The University of Hong Kong
Li Ka Shing Faculty of Medicine
香港大學李嘉誠醫學院

Press Conference
April 18, 2018

Speaker

HKU Discovers the Application of Human Induced Pluripotent Stem Cells in Precision Medicine for Hereditary Diseases

Professor David Siu Chung-wah

Clinical Professor of Department of Medicine

What is Precision Medicine?

"An emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person."*

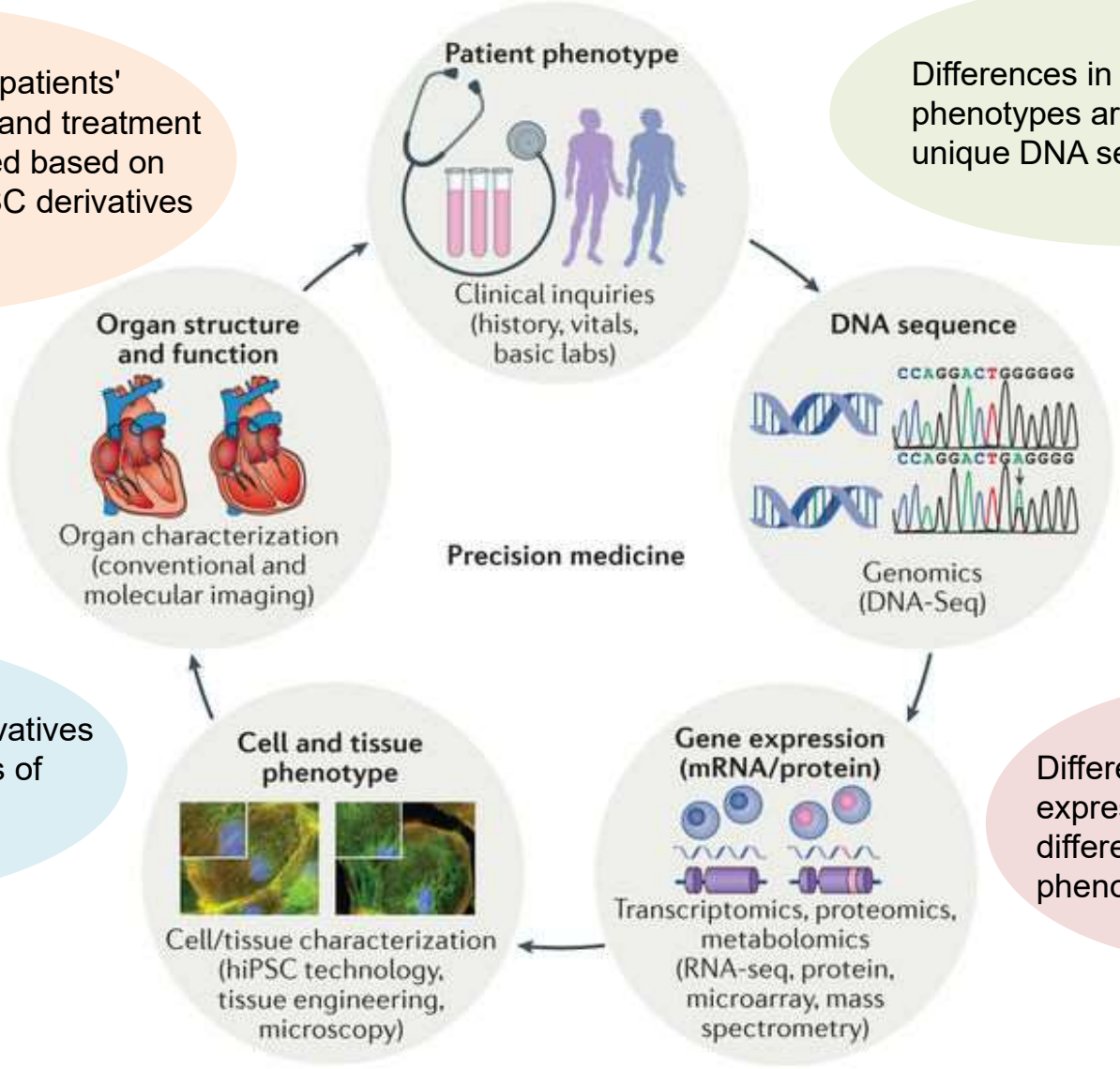
- Enables physicians to tailor medical treatment for each patient
- Supports the development of molecularly targeted drugs based on biologic pathways
- Identifies at-risk populations for targeted prevention prior to disease onset

*NIH - https://ghr.nlm.nih.gov/primer/precisionmedicine/definition

Role of Human Induced Pluripotent Stem Cell (hiPSC) Technology in Precision Medicine

In precision medicine, the patients' disease risks, prognoses, and treatment responses can be predicted based on the behaviors of their hiPSC derivatives in cell culture

Differences in patients' clinical phenotypes are the result of their unique DNA sequences

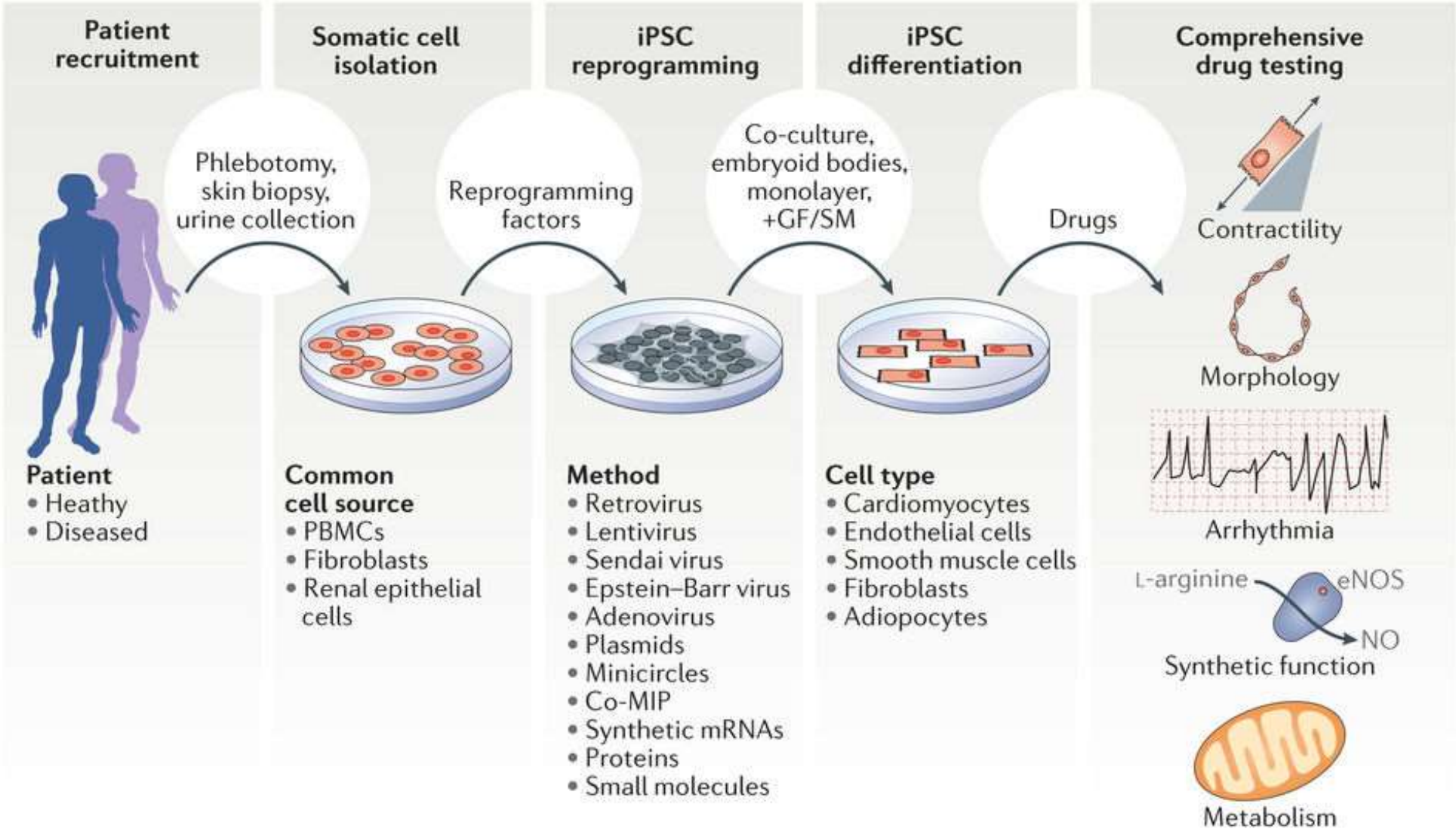


Patient-specific hiPSC derivatives recapitulate the phenotypes of their *in vivo* counterparts

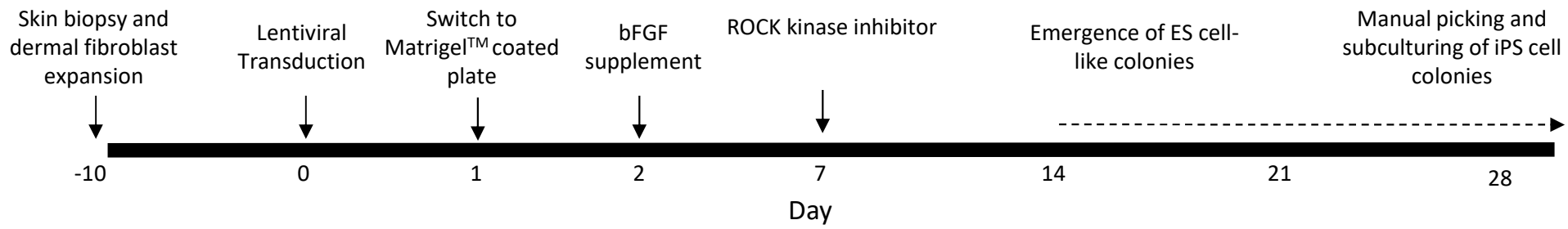
Differential gene expression patterns cause different cellular and tissue phenotypes

