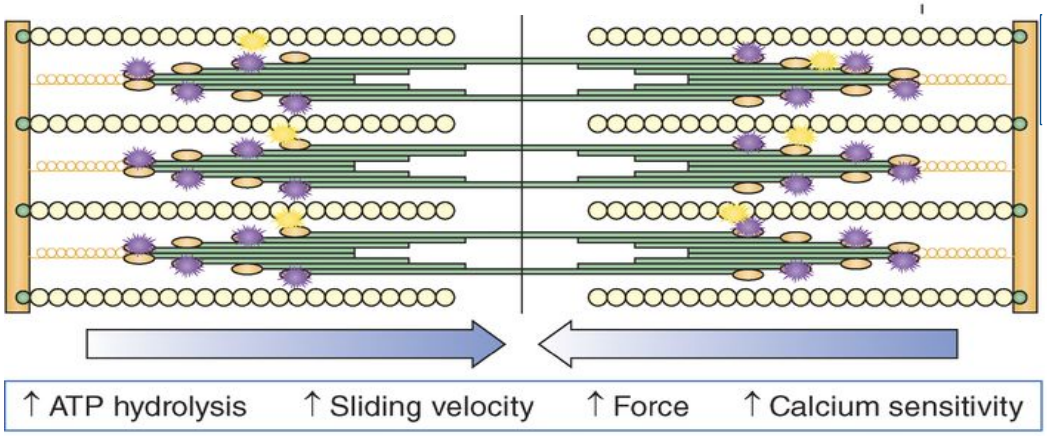




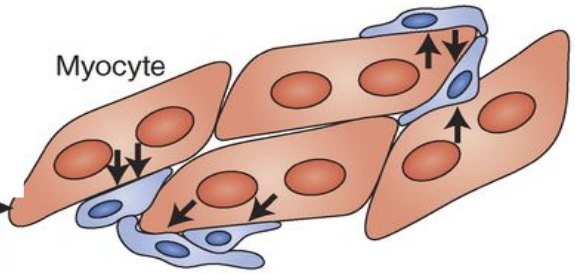




Sarcomere Missense Proteins

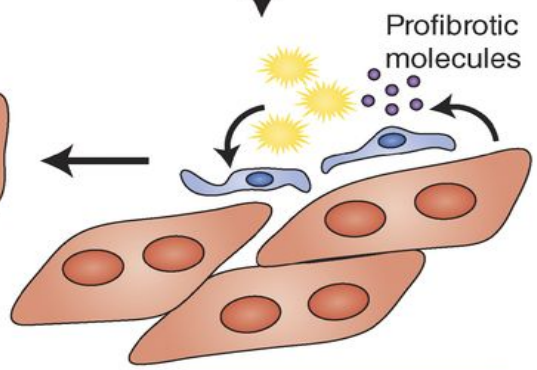


↑ Biomechanical power

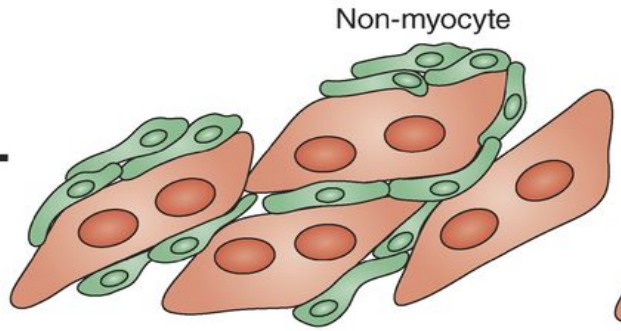


↑ Tgf-β
↑ Ca^{++} signals

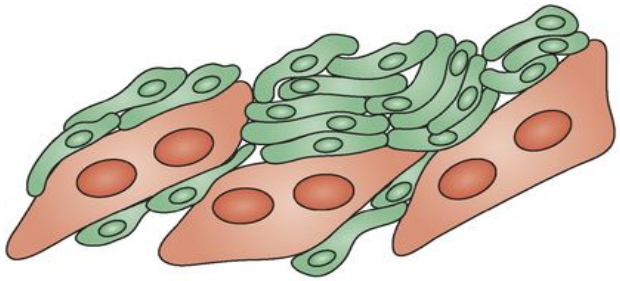
Therapeutic Trials in HCM:
DELIGHT
VANISH



Non-myocyte activation

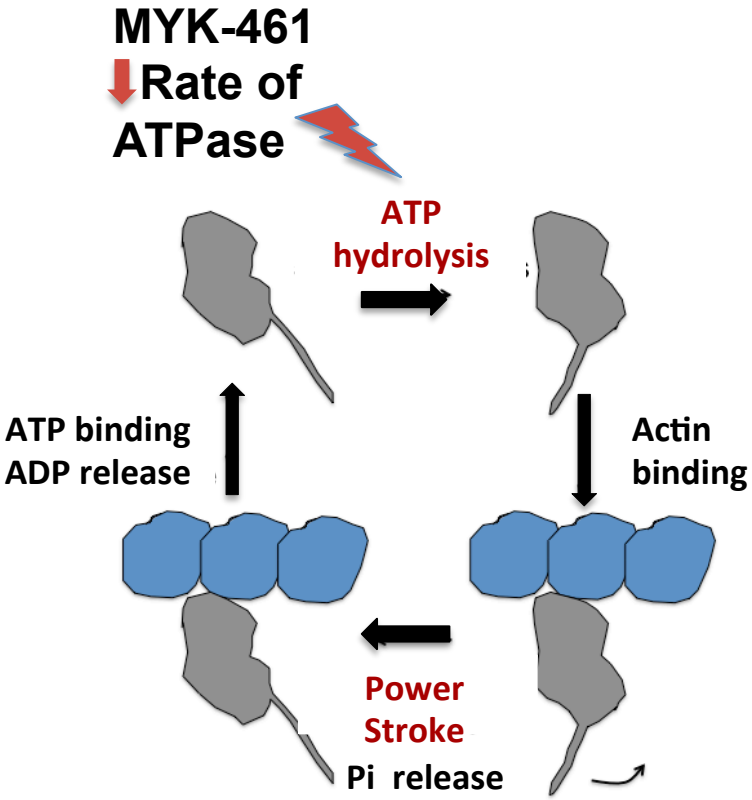


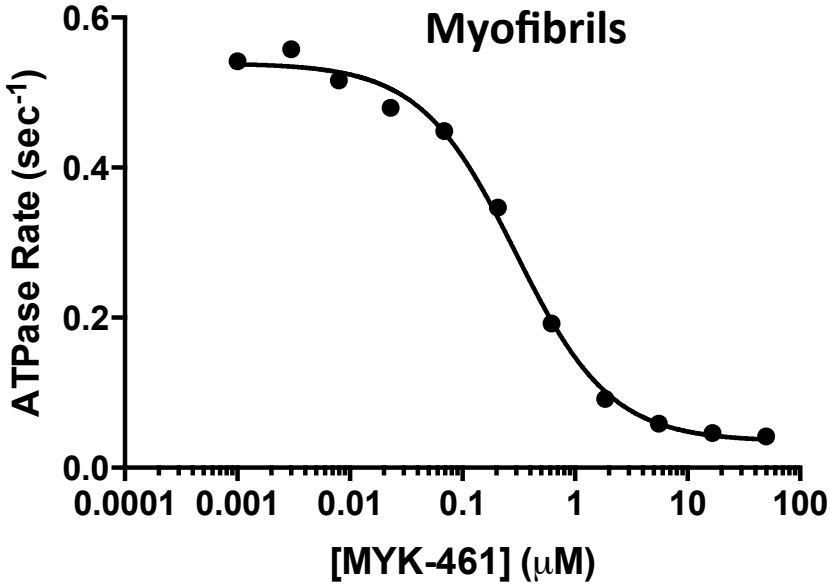
Non-myocyte proliferation and matrix expansion

