Sudden Cardiac Death Prevention with Molecular Diagnosis

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9-20-2017

Objectives

- To review the common and uncommon causes of sudden cardiac death (SCD)/cardiac arrest
- To understand the genetic basis of these conditions
- To understand the role of provocative diagnostic testing in unmasking the causes of cardiac arrest
- To explore the role of directed genetics testing in patient and family screening, and the future impact of genome-wide screening on propensity of sudden death

A story……..

- Around midnight on New Year's Eve, 2002, Kayla Burt, a sophomore guard on the University of Washington's women's basketball team, had just finished watching movies with friends and was getting ready for bed. The last thing that Burt recalls from that night was that, while brushing her teeth, she found herself chewing on the brush instead of going through the usual motions. Then everything went black. Burt collapsed as her heart stopped beating.
- When Burt's teammates saw her on the floor, they called 911. The emergency responders resuscitated Burt from what was diagnosed as sudden cardiac arrest, stabilizing her heartbeat before she faded into a coma. Burt survived.
- Her cardiologist diagnosed her with Long QT syndrome, an electrical abnormality of the heart that can cause life-threatening arrhythmias, or irregular heartbeats.

Sudden Cardiac Death/Arrest

- Sudden cardiac death (SCD/SCA) is an unexpected death due to cardiac causes that occurs in a short time period (generally within 1 hour of symptom onset) in a person with known/unknown cardiac disease
- Less than 5% of the patients will survive an episode of SCD
- The focus is on prevention! (for potential victims or family members)

Epidemiology of SCD

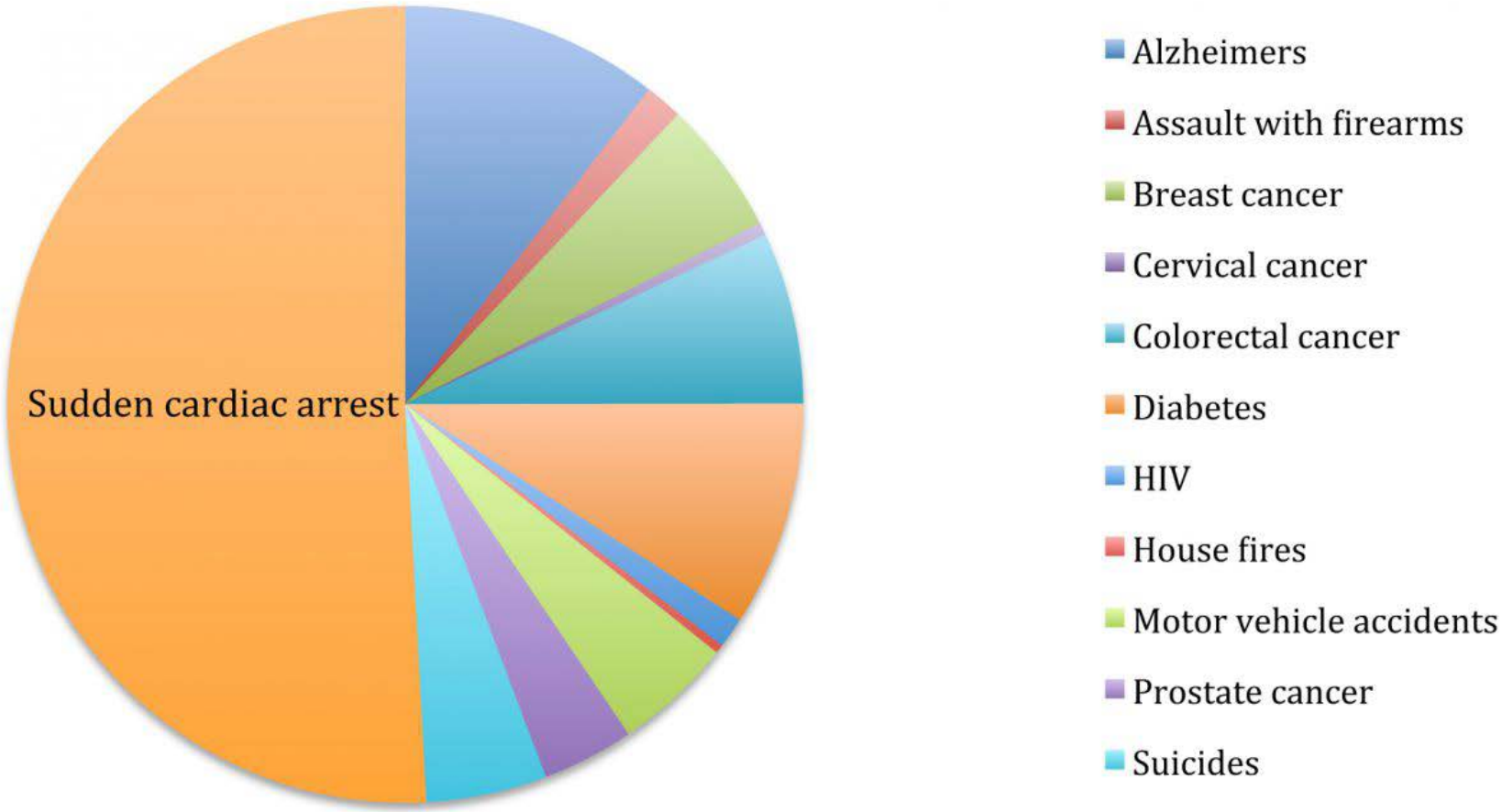
- Approx. 500,000 cases in U.S.A. per annum
- Ages of peak disease: 6 mos – 65 yrs
  - Incidence: 1-3 per 100,000 in those 1-35 yrs of age
  - Incidence: 10-75 per 100,000 in those 36-64 yrs of age
- May be the first presentation of cardiovascular disease in 25% of patients