Nature Reviews | Cardiology

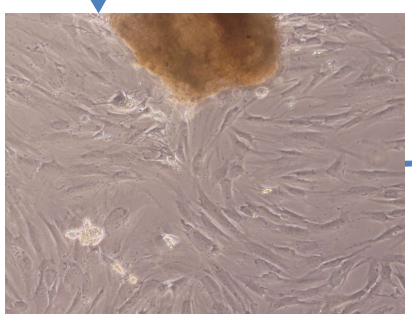
Human Induced Pluripotent Stem Cell (hiPSC)-Based Platforms for Drug Development



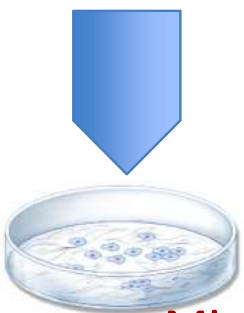
Animal Product-Free hiPSC Production



Reprogramming genes
ROCK kinase inhibitor

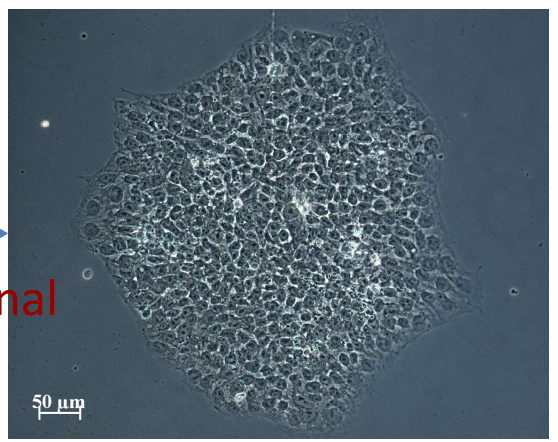


Skin cells



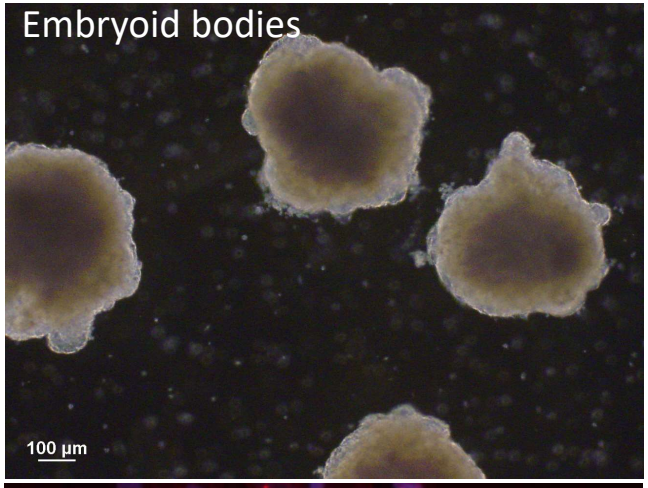
Micro-dissection & clonal expansion

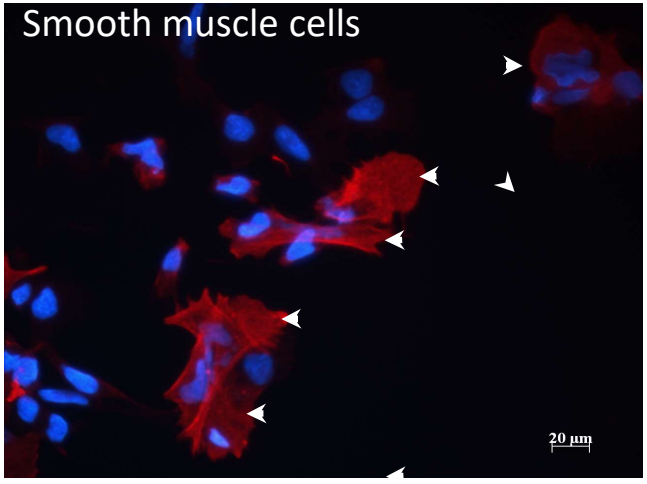
hiPSCs

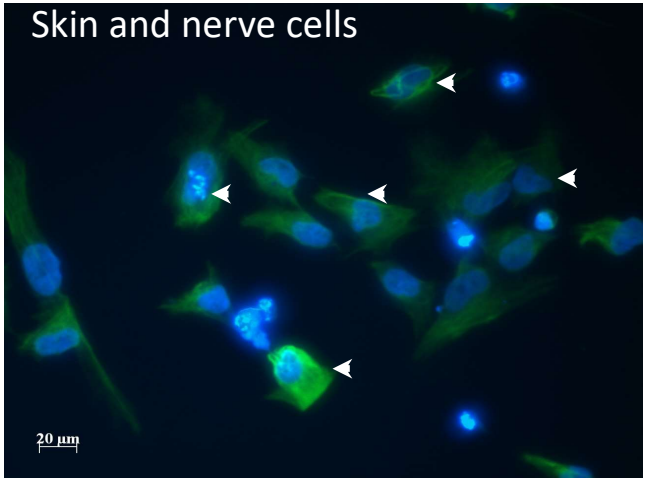


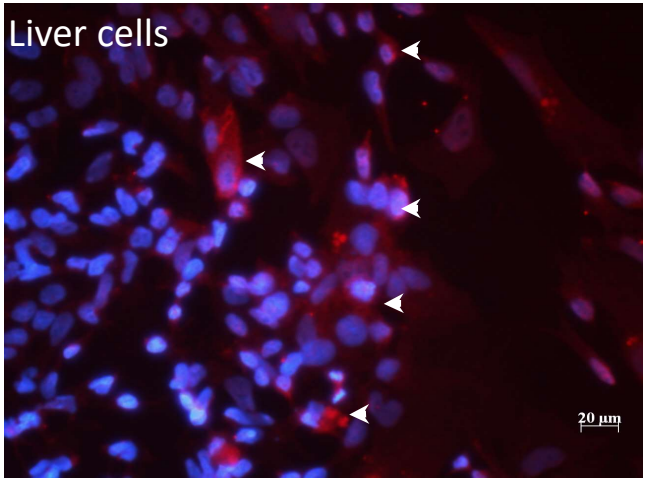
Lai WH, ... Tse HF, Siu CW: Cell Reprogram. 2010 Dec;12(6):641-53.

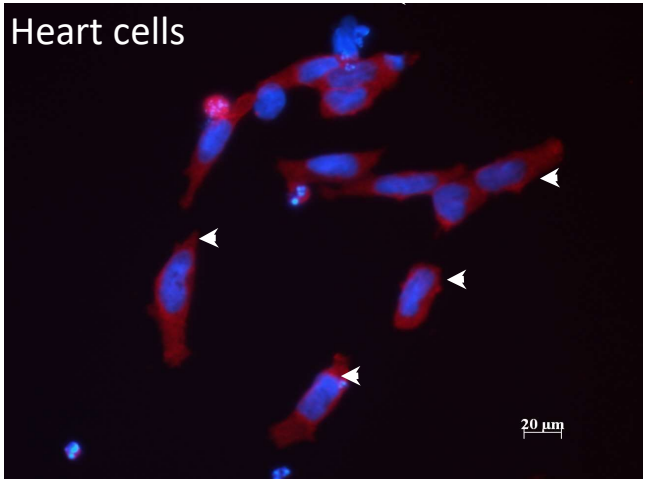
Branching into various cell types of the body