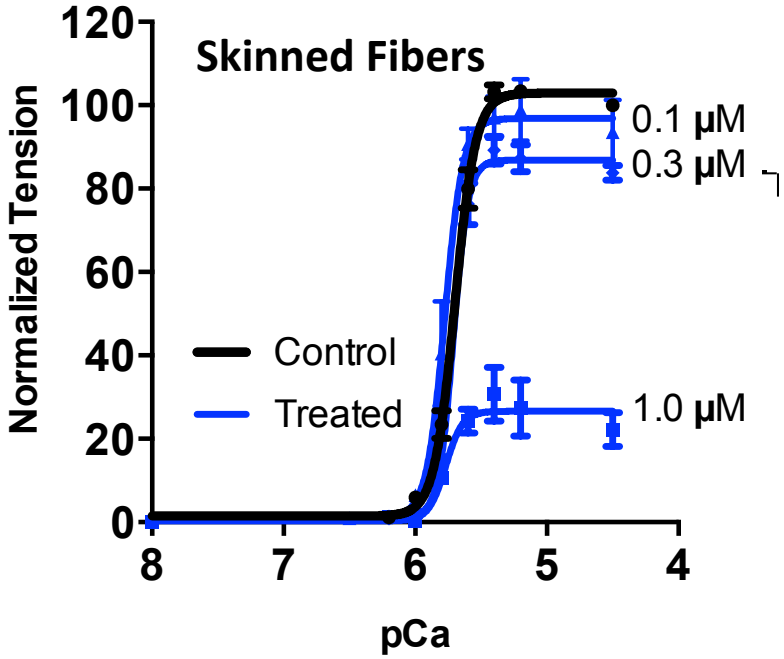


↑ Myocyte death
↑ Focal and interstitial fibrosis

Pharmacologic Reduction of Sarcomere Power



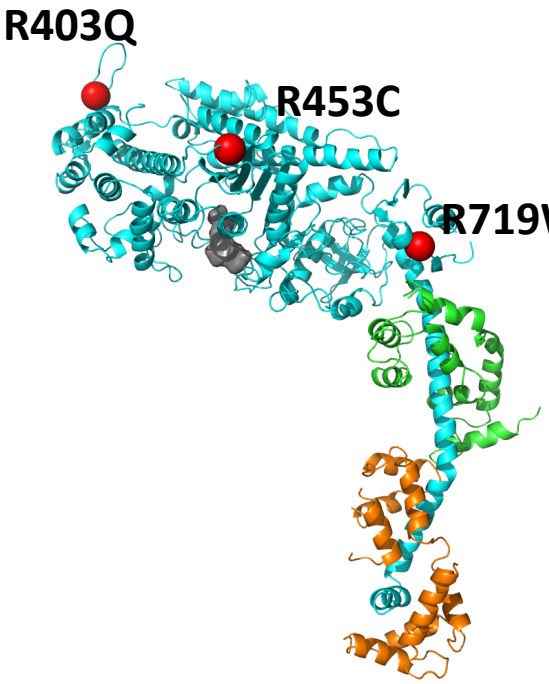


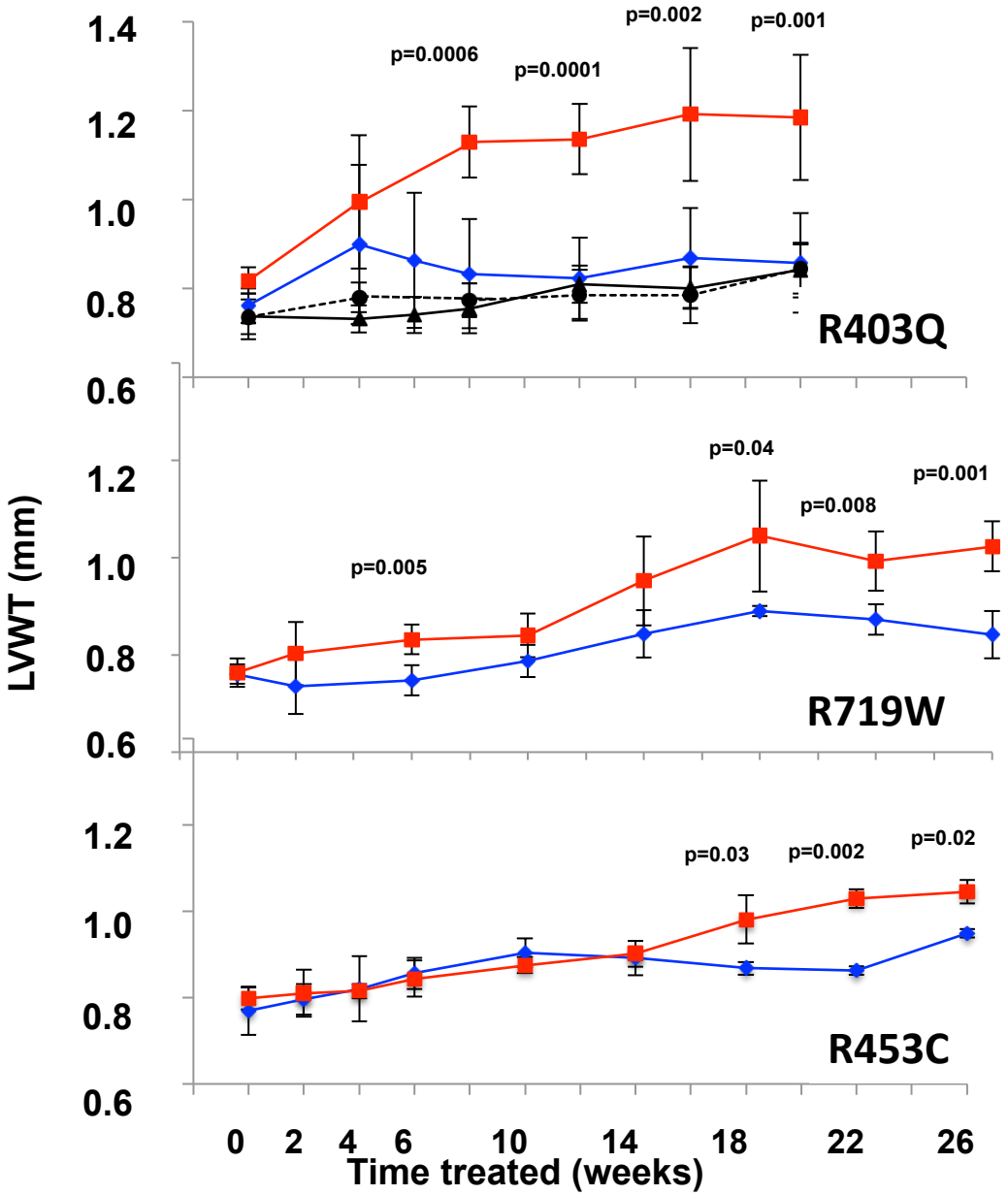


Green et al, Science 2015

MYK 461 (2.5mg/kg): 10-15% ⬇ Fractional Shortening

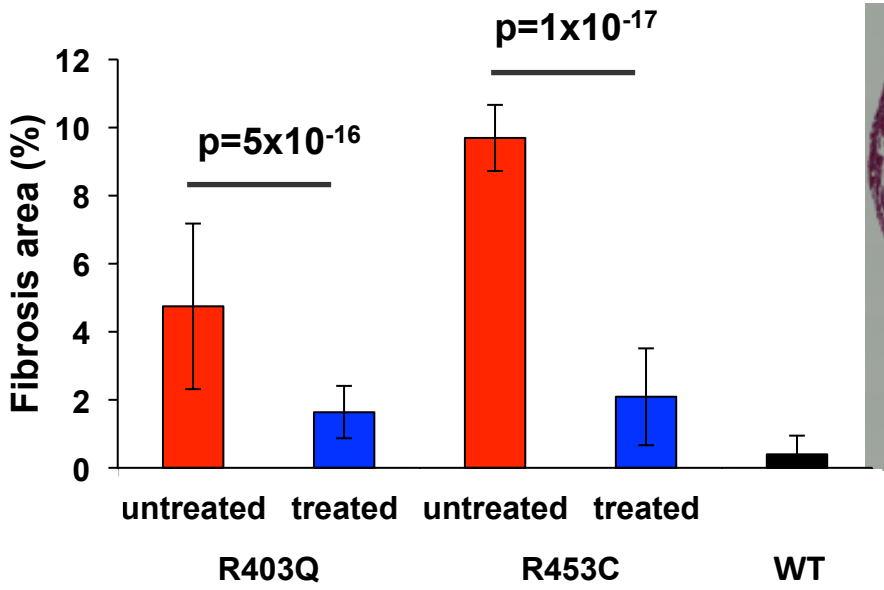
Preclinical HCM Mice (Treated Untreated) & WT (–Treated, ---Untreteated)