Circulation, 2015; 123:1911-1918

American Heart Association.

Most Common Cause of Death

Accounts for 10-15% of natural deaths and 50% deaths from cardiac causes.



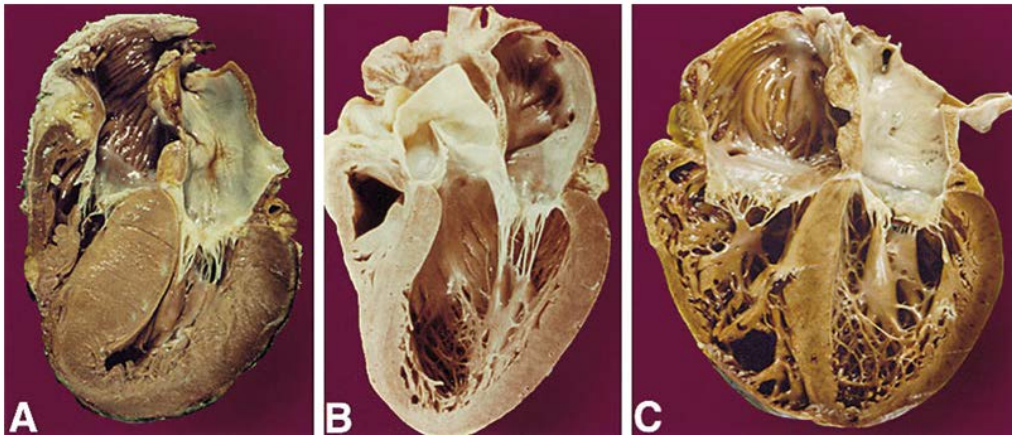
Importance and Relevance

- Higher in men than in women
- Increases with age due to the higher prevalence of CAD in older age

Causes of SCD

- Older (Over 35 yrs of age)
  - Coronary Heart Disease (‘hardening of the arteries’): multi-factored, genetics and environmental, life style
- Young (Under 35 yrs)
  - Cardiomyopathies (heart muscle disorder)
  - Congenital Heart Disease (‘hole in heart’, ‘blue baby’)
  - Channelopathies: ‘Structurally Normal Heart’ (ion channel disorders, conduction disease) such as Long QT
  - Myocarditis (infection or inflammation of heart muscle)
  - Substance abuse

Sudden Cardiac Death Syndrome < 35yrs



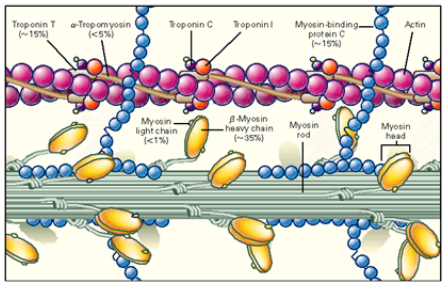
HCM Normal DCM

- Hypertrophic and dilated cardiomyopathies, Long QT
- Prevalence 1:500 (HCM)-1:10,000 (LQT)
- Altered cardiac morphology (H/DCM)
- Arrhythmia (LQT)
- Leads to syncope, palpitation, heart failure, sudden death