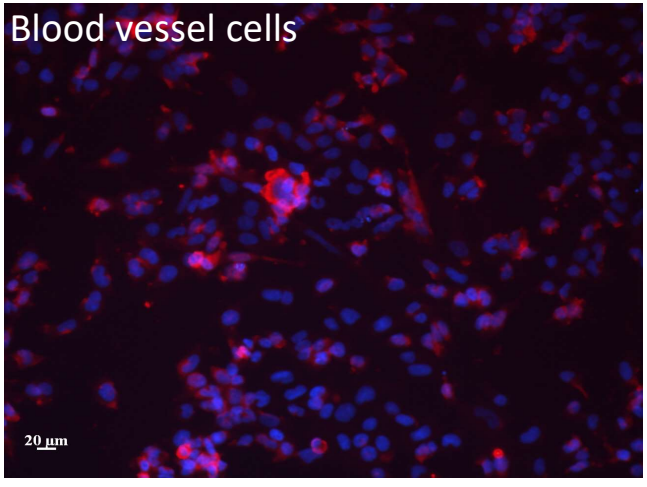






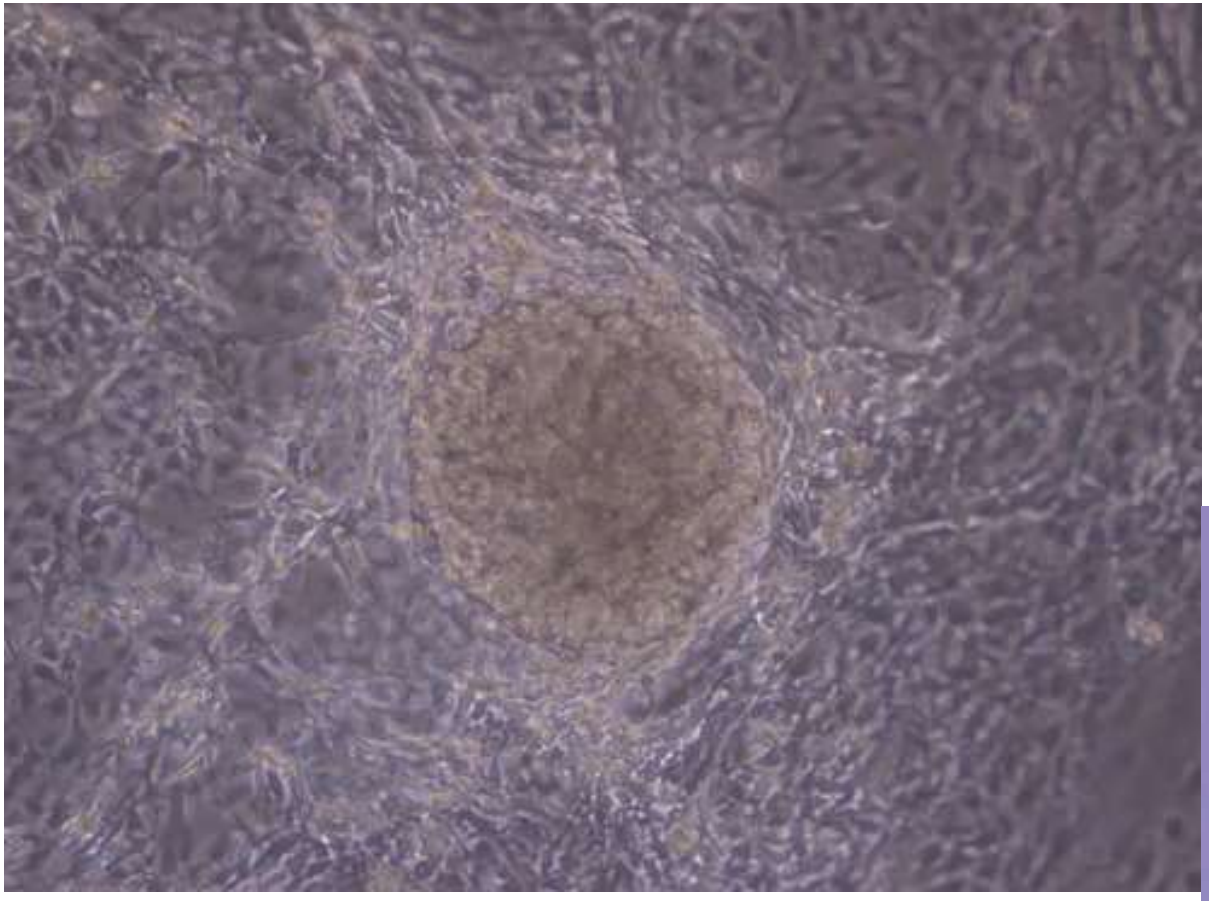


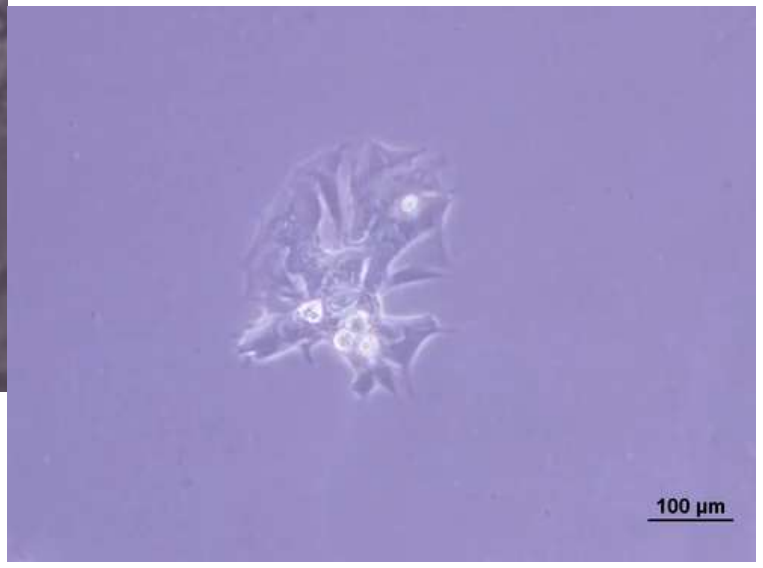




Lai WH, ... Tse HF, Siu CW: Cell Reprogram. 2010 Dec;12(6):641-53.

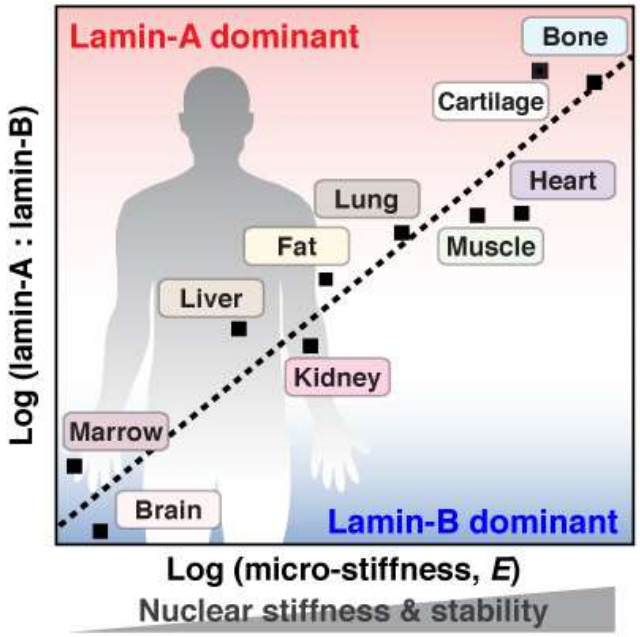
Beating Heart Muscle Generated from hiPSC

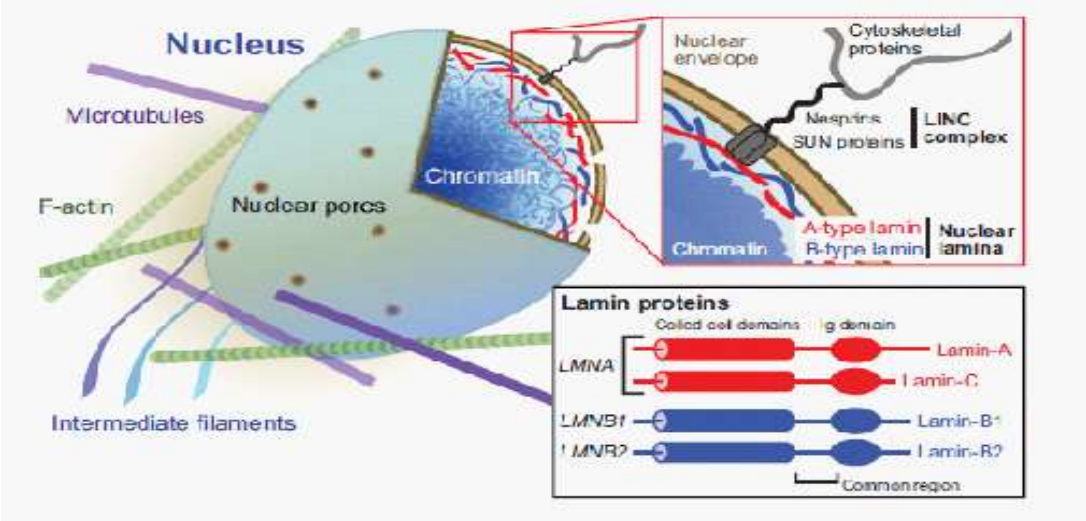




Laminopathies

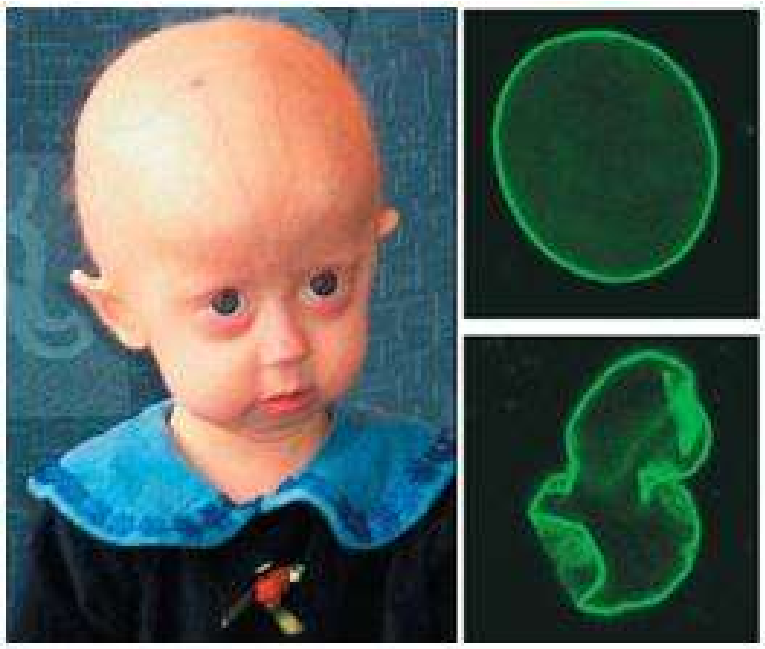
- Alternative splicing of *LMNA* gene generates lamin A and C, the intermediate filament protein of nuclear lamina.
- Serve as a matrix to maintain chromosome and genome integrity
- **Mutations in *LMNA*** referred to as “laminopathies, which cause Hutchinson Gilford Progeria (premature aging syndrome) and muscular dystrophy, to familial dilated cardiomyopathy (DCM).