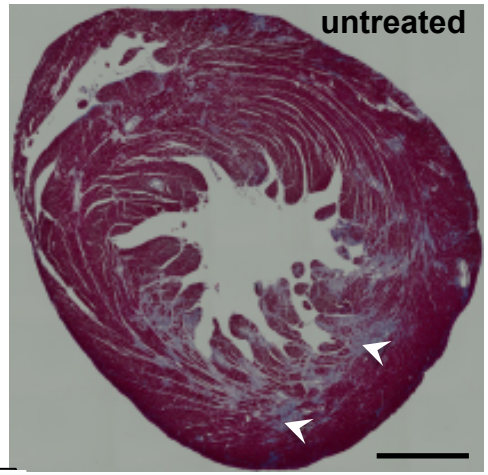


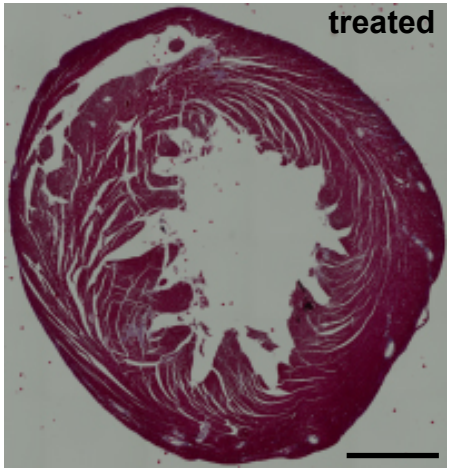


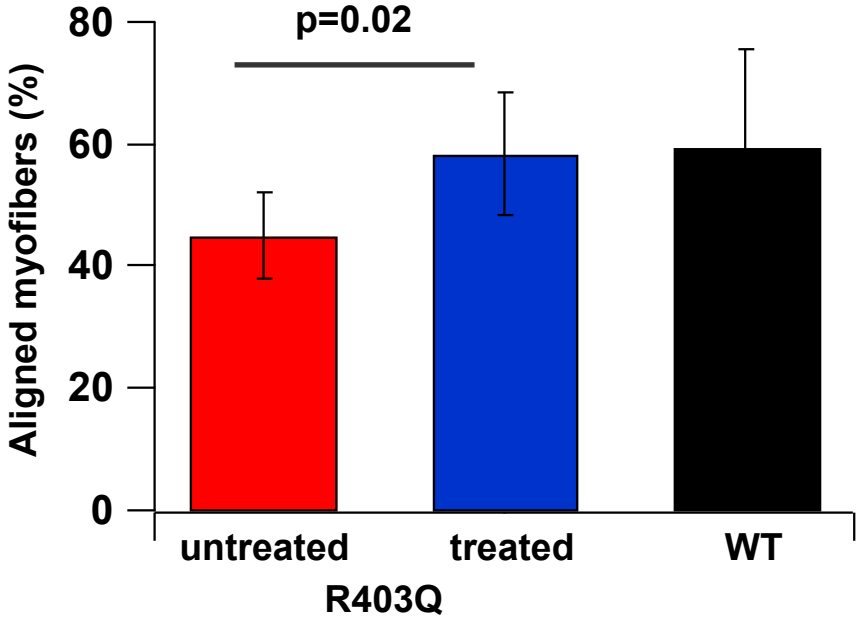
Green et al, Science 2015

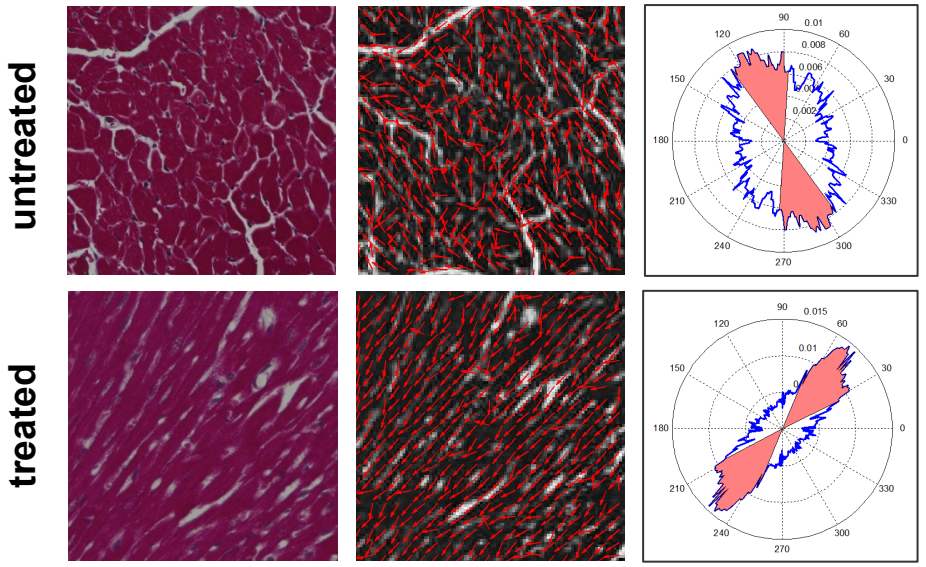
Reduced Fibrosis and Disarray





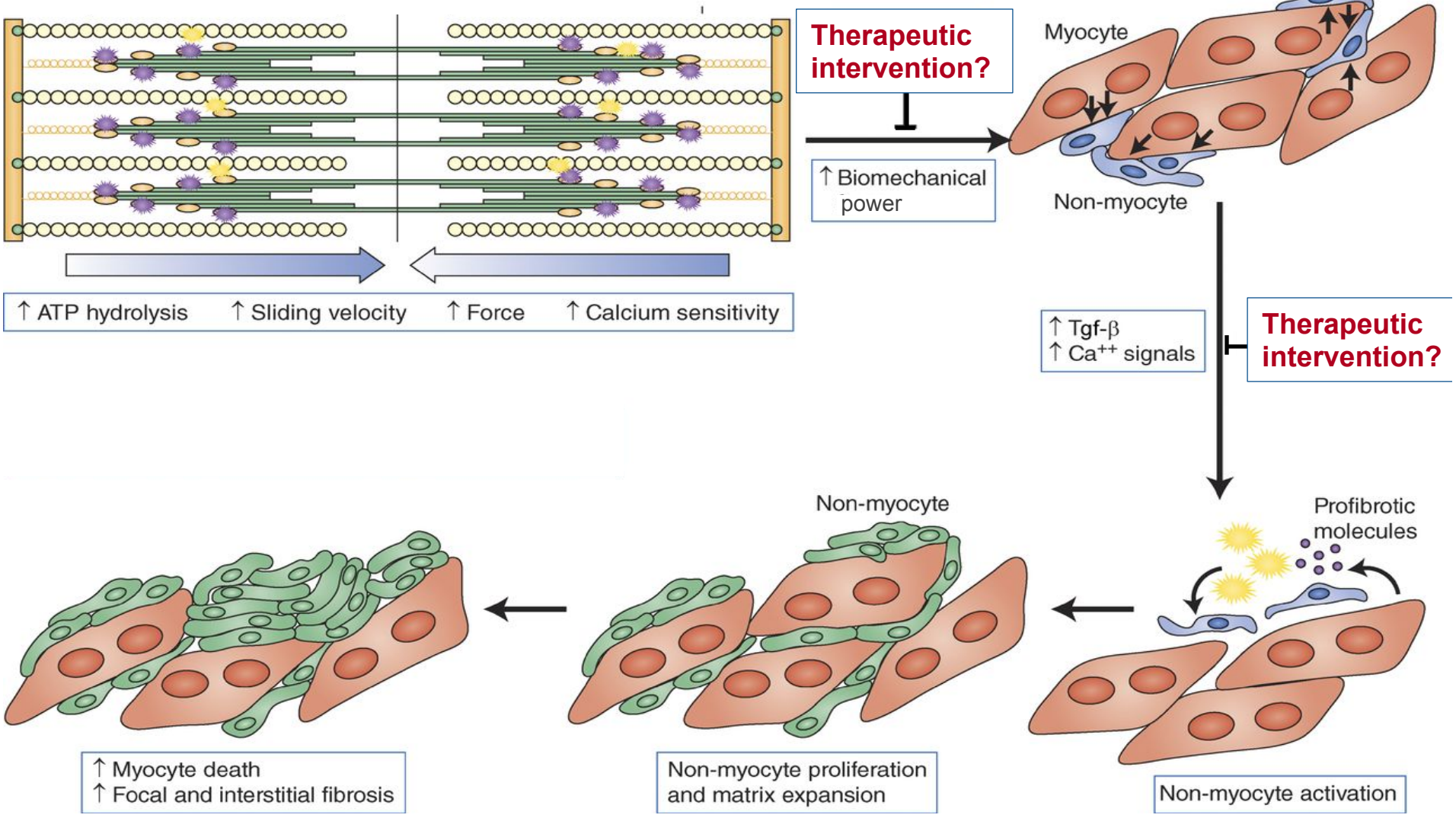




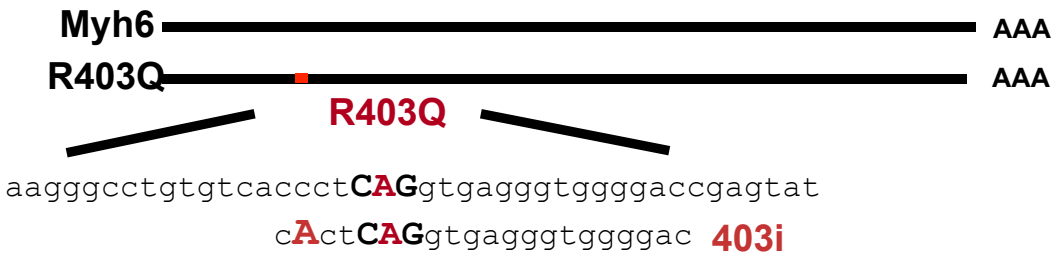


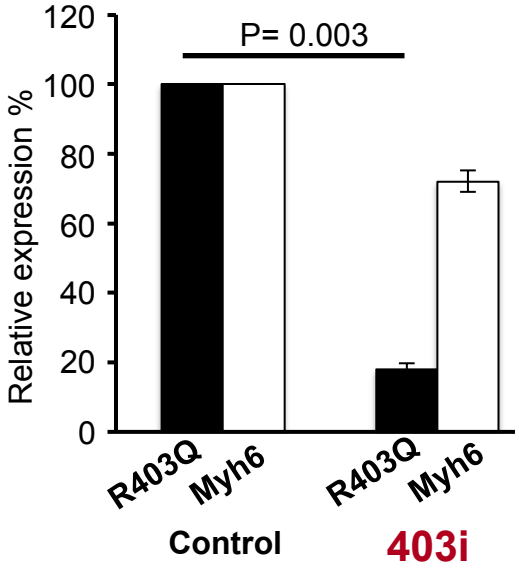
Green et al, Science 2015

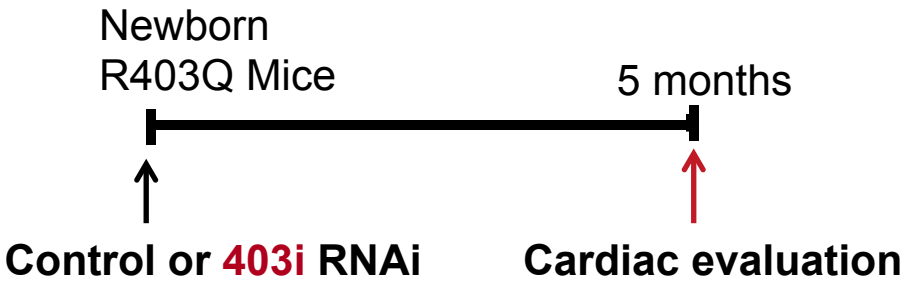
Sarcomere Missense Proteins



Selectively Silence HCM Transcripts

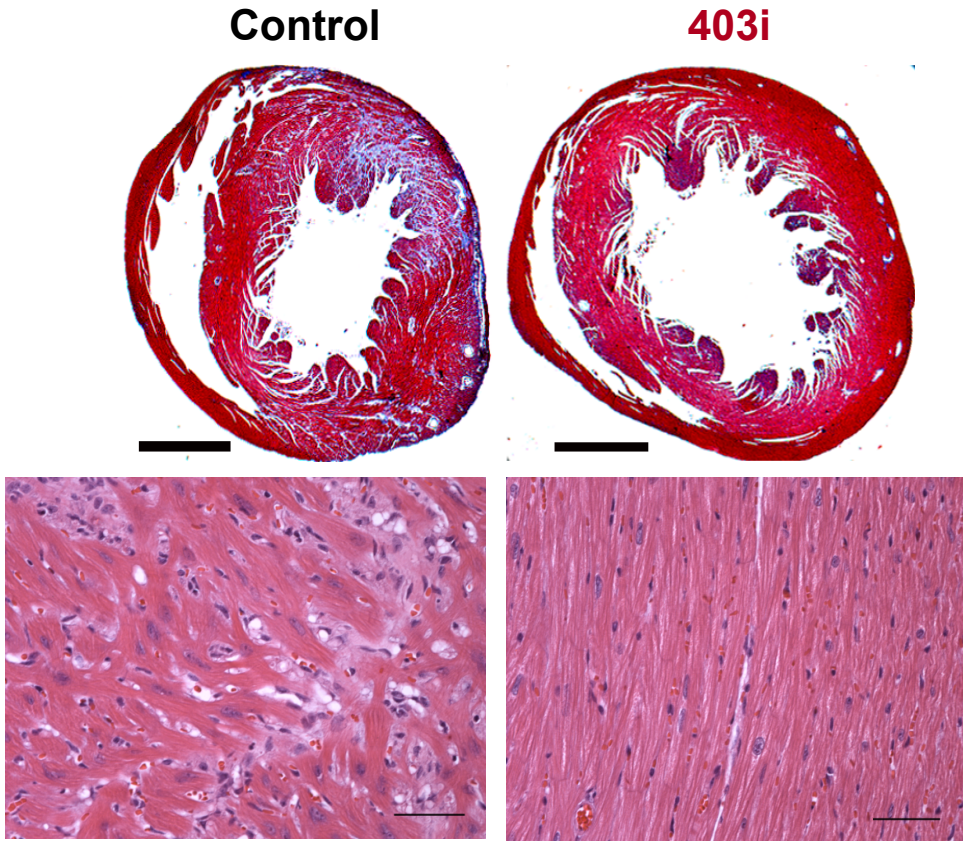


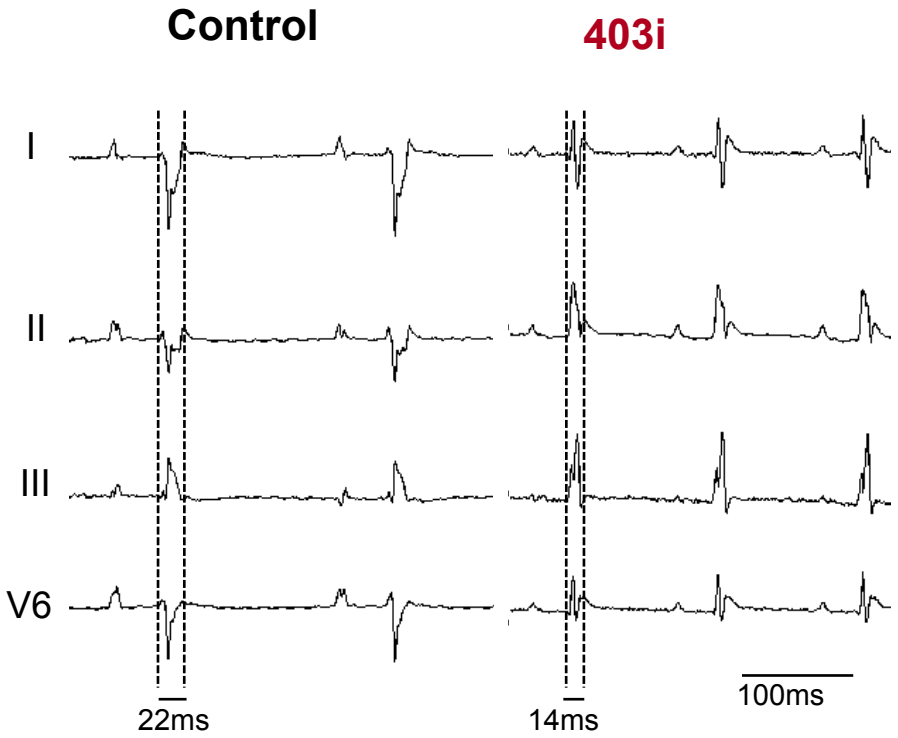


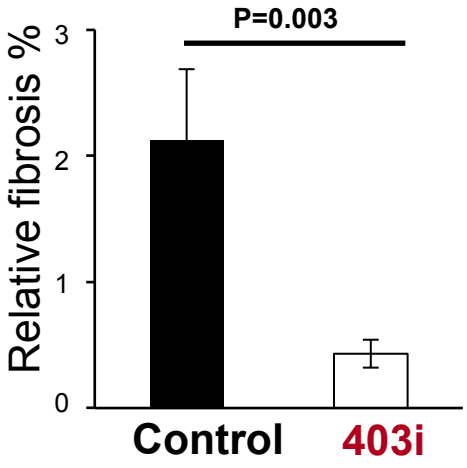


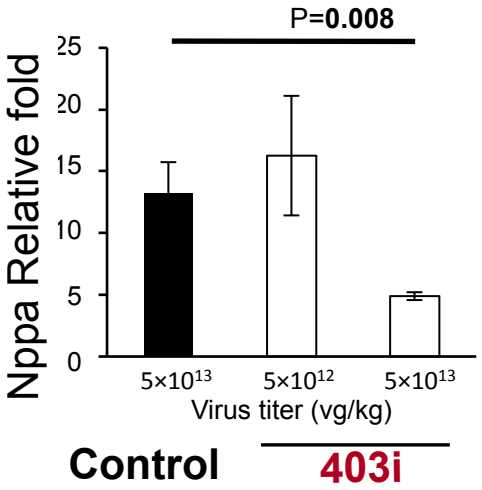
Mutant	RNAi	Age	LVDD	P	LVWT	P	FS (%)	P
403	Control	8 wks	2.53 ± 0.41		1.52 ± 0.25		32.68 ±	
403	403i	8 wks	3.04 ± 0.21	0.01	0.84 ±0.10	1.9E-05	40.37 ±	0.04
WT	None	6	3.75 ± 0.28		0.74 ± 0.05		30.01 ±	