Genetics Basis of SCD

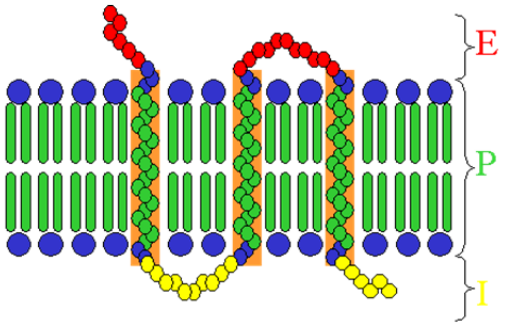
- Inherited single gene disorders
- Autosomal dominant
- Heterogeneous
- Known gene account for 70-80% cases
- Opportunity for genetic testing

H/DCM



MYH7
MYBPC3
TNNT2

LQT



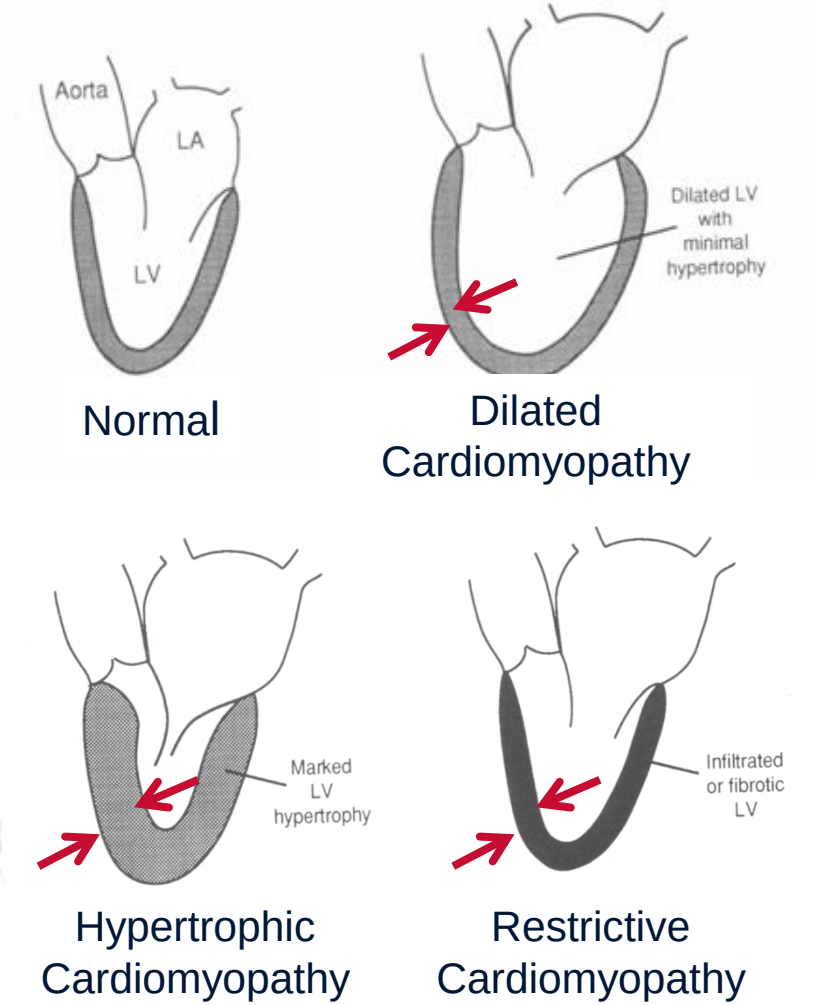
KCNQ1
KCNH2
KCNE1
KCNE2
SCN5A

What is Cardiomyopathy?