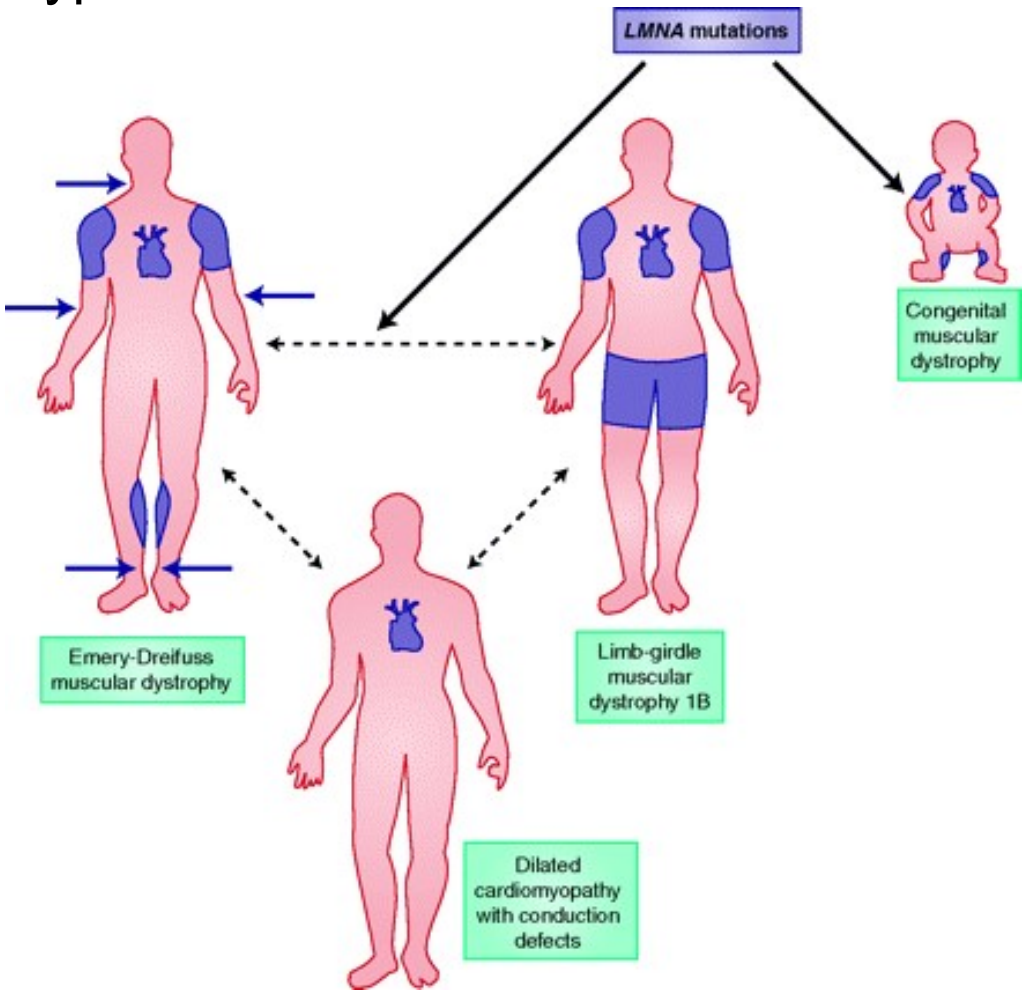


Laminopathies

Laminopathies are rare human degenerative disorders with a wide spectrum of clinical phenotypes.



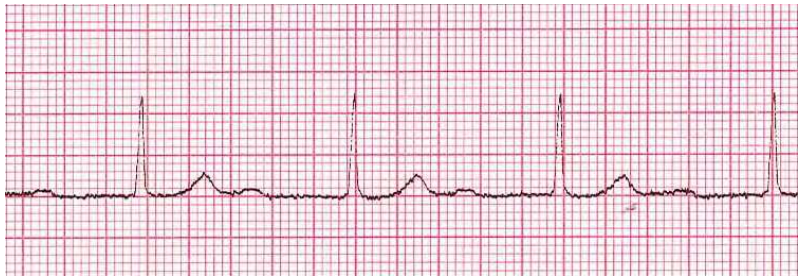
Hutchinson-Gilford Progeria Syndrome
In HGPS patients the cell nucleus has dramatically aberrant morphology (Scaffidi *et a*l., 2005).



LAMIN A/C Related Cardiomyopathy

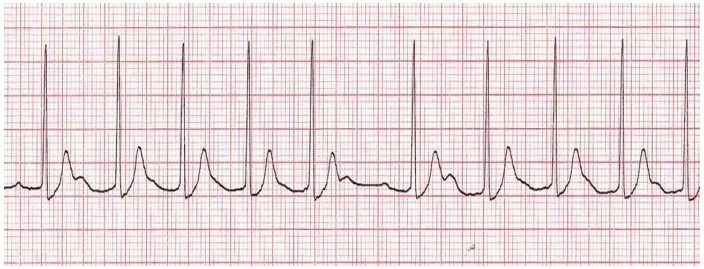
Asymptomatic Conduction block
1st degree AV block
•Sick Sinus Rhythm

0-20 years



2nd to 3rd AV nodal block

20-40 years



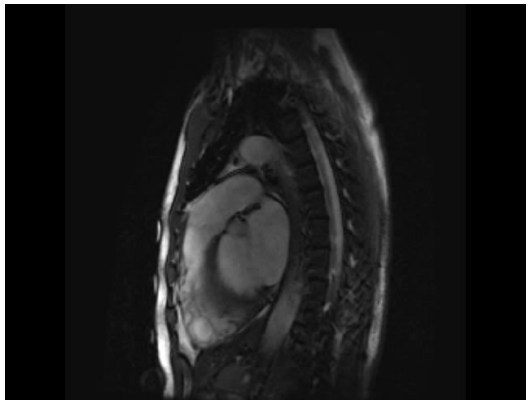
Atrial fibrillation

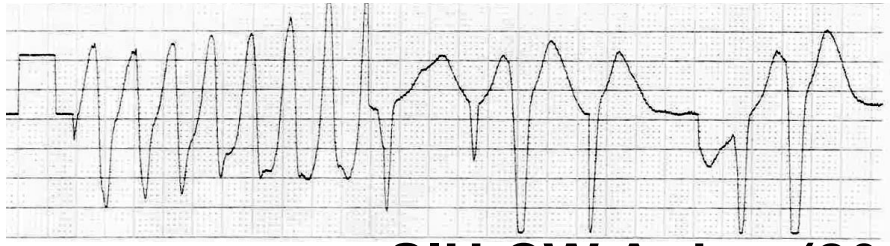
30-60 years

Cardiomyopathy

VT/VF

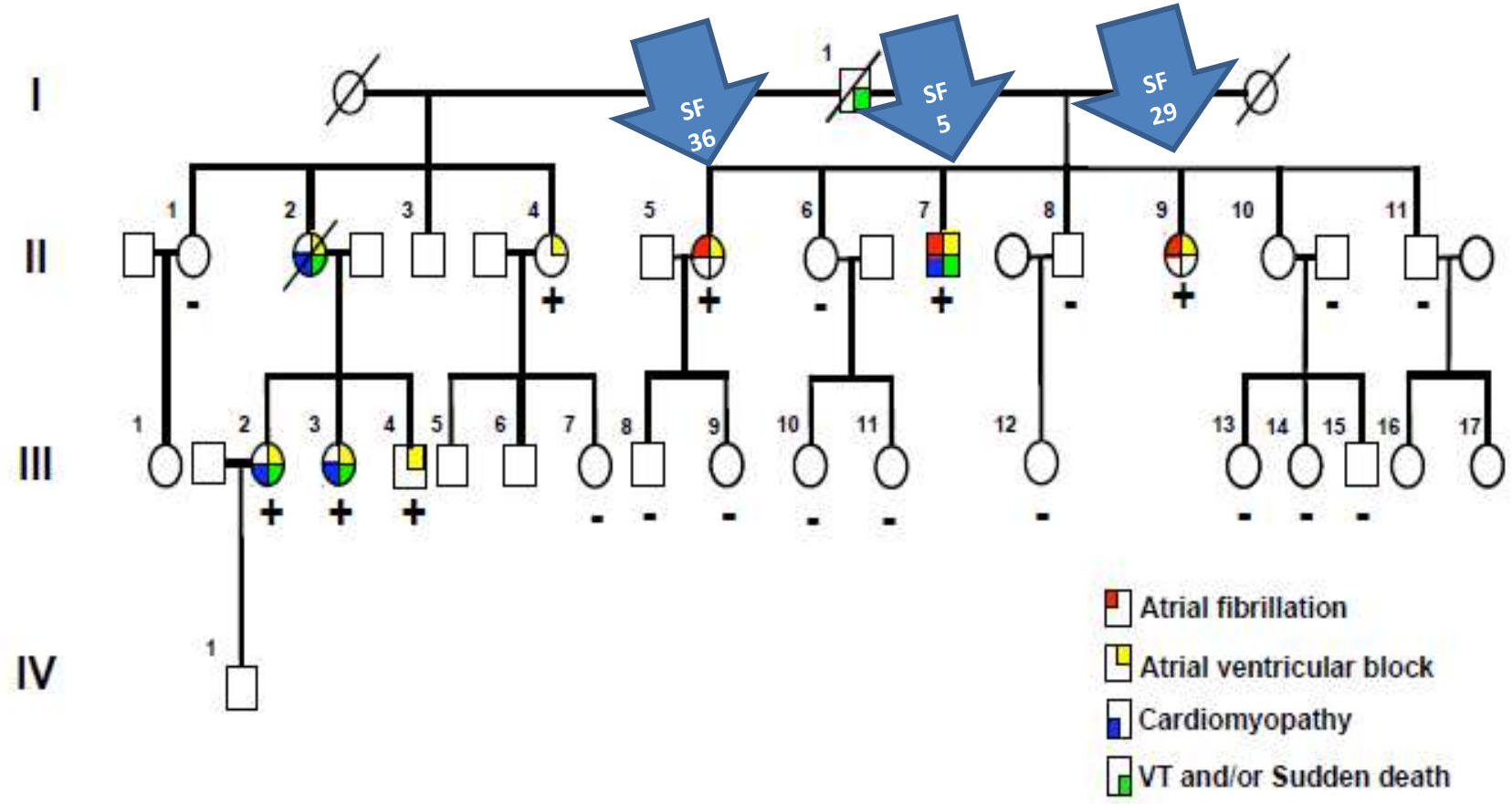
40-60 years





SIU CW Aging (2011)

Pedigree of LMNA$^{R225X/WT}$ probands with two females (II-5 & II-9) and one male (II-7)