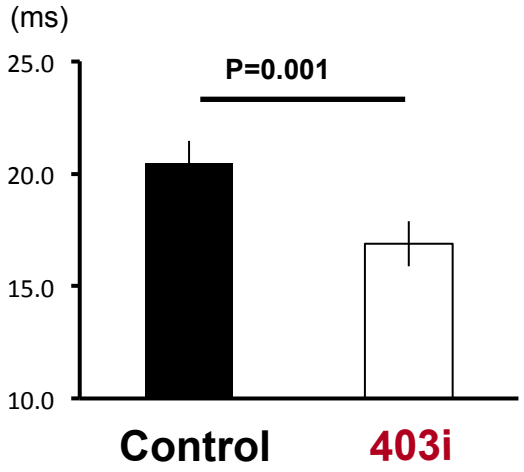
Jian et al, Science 2013



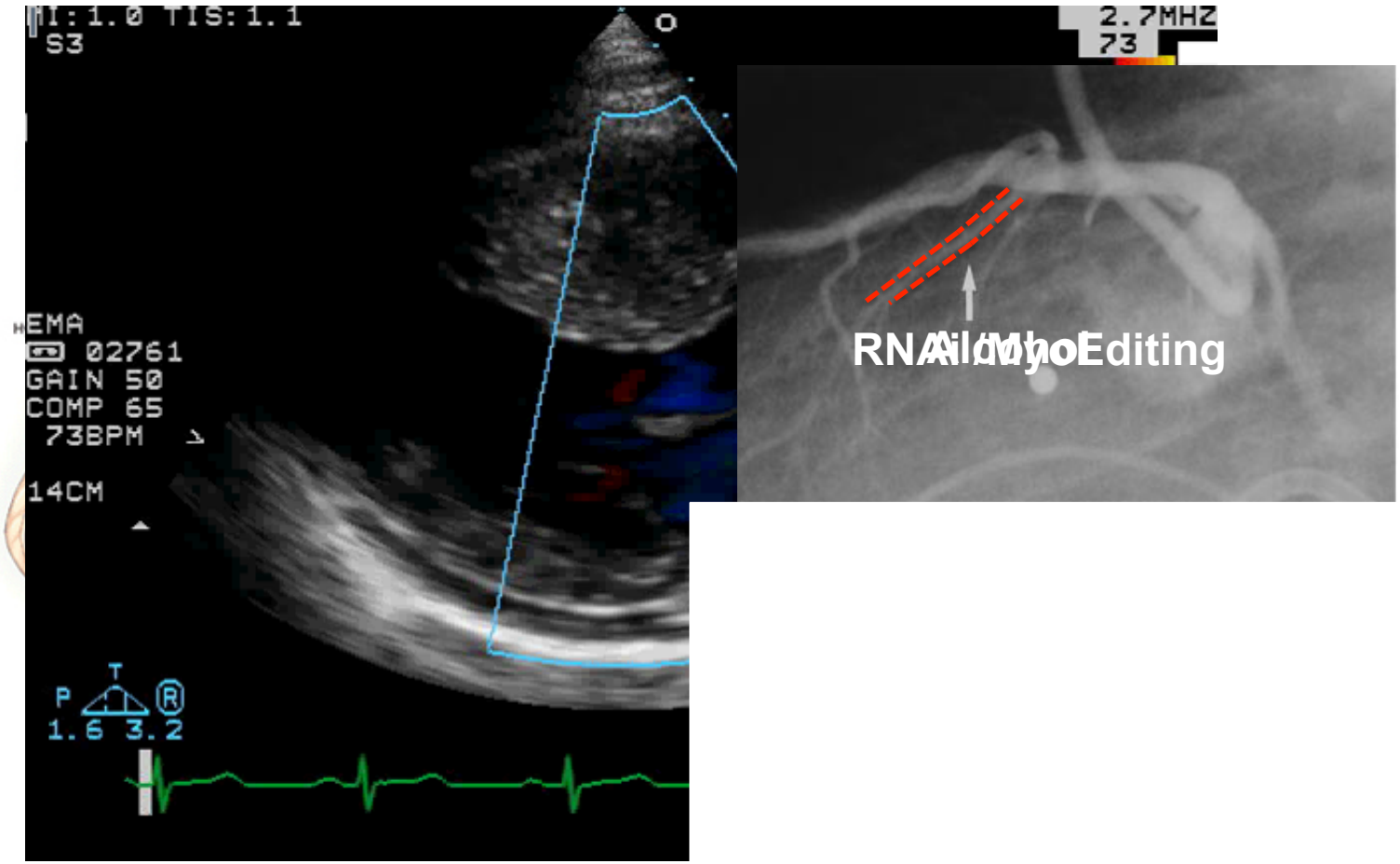




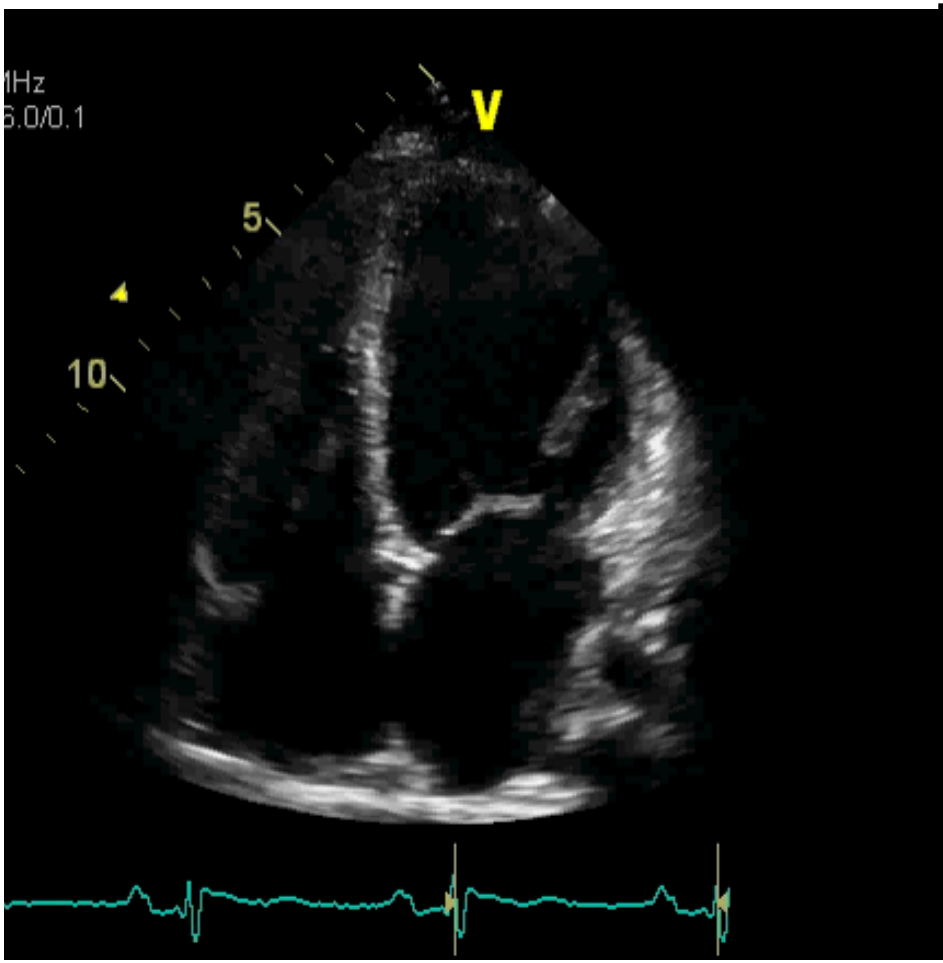


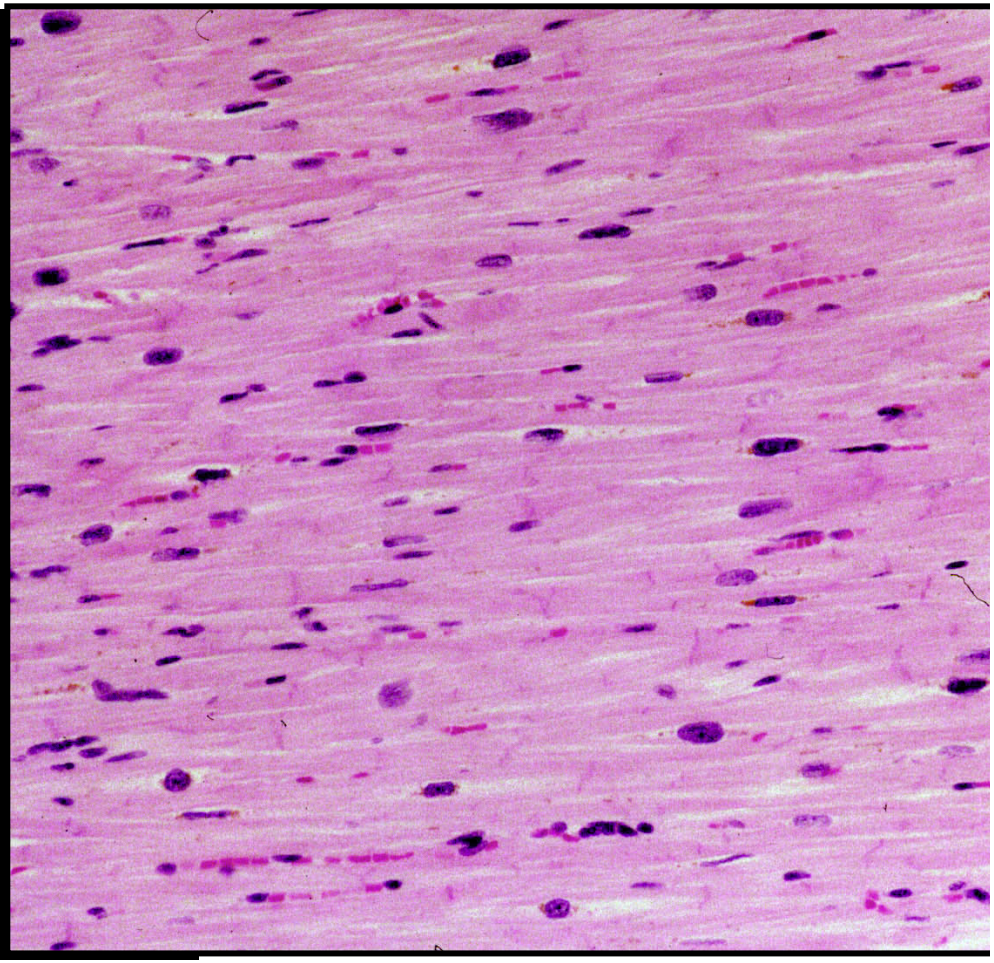


Jian et al, Science 2013


TI: 1.0 TIS: 1.1
S3
2.7MHZ
73
EMA
02761
GAIN 50
COMP 65
73BPM
14CM
P
T
R
1.6 3.2
RNA Editing

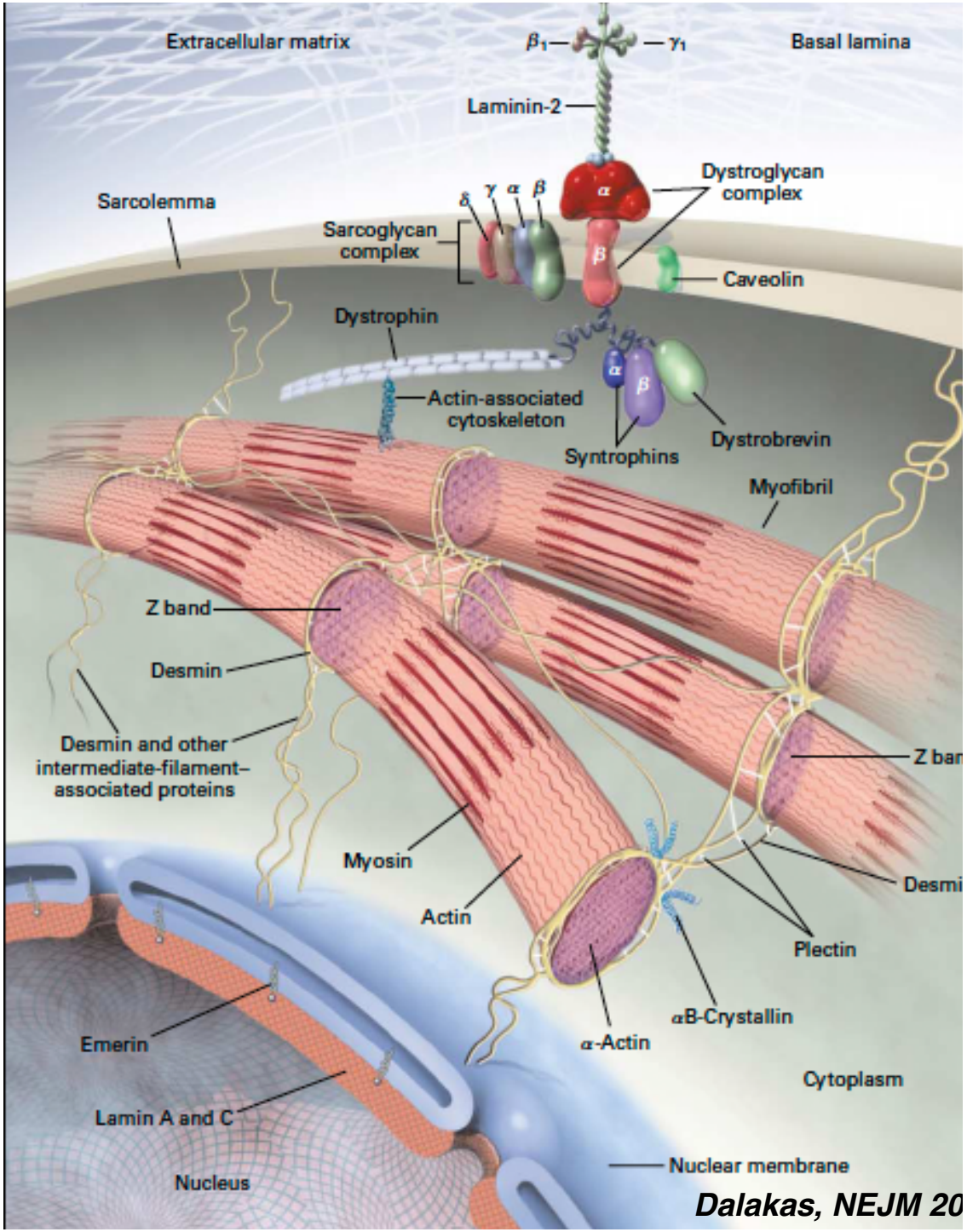
Dilated Cardiomyopathy: Morphology/Histopathology Unable to Distinguish Etiology



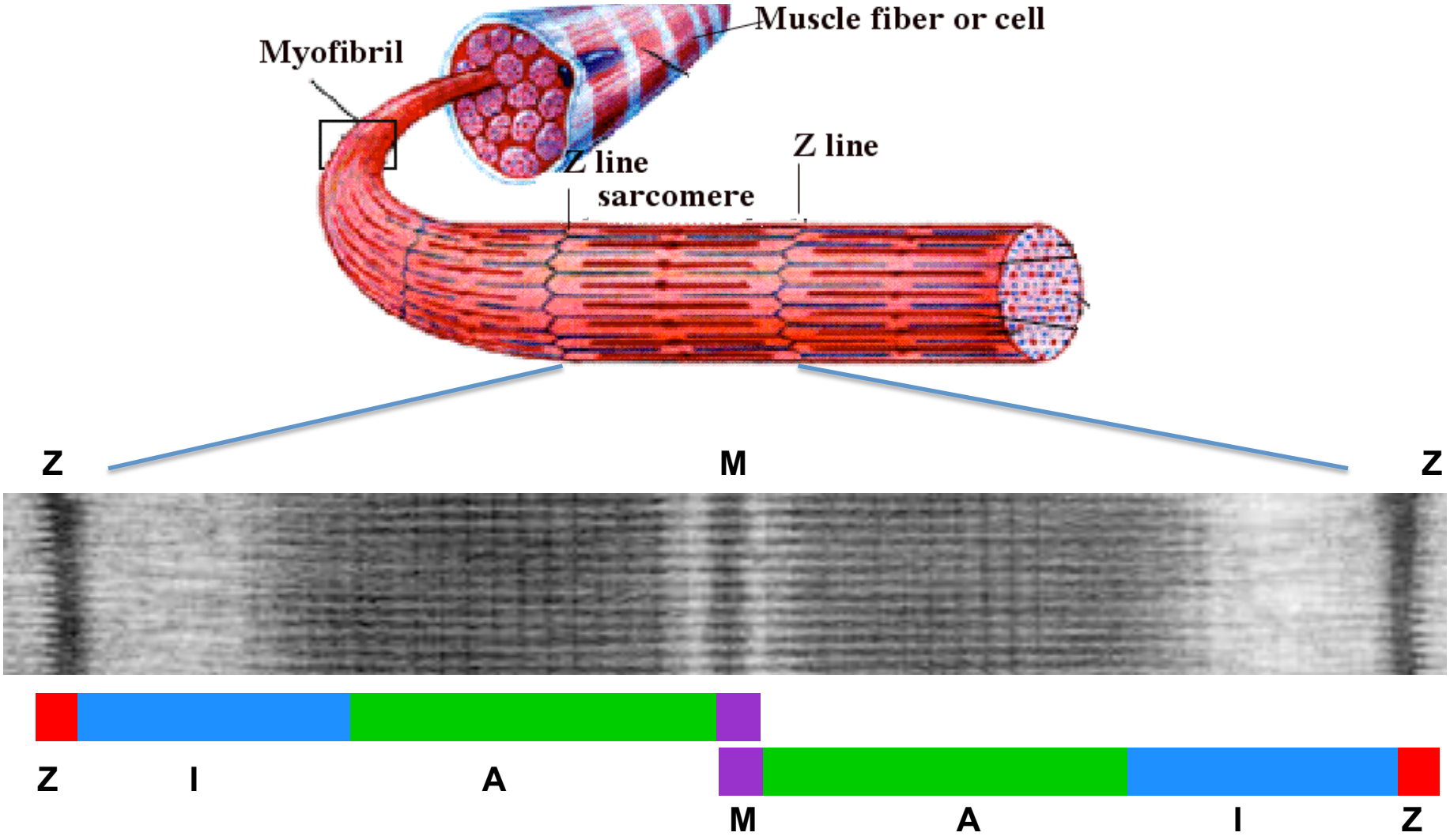


Genetic Causes of Dilated Cardiomyopathy

ATP-binding Casette
Apolipoprotein A-1
αB Crystallin
Dystrophin/Glycoproteins
Fukutin
Intermediate Filaments
Nuclear Lamina
Presenlin-1,2
RNA-binding motif protein 20
Sarcomere Proteins
Tafazzin
Transcriptional Regulators
Voltage gated Na+ channel
Z-disc Proteins



Titin (TTN)



- Giant protein spans ½ Sarcomere (1 μm)
- Contains 34,350 amino acids encoded in >100,000 bp
- Variants are Prevalent: 1 rare missense residue / person

"It's true that more is not necessarily better, Edward, but it frequently is."

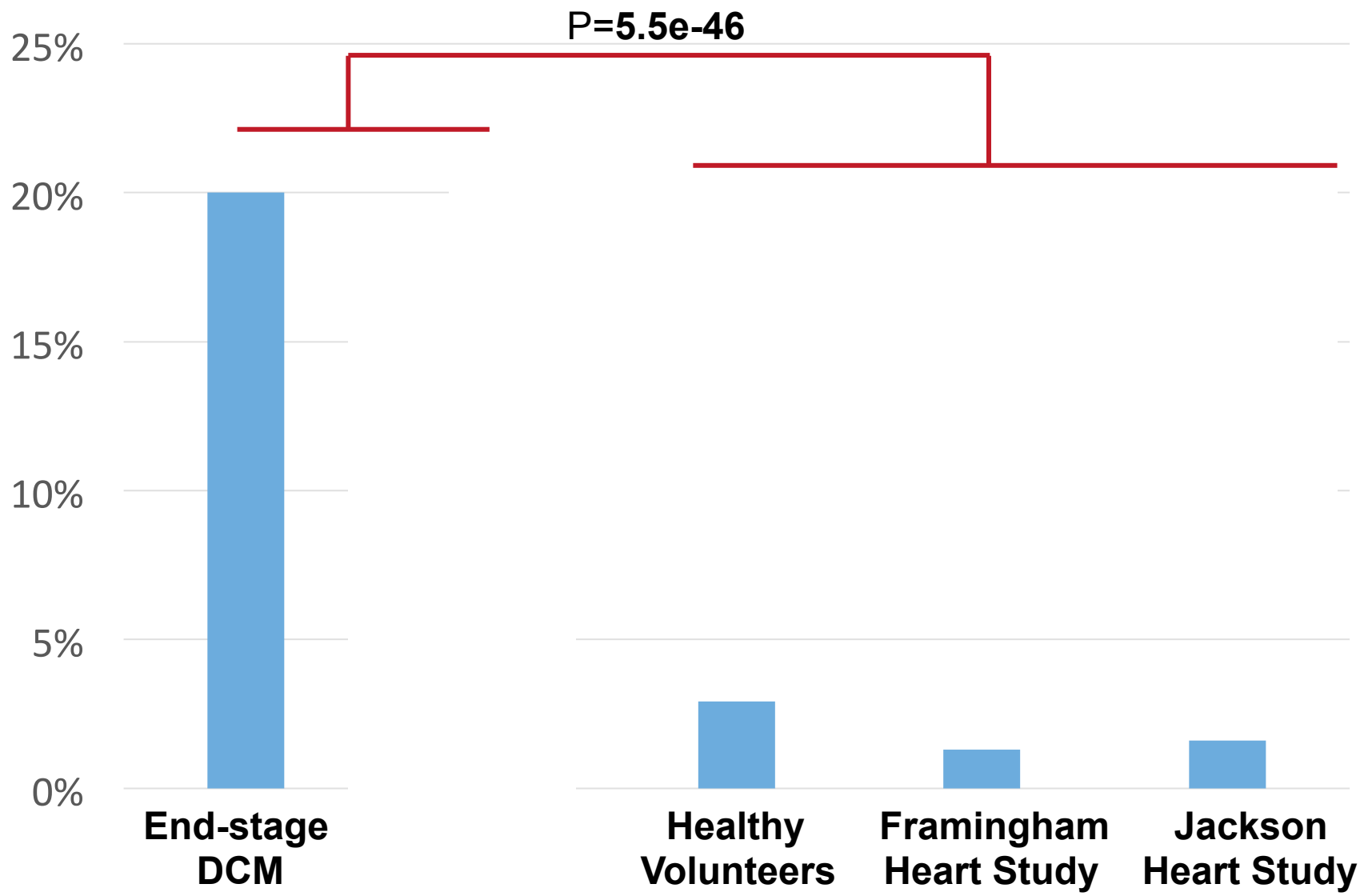
Titin Sequenced in 5267 Individuals with Spectrum of Cardiac Physiology in Health & Disease

- 308 Intensively Phenotyped Healthy Volunteers
- 3603 Jackson and Framingham Heart Study Subjects
- 231 Mutation-negative HCM Patients
- 374 Prospective ambulatory DCM Patients
- 155 End-stage DCM Patients listed for Transplant
- Replication cohorts: 160 DCM Patients and 667 Controls
- DCM tissues for protein and RNA studies
- Restrict analyses to Truncating Variants: TTNtv

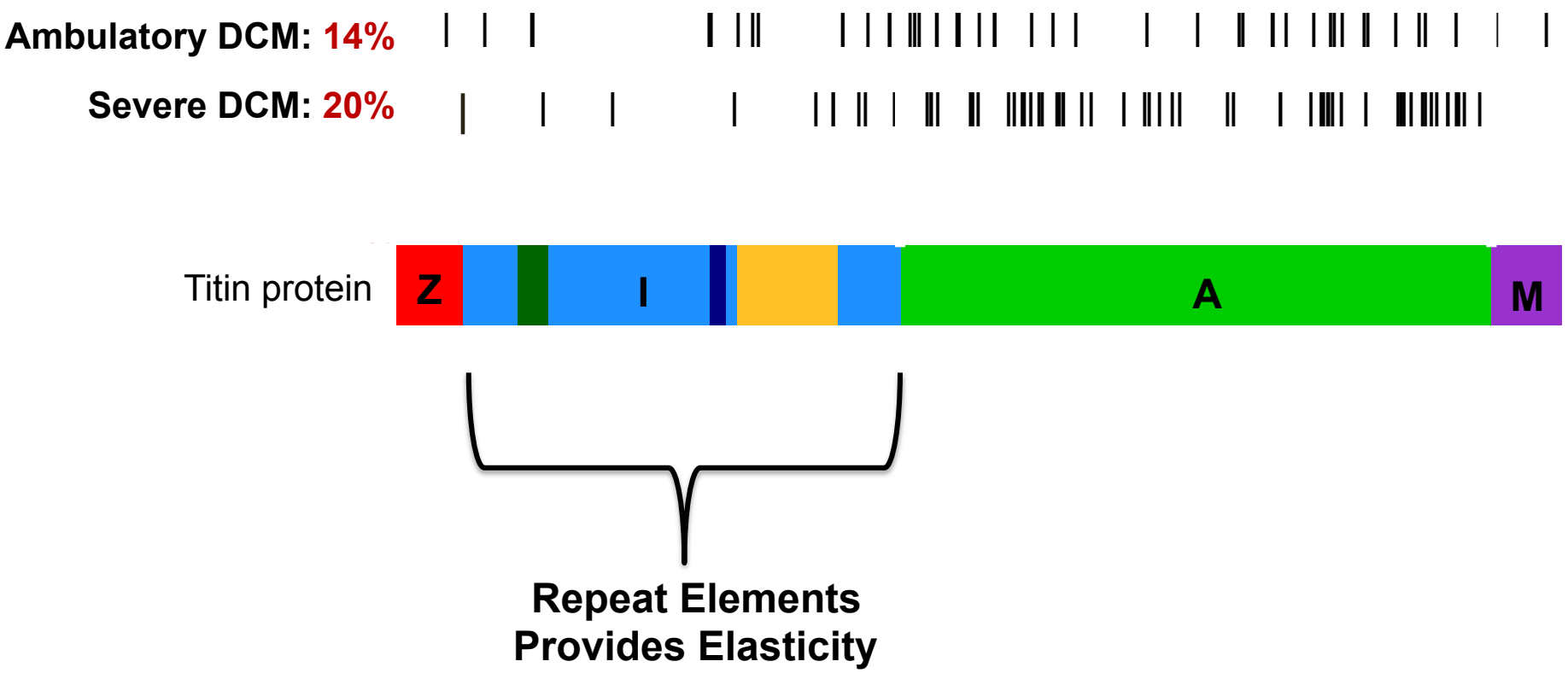
TTNtv: Most Common Genetic Cause of Unselected DCM

TTNtv: Most 20% End-stage DCM *Herman et al (2012) N.Engl.J.Med.*

14% Cases ($P=1.7x10^{-25}$) *Roberts et al (2015) Sci. Transl. Med.*



Distribution of TTNtv Mutations

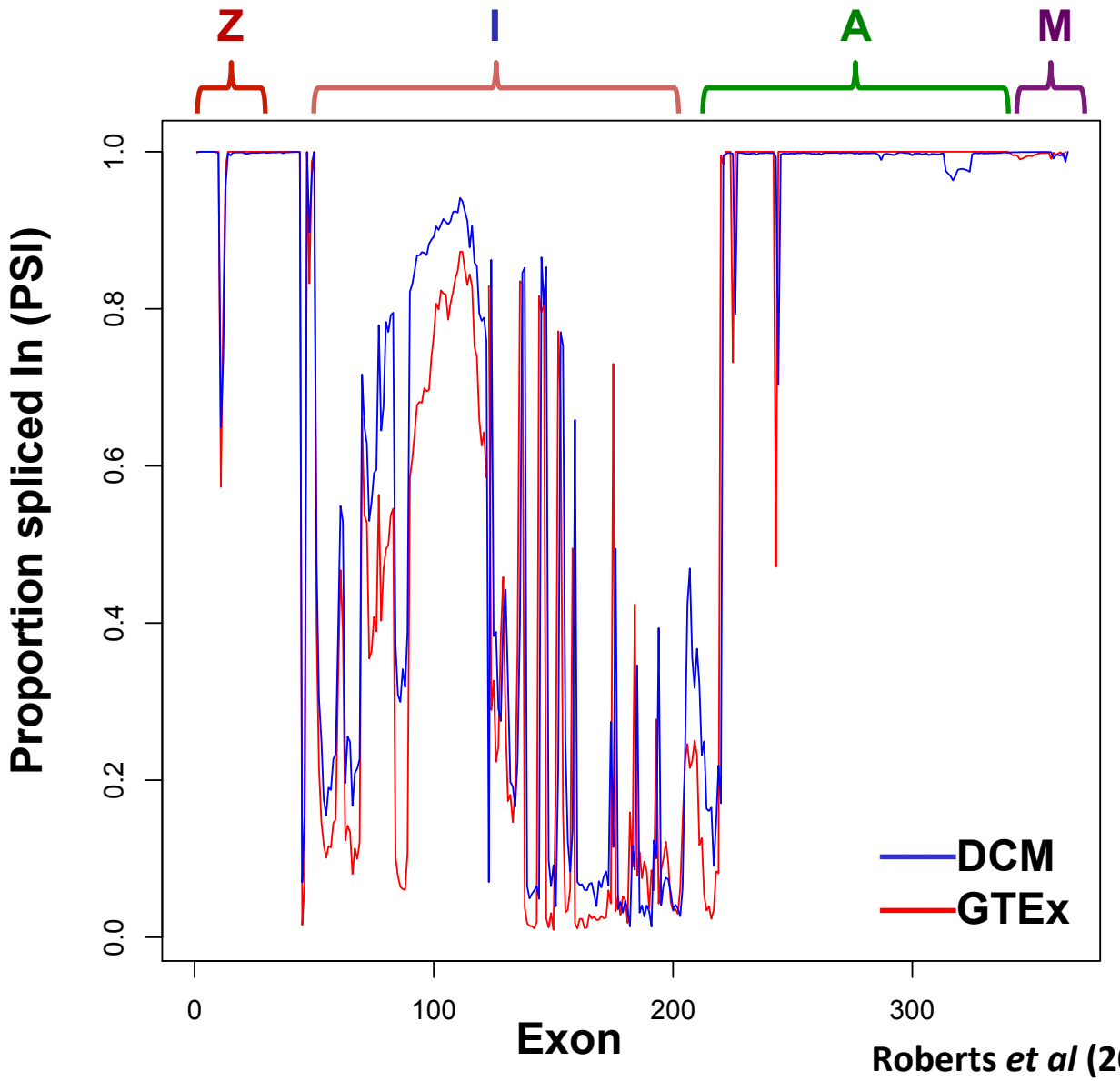


Roberts *et al* (2015) Sci Transl Med

***TTN* Splicing:** 85% Symmetric Exons (Exome-wide; P=1.2e11)

Exon symmetry ≈ PSI; Asymmetric exons, PSI >0.99

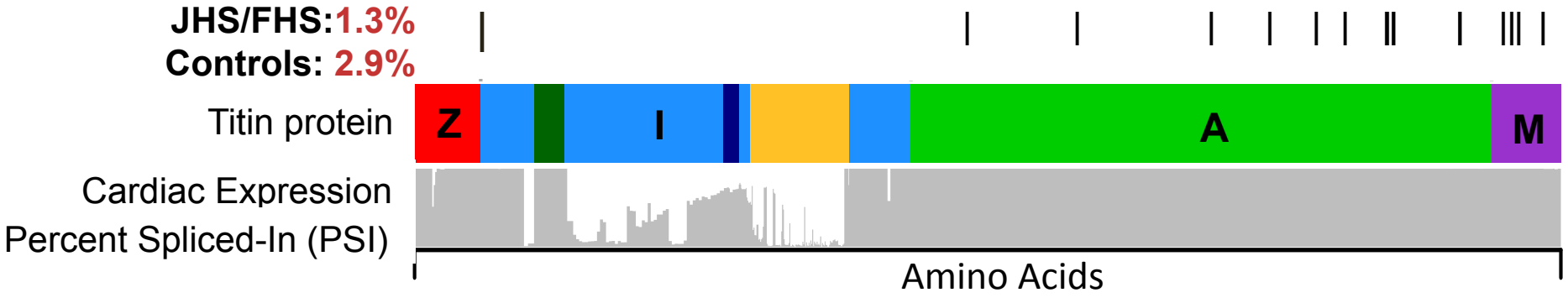
I-band Exons: Lowest PSI + Greatest Symmetry