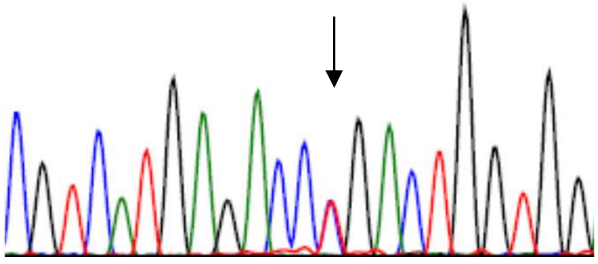


Schematic diagram of LMNA/C structure presenting mutation sites of our enrolled patients

TGA premature stop

R225X heterozygous

C to T nonsense mutation



WT: CGTCATGAGACCCGACTGGTGG

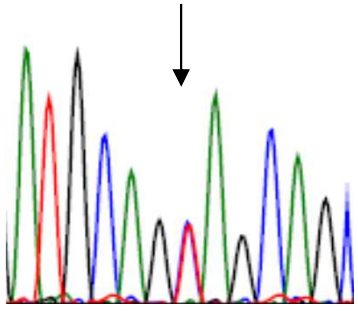
MT: CGTCATGAGACCTGACTGGTGG

stop

TAG premature stop

Q354X heterozygous

C to T nonsense mutation



WT: ATGCAGCAGCAG

Q

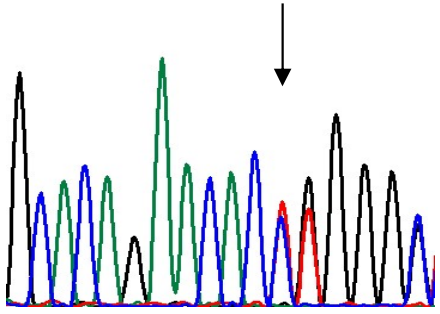
MT: ATGCAGTAGCAG

stop

Frameshift

T518fs heterozygous

frameshift mutation

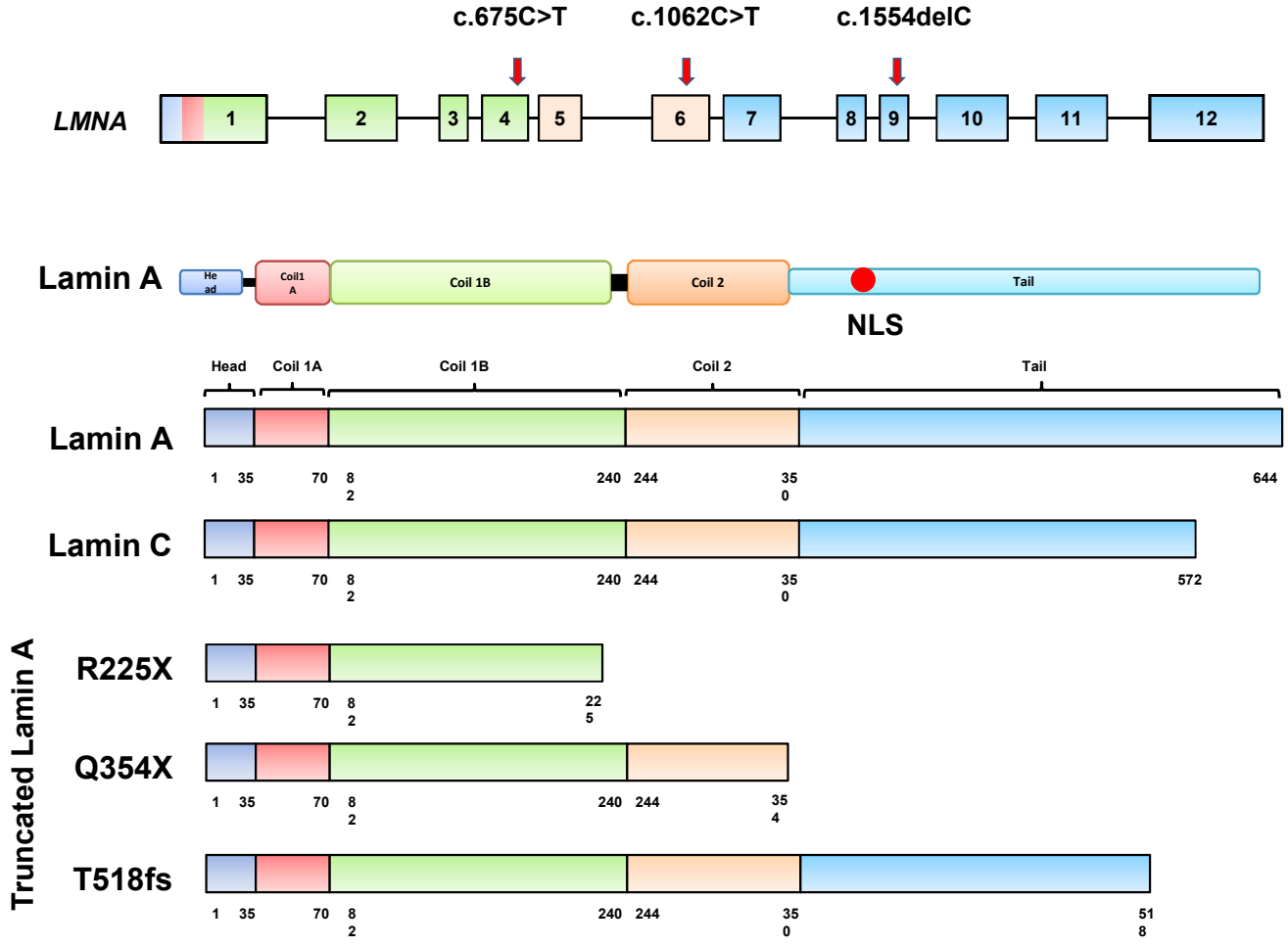


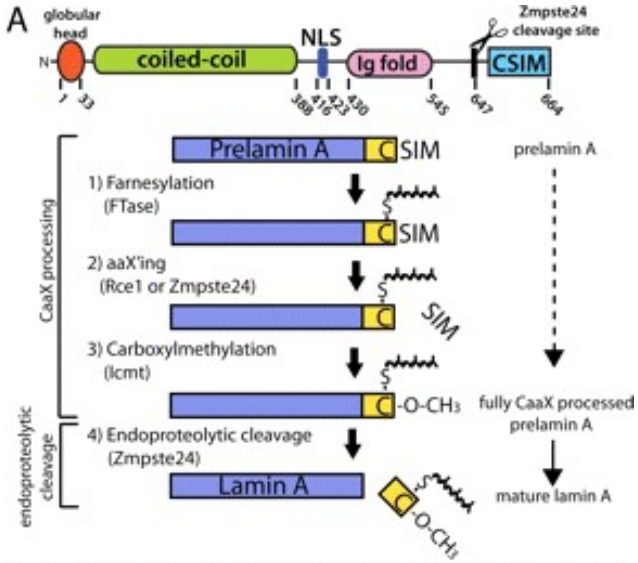
WT:GCACAGAACACCTGGGGC

MT:GCACAGAACACTGGGGC

Deletion of 'c'

Schematic Diagram Illustrating the *LMNA* Mutations Involved in Current Study: Nonsense and Frame-Shift Mutations in *LMNA*





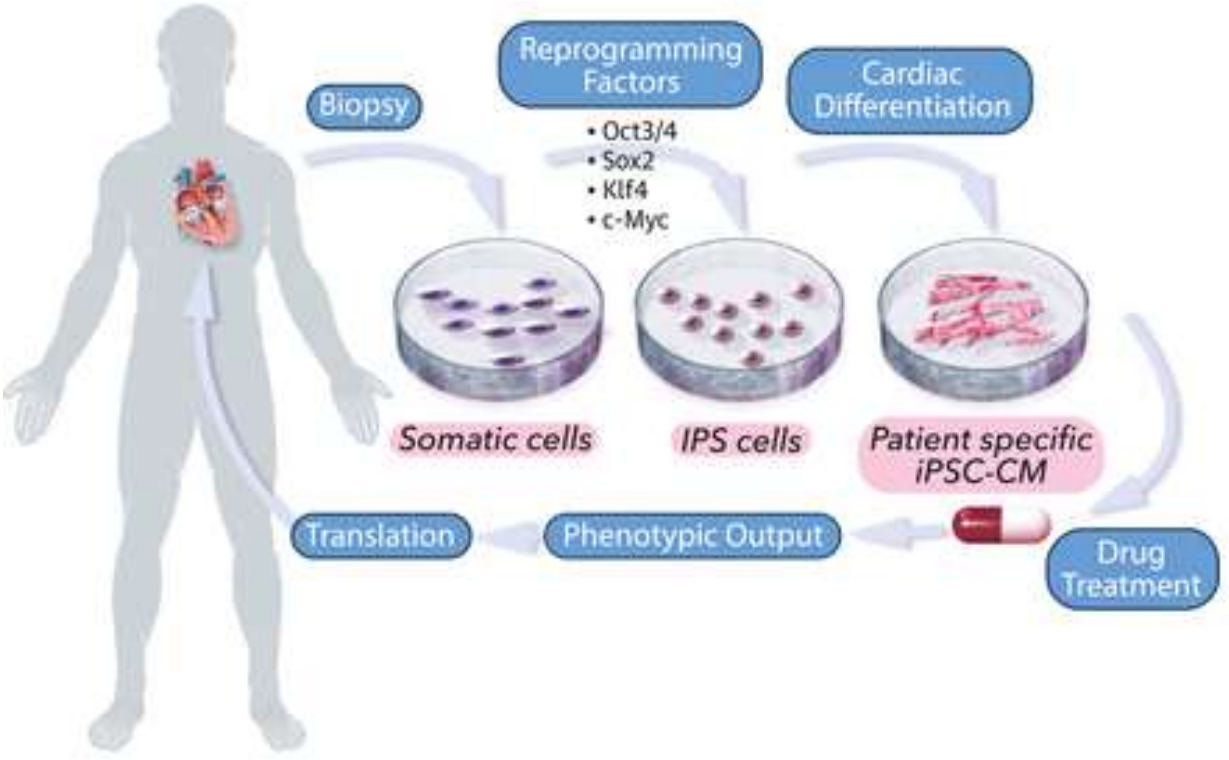
Cardiac Manifestations (age of diagnosis in years)							
Affected subjects	Sex	heart block	AF	VT/VF	Cardiomyopathy	AICD/Pacemaker	**Age of death**
SF5* (II-7) LMNA$^{R225X/WT}$	M	CHB (49)	+ (49)	+ (50)	-	PPM (49);AICD (52)	**(57)**
SF29*(II-9) LMNA$^{R225X/WT}$	F	CHB (48)	+ (48)	-	-	PPM (49);AICD (53)	**-**
SF36*(II-5) LMNA$^{R225X/WT}$	F	CHB (51)	+ (52)	+ (60)	+ DCM (60)	ICD (60)	**-**
SF11 LMNA$^{frameshift/WT}$	M	3° HB (46)	+ (49)	-	-	Pacemaker (50)	**-**
SF26 LMNA$^{E422X/WT}$	M	CHB (50)	+ (54)	+ (54)	+ DCM (57)	ICD (?)	**(64)**
SF30 LMNA$^{Q534X/WT}$	M	CHB (50)	+ (50)	+ (56)	+ DCM (50)	PPM: AICD (50)	(56)
SF39 LMNA$^{T918fs/WT}$	M	CHB (43)	+ (43)	+ (47))	+ DCM (47)	AICD (47)	-

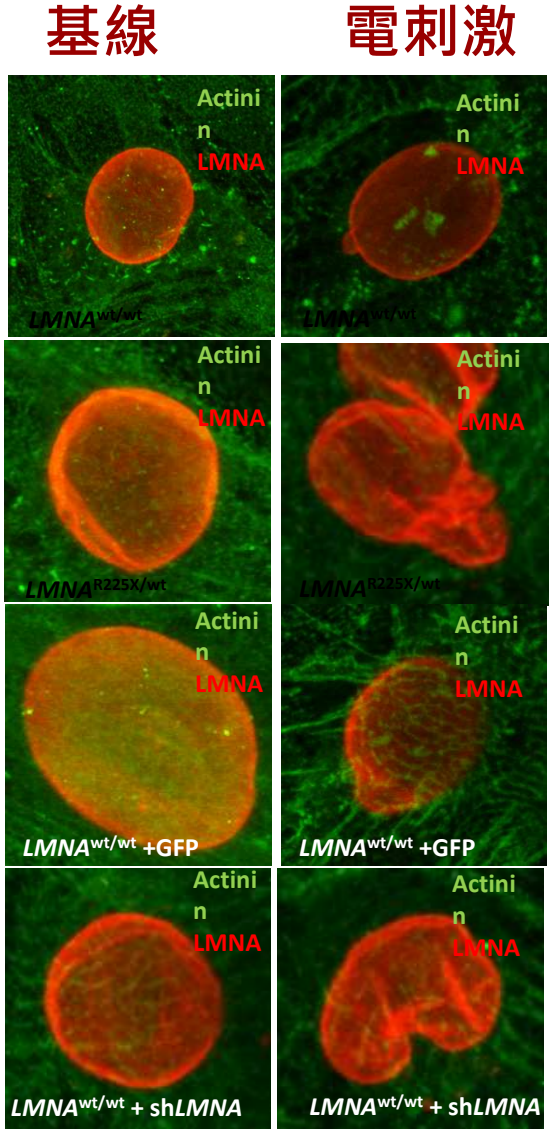
Table 1. Cardiac manifestations in affected subjects bearing *LMNA* mutation in present study

Abbreviations: 1° HB: first degree heart block; 2° HB: second degree heart block; 3° HB: third degree heart block; AF: Atrial fibrillation; AICD: automatic implantable cardioverter defibrillator; PPB: permanent pacemaker; AV block: atrio-ventricular block; CHB: complete heart block; PR: P-R interval; VT: ventricular tachyarrhythmia, DCM: dilated cardiomyopathy.

* Three probands were come from the same family.

hiPSC Heart muscle cells from patients with Cardiac Laminopathy exhibit nuclear blebbing upon electrical stimulation

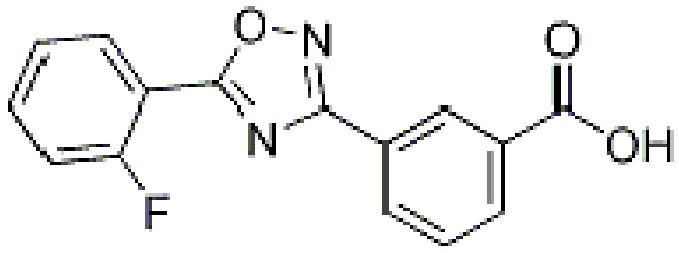


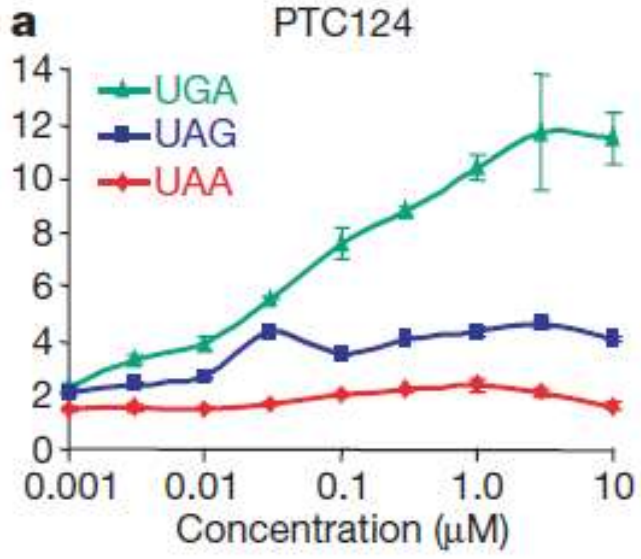


SIU CW Aging (2011)

PTC124 (Ataluren)