Roberts *et al* (2015) Sci Transl Med

TTNtv in General Population / Controls

- **Usually Associated with Normal Cardiac Physiology**
- **TTNtv in high PSI exons increased DCM Risk (RR=16, p=0.008)**



***TTN*tv**

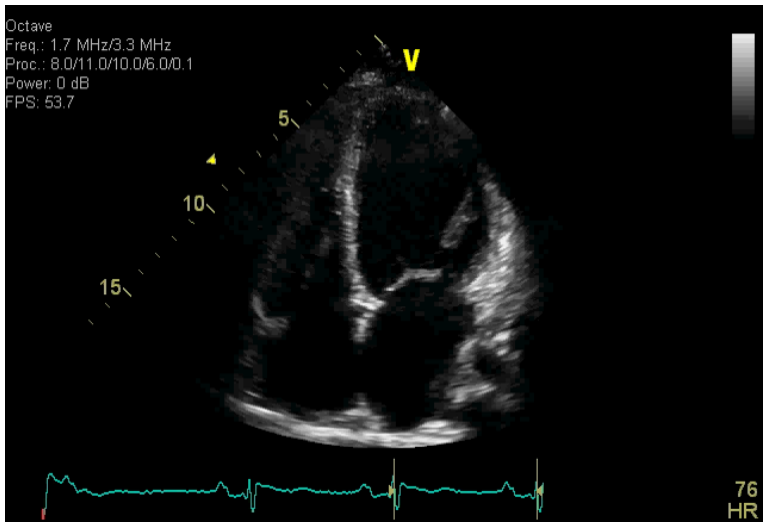
Controls: Low PSI (I-band) enrichment: (**vs DCM P=2.0e-4)**

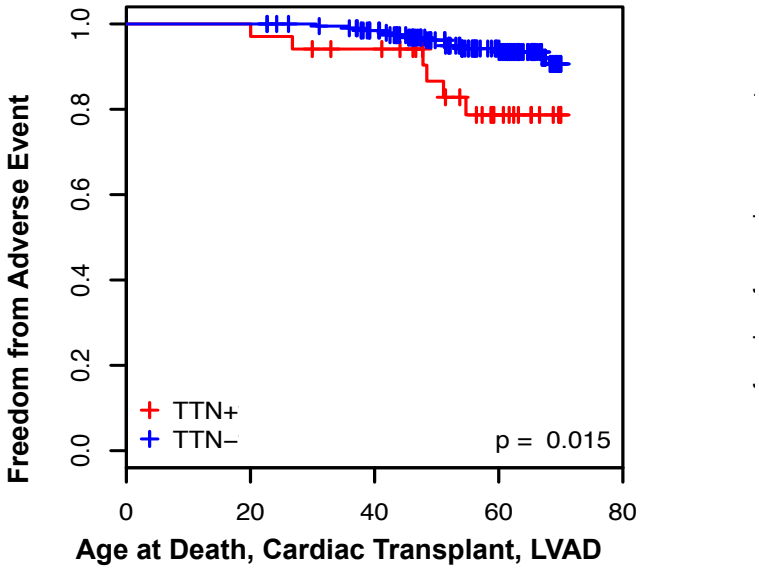
DCM: High PSI enrichment (**vs. Controls: P= 5.5.e-45)**

Validation: Independent cohorts: **P=3.e-21**

Roberts et al (2015) Sci. Transl. Med.

Clinical Manifestations of TTNtv^{+} DCM





TTNtv^{+} *vs* TTN^{-} DCM
LV Ejection Fraction: -18±7%, *P* = 0.006
LV Stroke volume: -22±8ml, *P* = 0.0017
LV Walls Thickness: -0.84 mm, P =0.0007
Sustained Ventricular Tachycardia: 47% vs 18%; P =0.003
Adverse Events: 10 Years Earlier in Men than Women; P= 4.5e-5

Roberts et al (2015) Sci. Transl. Med.

Peripartum Cardiomyopathy (PPCM)

Incidence: 1 in 300 (Nigeria and Haiti)
1 in 1000-4000 (US and Europe)
Risk Factors: Preeclampsia
Twin pregnancy
Advanced maternal age
Outcomes: Heart Failure Resolves
Death in 5-19%
Cardiac Tx: Accounts for 4% of Tx in US

***Zolt Arany MD PhD* (University of Pennsylvania)**
IMAC/IPAC Investigators

IMAC-2: Intervention in Myocarditis and Acute Cardiomyopathy-2
IPAC: Investigations in Pregnancy Associated Cardiomyopathy

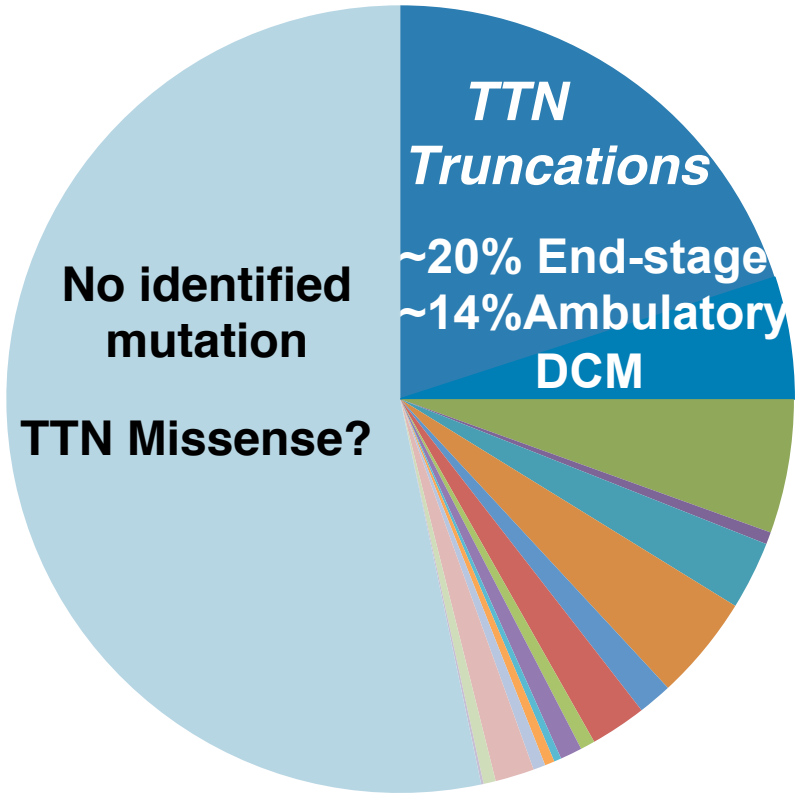
Genetic Analyses of 172 PPCM Cases

Age= 31 ± 6 years, 35% African ancestry
Number of Pregnancies: 2.8 ± 2; Twins =20%
Family Hx of DCM: 10%
Hypertension: 45%
Ejection Fraction = 33%

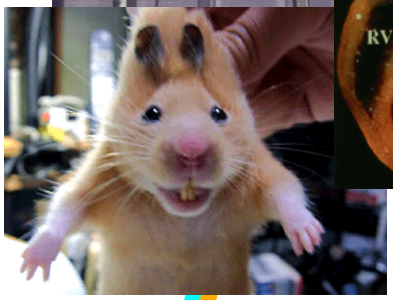
TTNtv^{+}: 26 (15%) P=1.3E-7 vs controls
9% Hypertensive (P=0.009 vs TTNtv^{-})
1Year Outcome (n=83): EF TTNtv^{+} < TTNtv^{-} (P=0.005)

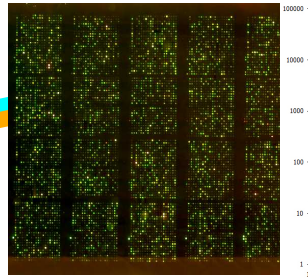
Ware et al NEJM, 2016

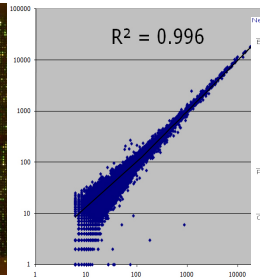
Gene involvement in DCM

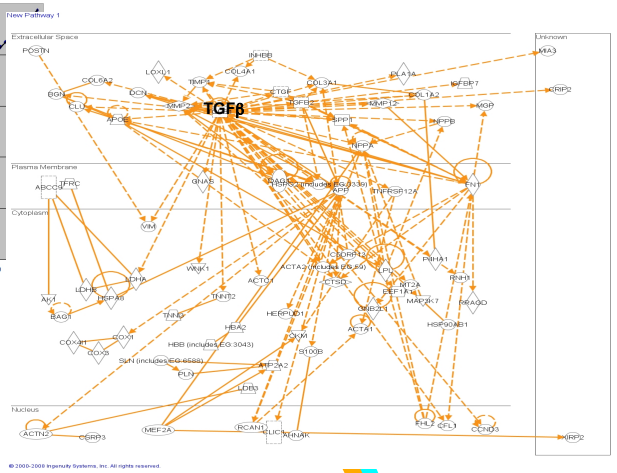


Adapted from Zimmerman et al Genet Med, 2009

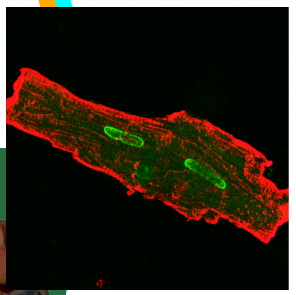


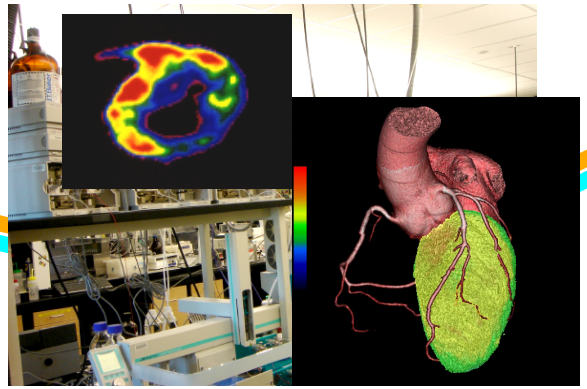


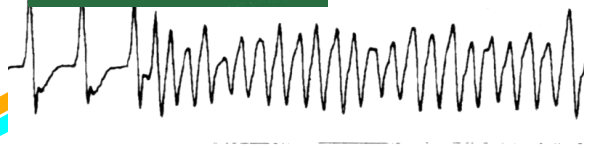




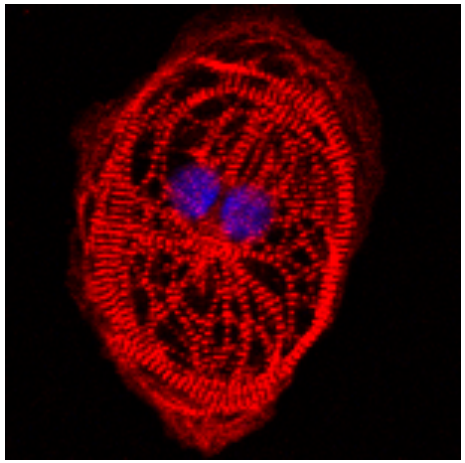
Models Inform
Cardiac Physiology
Disease Mechanism
Therapeutic Targets



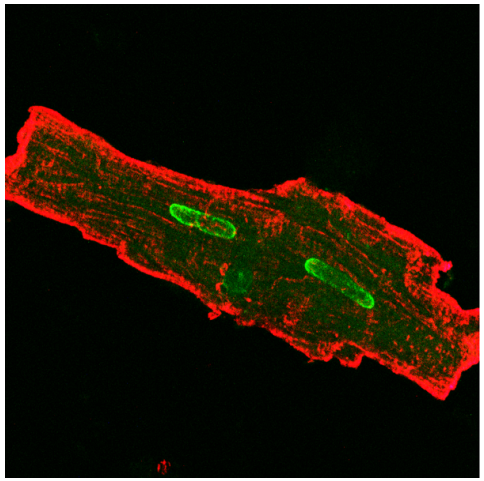




Functional Assessments of iPS-induced Cardiomyocytes

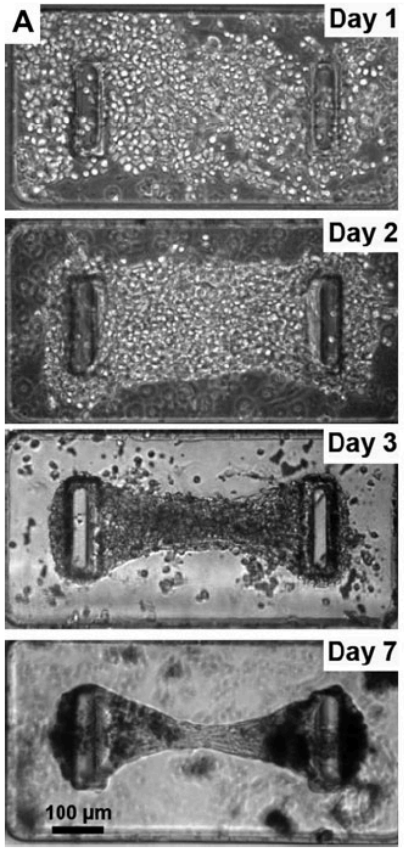


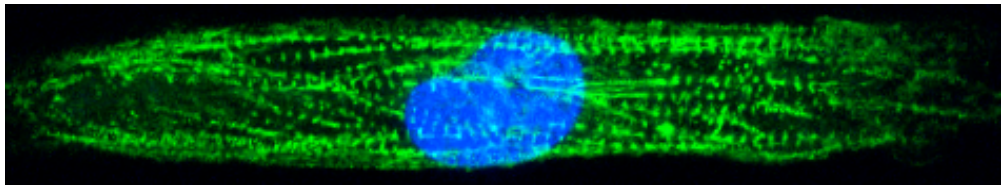
iPS cell derived CMs
MYH7:MYH6 0.8-1.2

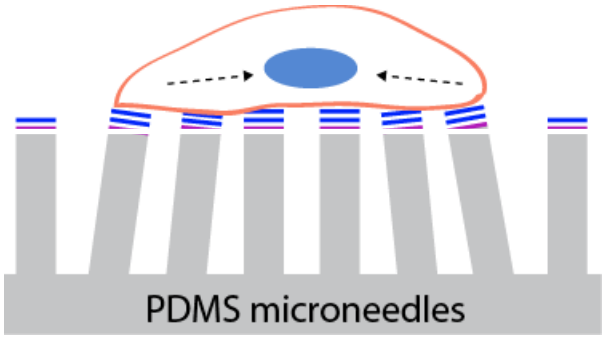


In Vivo CMs

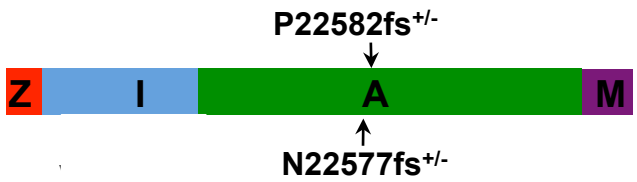
+10% MSCs
3D - tugs
~4 days
2D Patterned