PTC 124 Chemical Structure

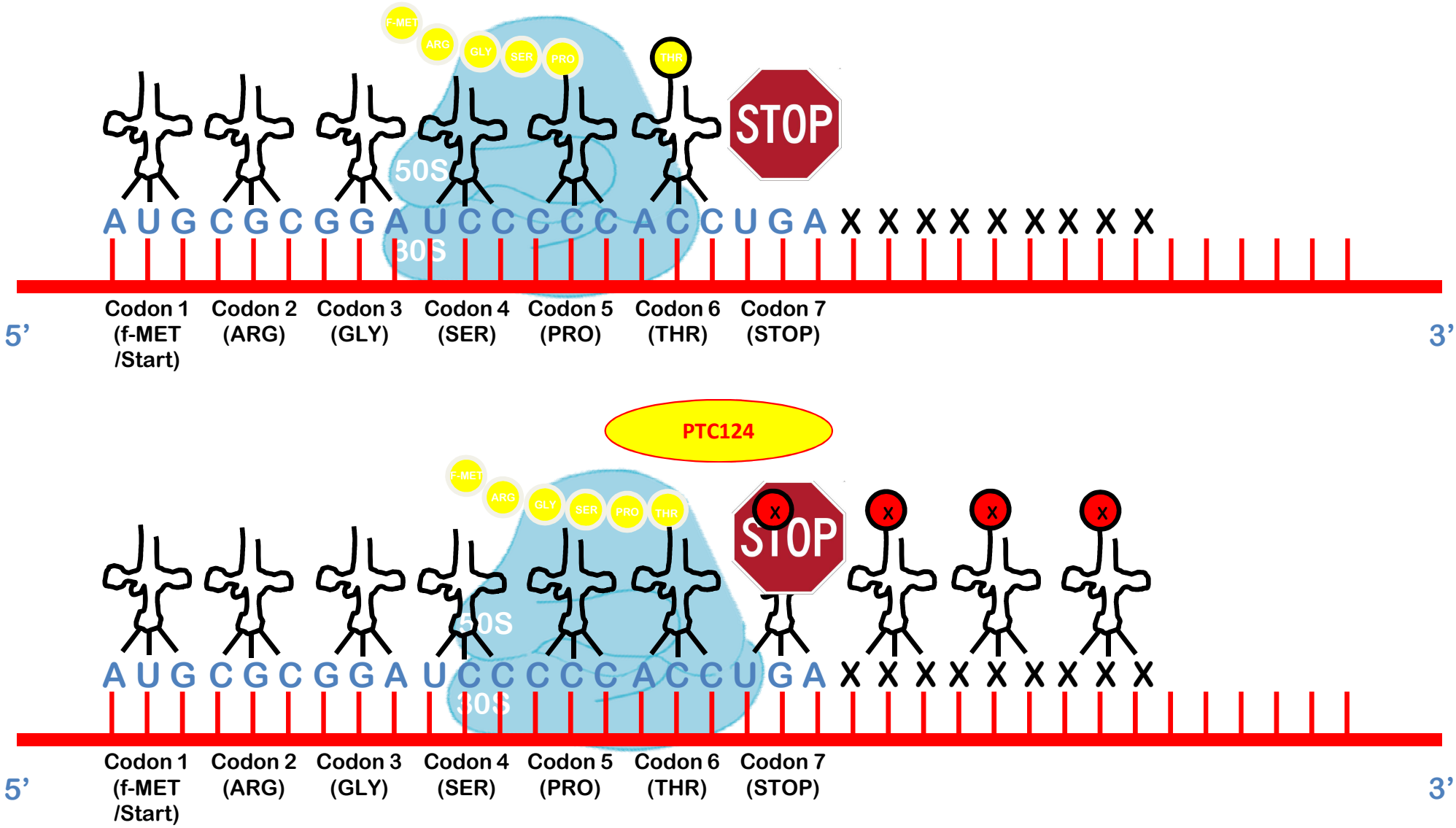




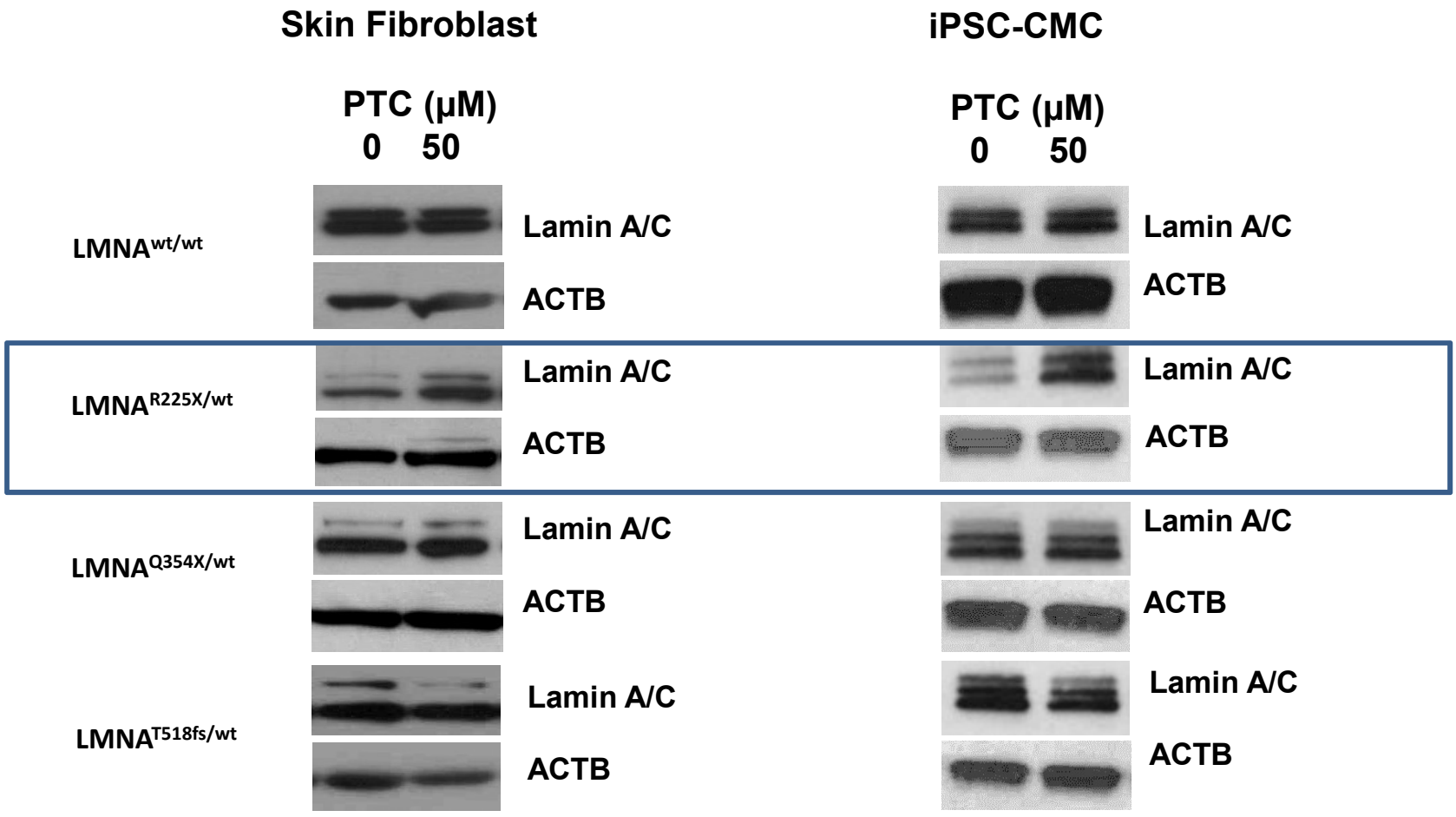
(Nature, Vol 447 2007)

- **PTC124 (Ataluren) is a novel small molecular CFTR-G542X nonsense allele inhibitor.**
- **In safety pharmacology studies in rats and dogs, oral administration of PTC124 (Ataluren) induces no adverse neurological, pulmonary, or cardiovascular effects at doses through 1500 mg/kg.**
- **In toxicology studies in rats and dogs at oral doses through 1500 mg/kg for 28 days, PTC124 (Ataluren) has shown good tolerability.**
- **FDA approved a new indication for orphan drug PTC 124 (made by PTC Therapeutics, Inc.), allowing its use in the treatment of Duchenne muscular dystrophy (MD) caused by a nonsense mutation in the dystrophin gene.**

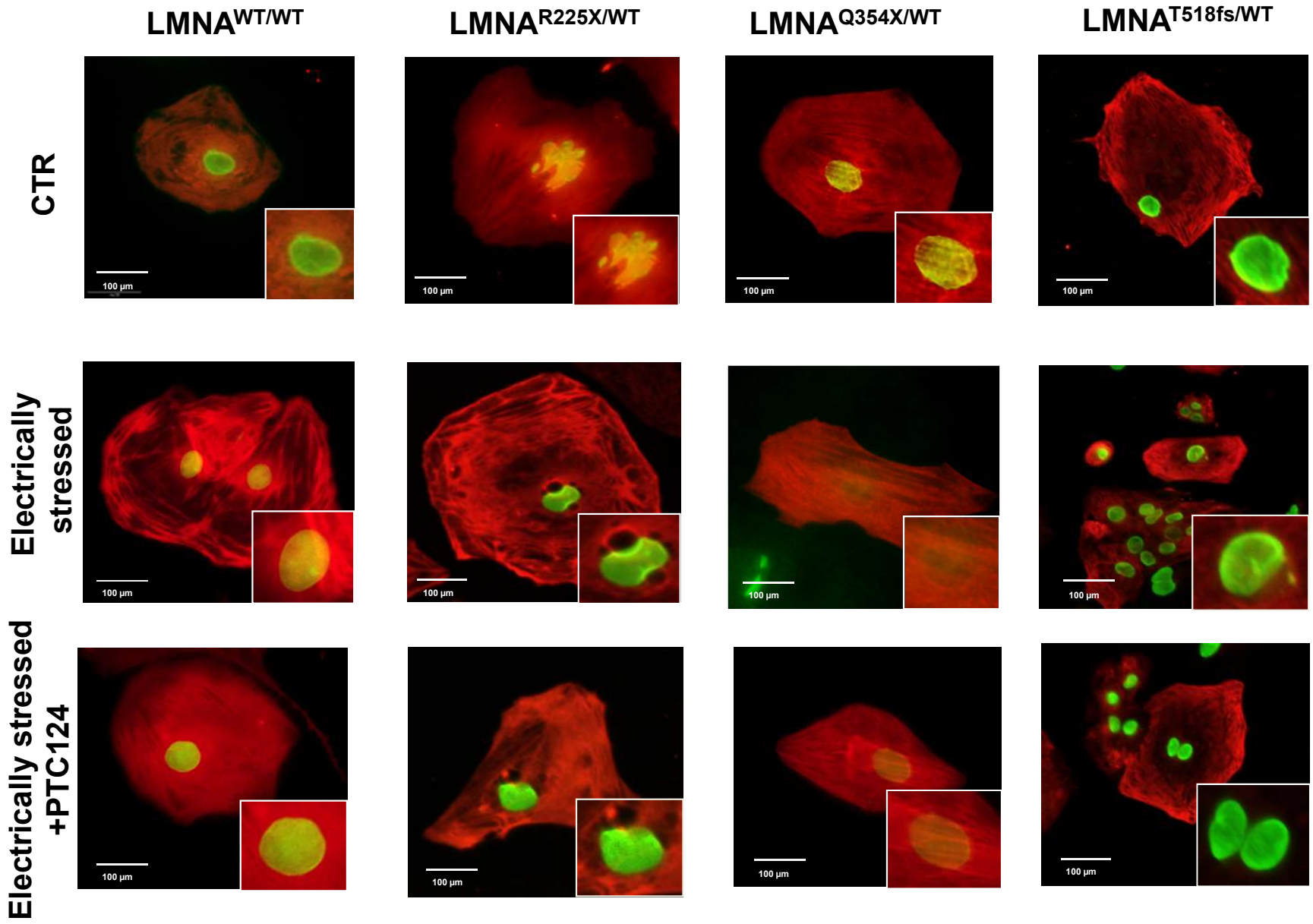
PTC124 reverses or alleviates nonsense mutation



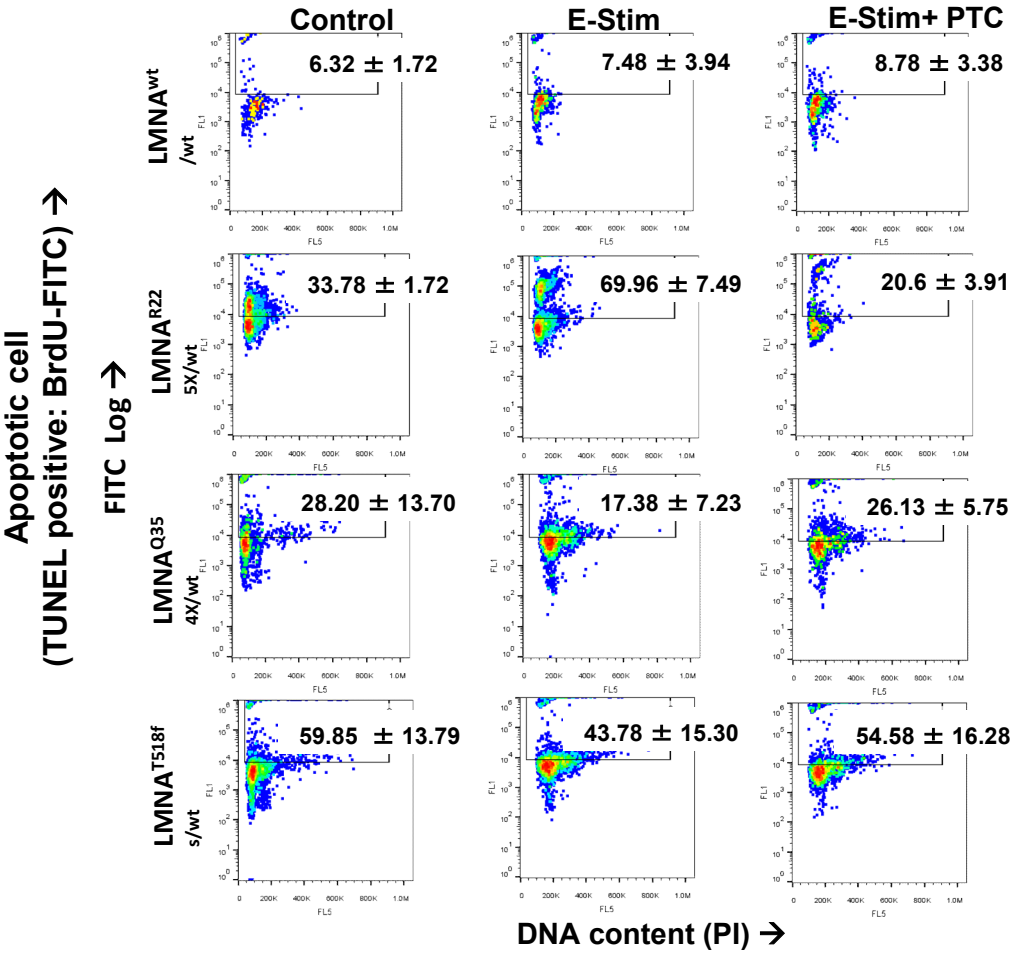
Effects of PTC124 on Expression of Lamin A/C Proteins in Dermal Fibroblasts and hiPSC-Derived Cardiomyocytes