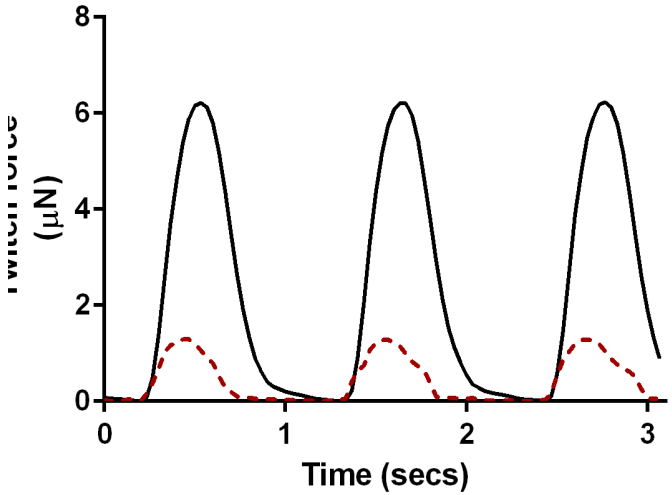




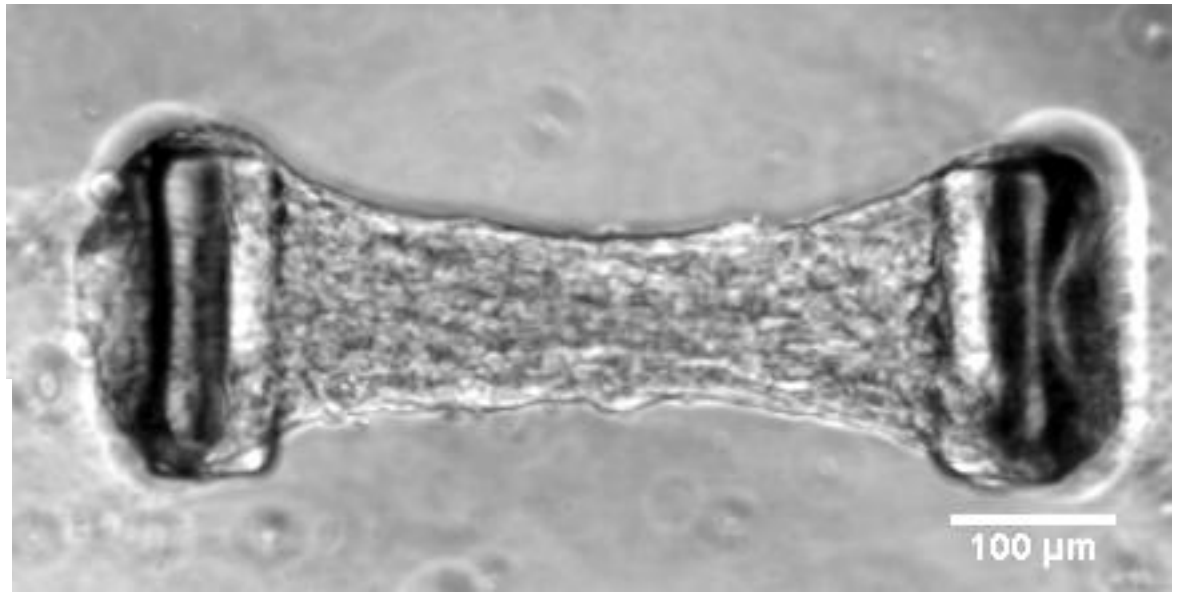
TTNtv: Diminished Contractile Function

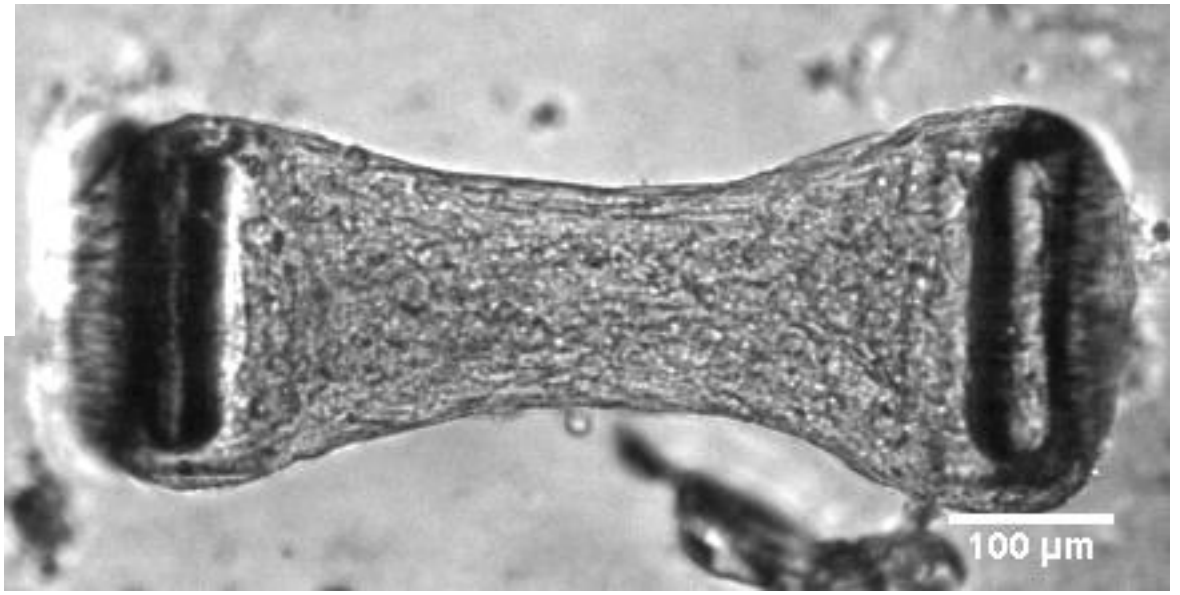


I-Band TTNtv



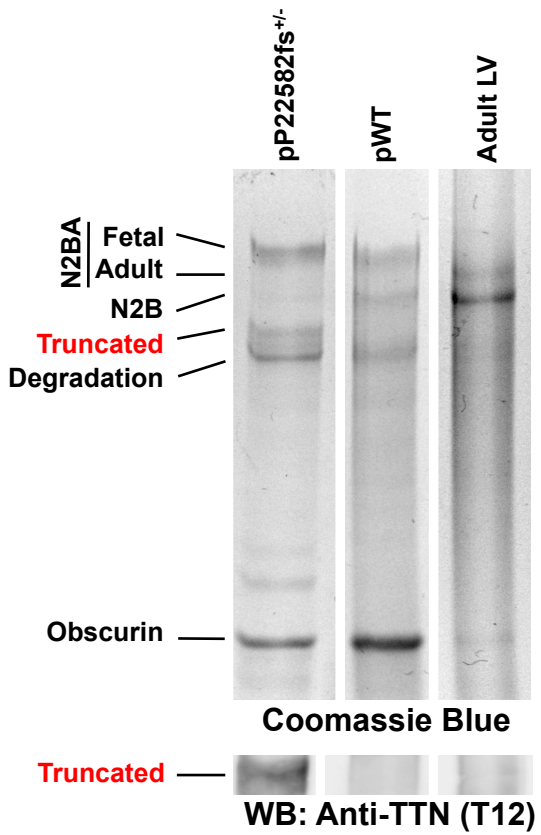
Exon 66 Usage
Adult LV: 18%
iPS-CM: 80%





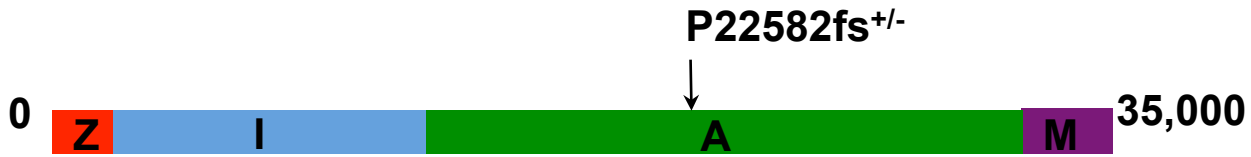
Hinson et al (2015) Science

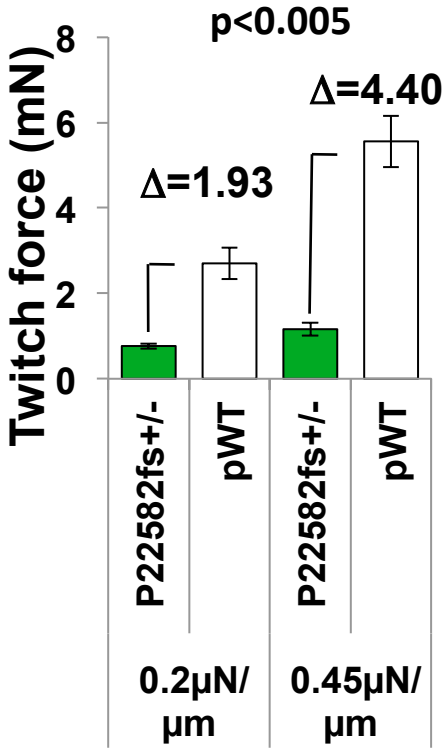
TTNtv Protein: Sometimes Stable in Cells but Impairs Sarcomerogenesis

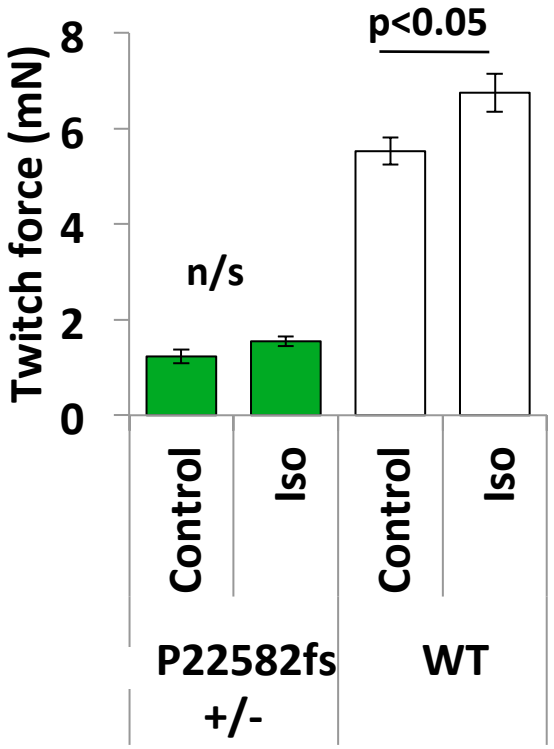


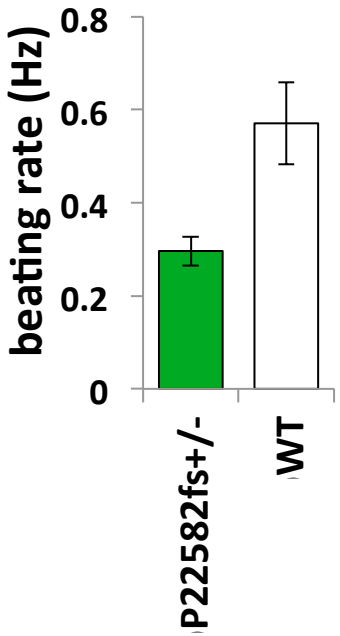
Hinson et al (2015) Science

A-band TTNtv Cardiomyocytes: Reduce Responses to Load & Adrenergic Stimuli



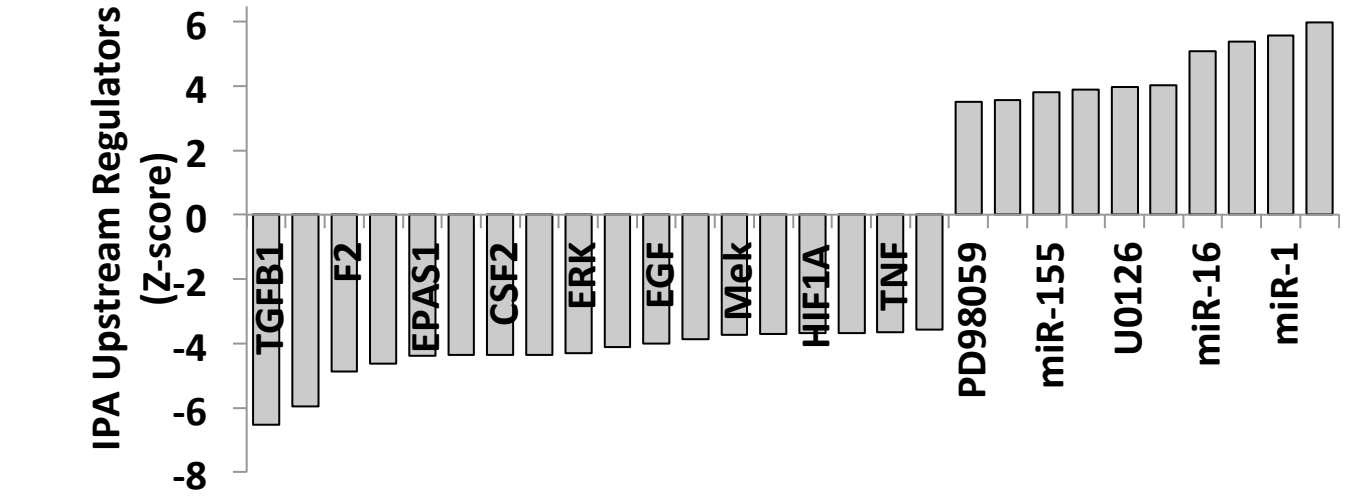


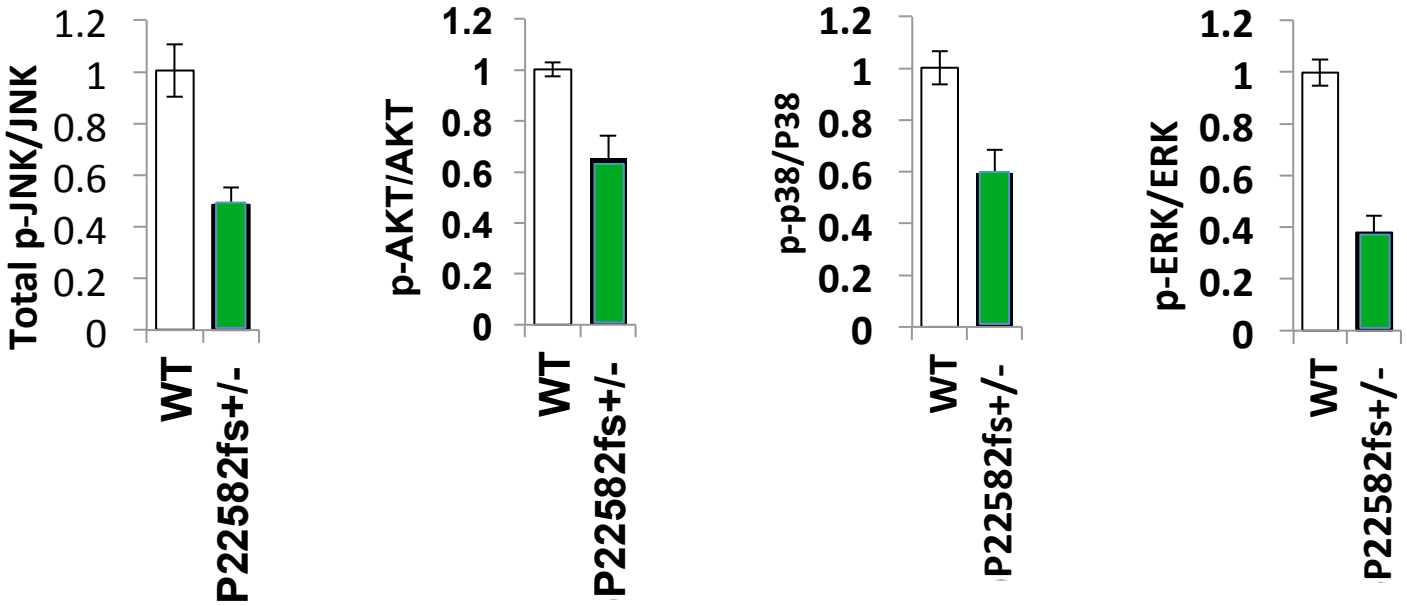




Hinson et al, Science 2015

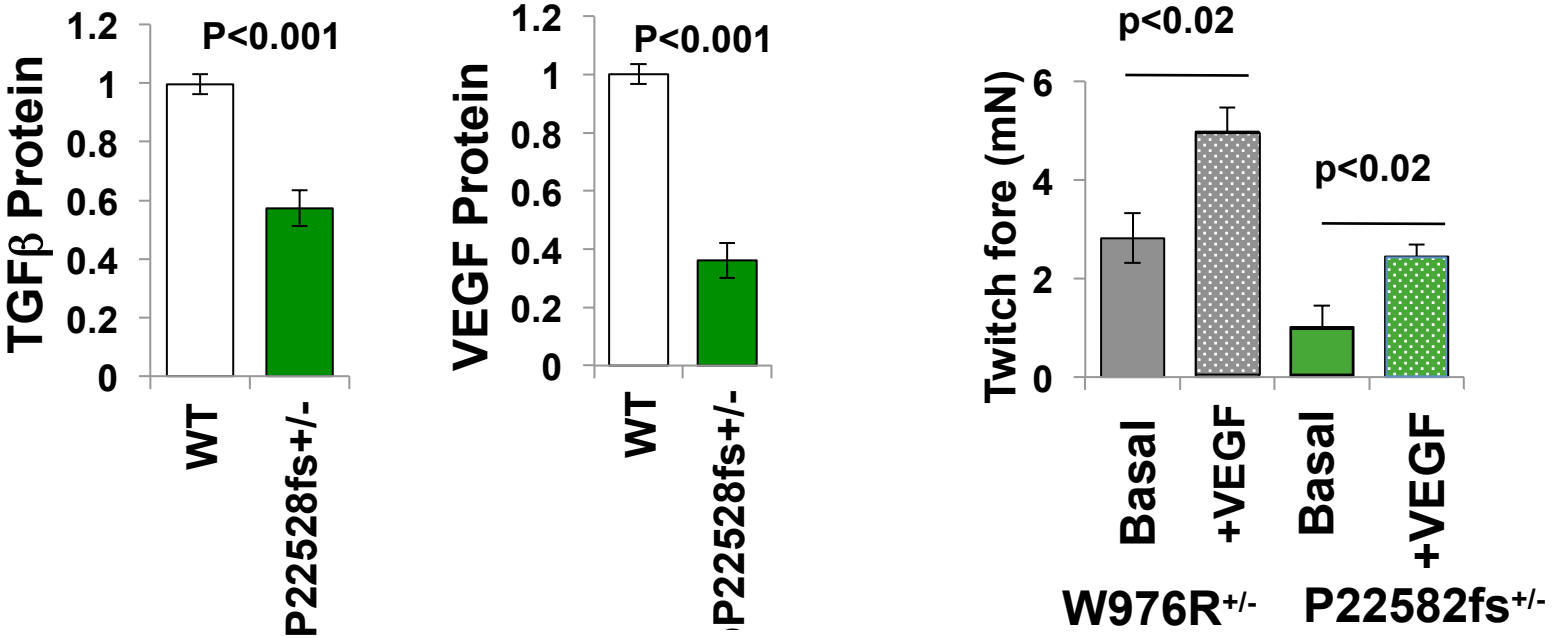
A-band TTNtv iPS-CMs:
Reduced Growth Factors & Signaling Molecules



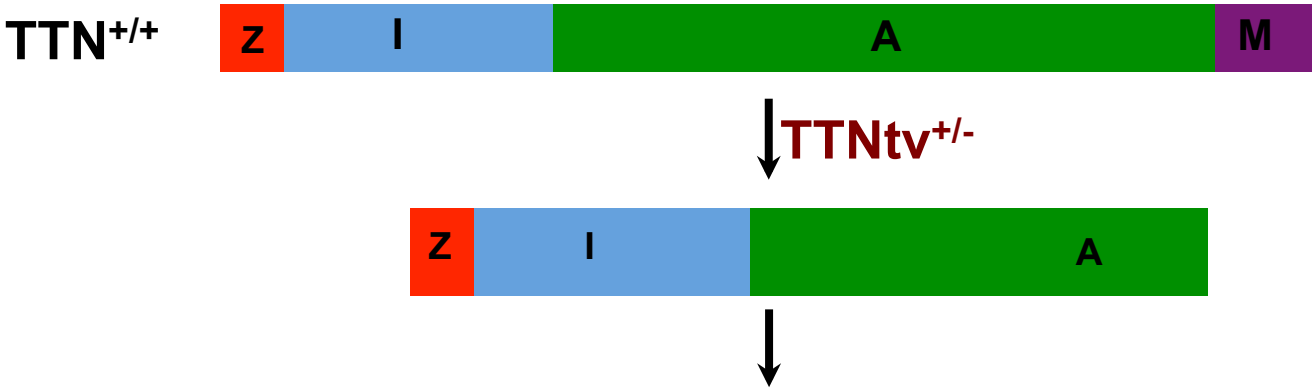


Hinson et al, Science 2015

Supplemental Growth Factors Improved Force Production by TTNtv iPS-CMs



Hinson et al (2015) Science



Haploinsufficiency of full length TTN

Disease severity ~ PSI of truncated exon
A-band (PSI 100%) >> I-band (mean PSI 69%)

Sarcomere Insufficiency

Failed Adaptation

↓ Responsiveness:
Mechanical load
Adrenergic stimulus

Failed Signaling

↓ Activation:
Growth factors/Regulators
(TGF-β, VEGF, MAPK, AKT)

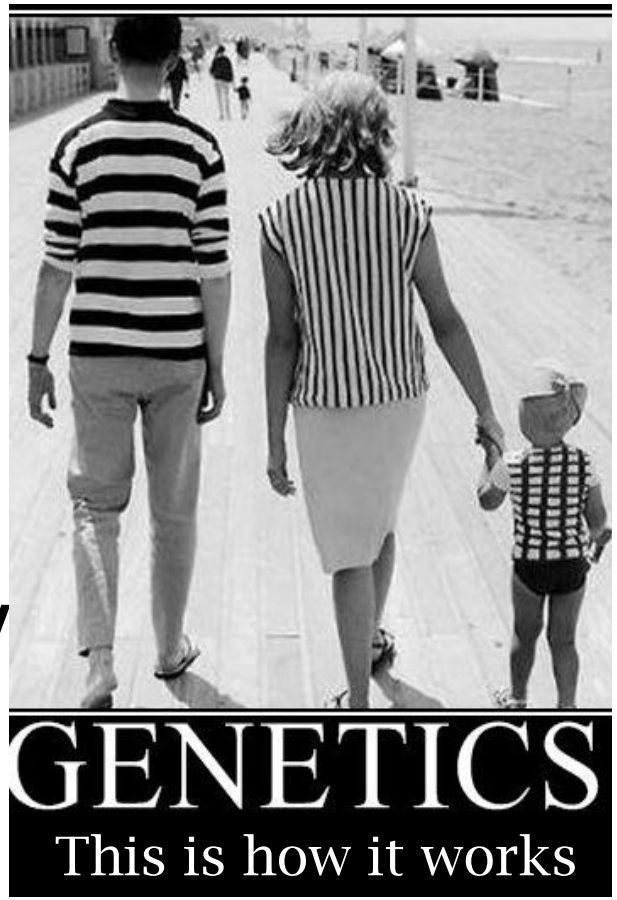
Dilated Cardiomyopathy and Heart Failure

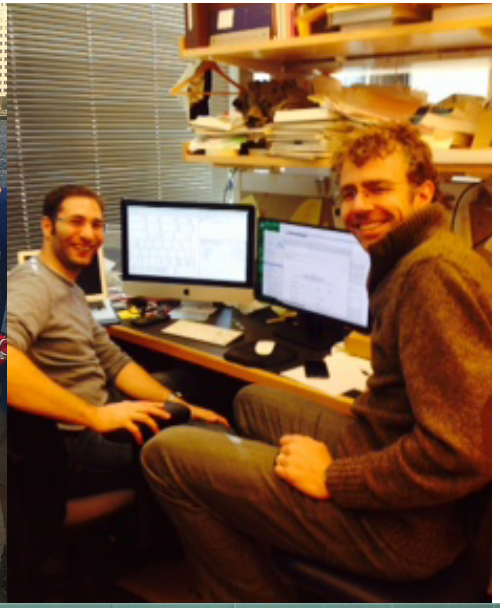
Insights From Genetic Heart Diseases

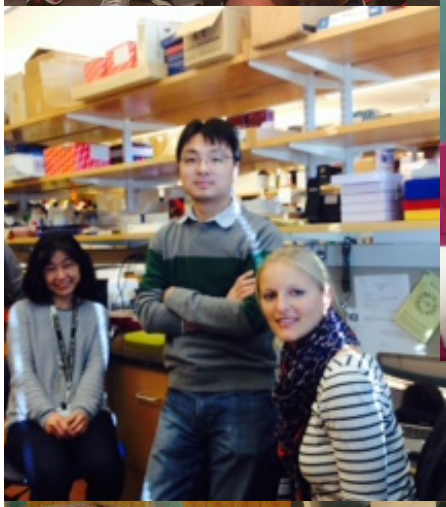
- **Molecules**: Sarcomere Proteins
  Prevalent Causes of DCM and HCM

- **Mutations**: Not Created Equal
  Variables = Type, Location, Exon Usage
  Exquisite Probes for Molecular Physiology

- **Mechanisms**: Informed by New Tools
  Provide Powerful Strategies to Identify
  New Treatment Strategies







Human Genetics

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