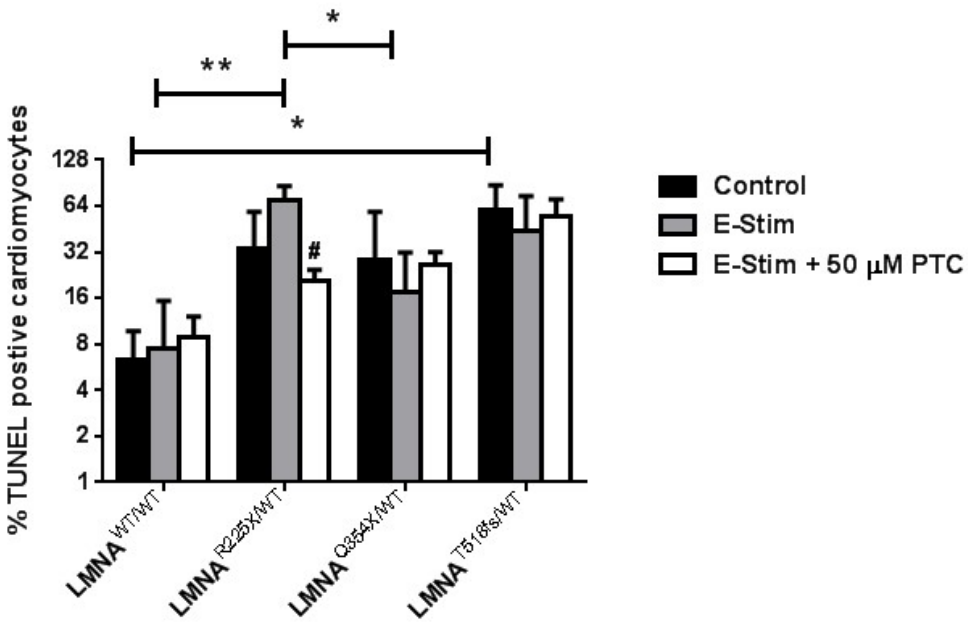


Nuclear blebbing in the hiPSC-derived cardiomyocytes



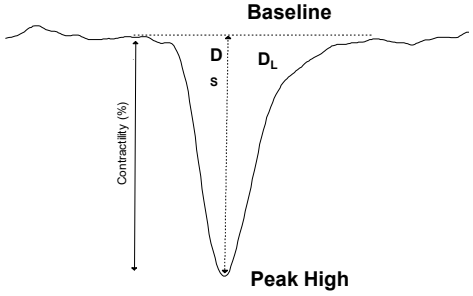
Evaluation of TUNEL-Positive Apoptotic Cell in Electrically Stressed and PTC124-Treated Cardiomyocytes





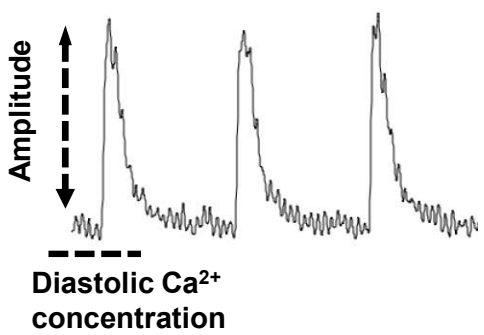
Simultaneous Recording of Cardiac Cell Contractile Force and Calcium Transients of Single Cardiomyocytes

Cell shortening: Video based edge detection used to record cell shortening

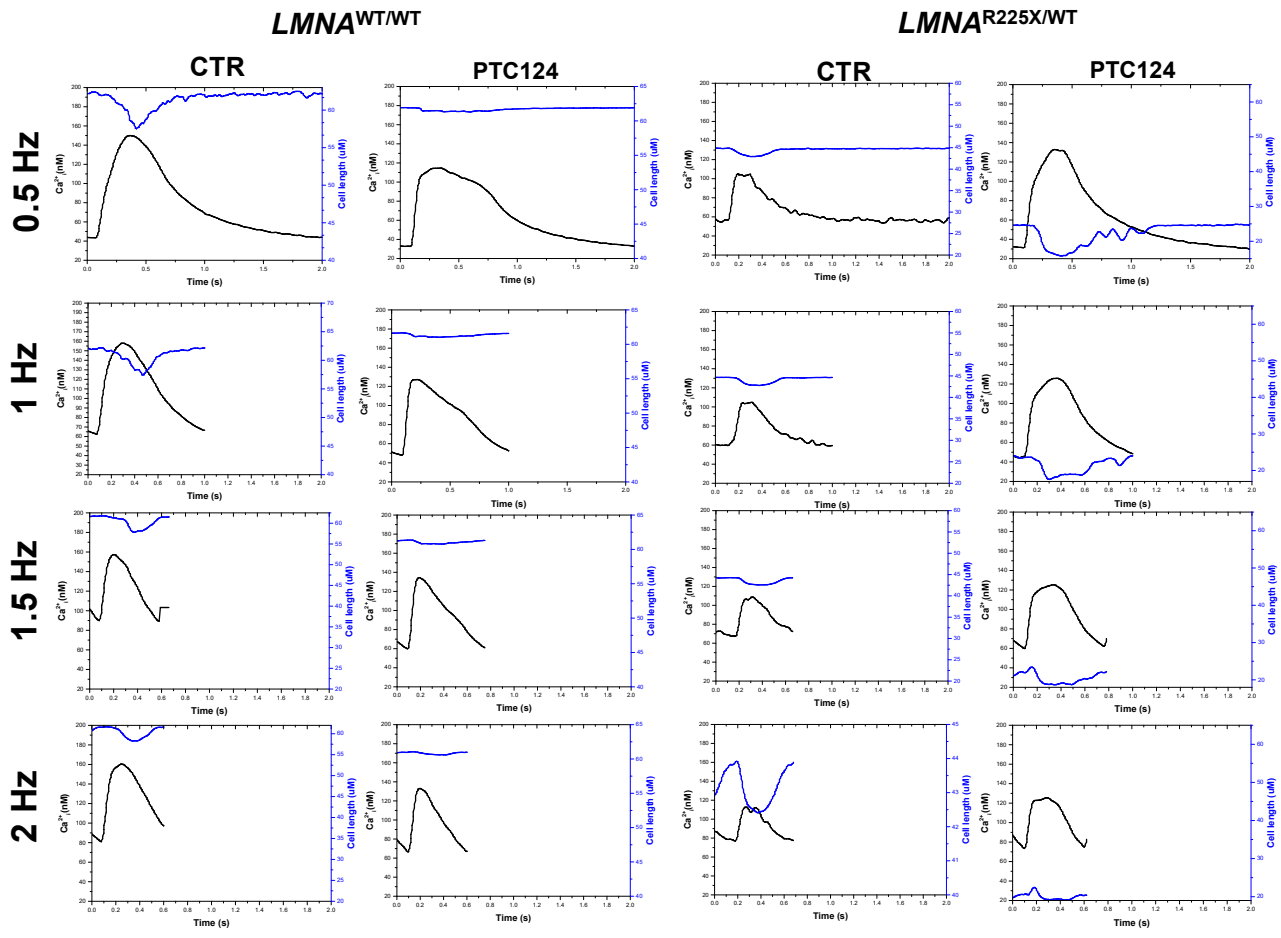


D_s: Duration of shortening
D_L: Duration of Lengthening

Ca^{2+} Transient: the calcium indicator, Fura-2 AM was loaded into cells



Field electrical pacing at 40 V at the frequency of 0.5 Hz, 1 Hz, 1.5 Hz and 2 Hz



Conclusions

- Precision medicine is a new trend in modern medicine. It aims to allow tailing disease treatment and prevention according to individual variability in genes, environment, and lifestyle for each person.
- hiPSC technology provides an unique platform for clinicians and scientists to study the underlying mechanisms of various diseases.
- More importantly, while the clinical manifestations are very similar in patients with the same disease, hiPSC technology allow better prediction to clinical responses prior to real clinical trials. This is particularly important for rare diseases, whose number of patients often too small for meaningful clinical trials.

Conclusions

- In fact, since 2008, we have generated more than 20 disease-specific hiPSC line. This hiPSC bank is a unique platform for innovative biomedical research and drug development in Hong Kong and Mainland China.
- The present work demonstrate the feasibility of hiPSC technology in precision medicine for rare disease, representing a step forwards to its clinical applications.