- Cardio=heart, Myo=muscle, Pathy=disease
- Cardiomyopathy: disease of heart muscle

Anatomy & Physiology of Cardiomyopathy

- Dilated
  - Muscle weakness
  - Enlarged
  - Systolic Dysfunction
- Hypertrophic
  - Thickened
  - Diastolic dysfunction
- Restrictive
  - Diastolic dysfunction



Circ 93:841, 1996

CM: Specific Etiologies

- Ischemic
- Valvular
- Hypertensive
- Inflammatory
- Metabolic
- Inherited
- Toxic reactions
- Peri-partum

Inherited Cardiomyopathy

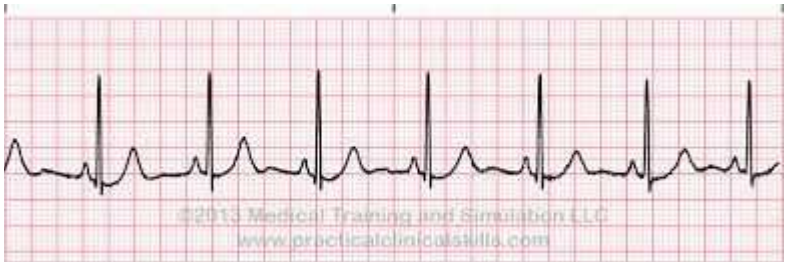
- Hypertrophic cardiomyopathy
  - ~ 55% of cases with autosomal dominant transmission
  - Mutations in 1 of 4 genes encoding proteins of cardiac sarcomere
  - *MHY7, MYBP3, TNNT, TNNI3*
- Dilated cardiomyopathy
  - 50% are familial
  - Inheritance pattern: autosomal dominant, recessive, X-linked and mitochondrial
  - Up to 30 genes, 20% of DCM having mutation in *TTN*

Cardiac Arrhythmias

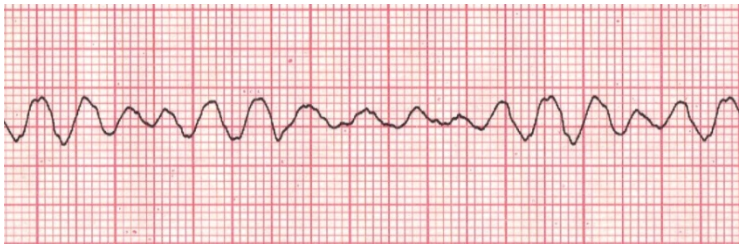
Arrhythmia: variation in normal rhythm

- Ventricular
  - Ventricular tachycardia (VT)
  - Ventricular fibrillation (VF)
  - Ventricular premature beats
- Atrial
  - Atrial fibrillation (AF)
  - Atrial flutter
  - Atrioventricular nodal re-entrant tachycardia (AVNRT)
  - Atrioventricular re-entrant tachycardia (AVRT)
  - Atrial tachycardia (AT)
  - Sinus tachycardia
  - Inappropriate sinus tachycardia
  - Atrial premature beats

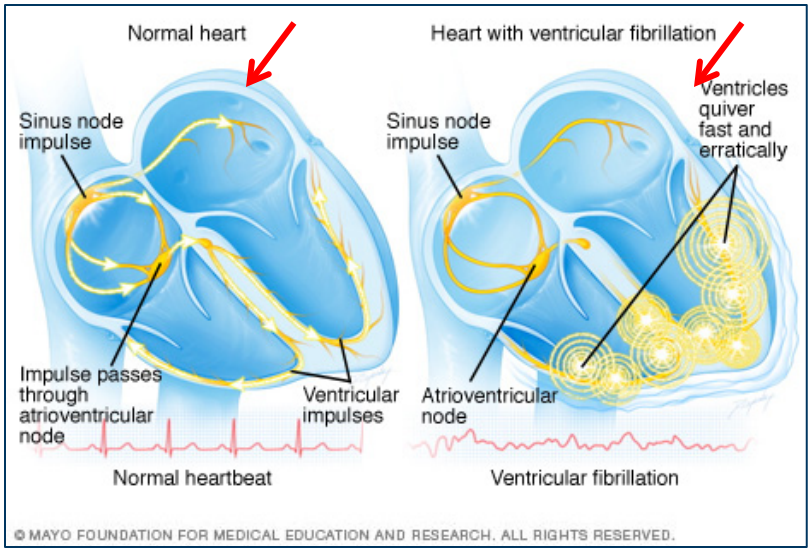
Ventricular Fibrillation



Normal



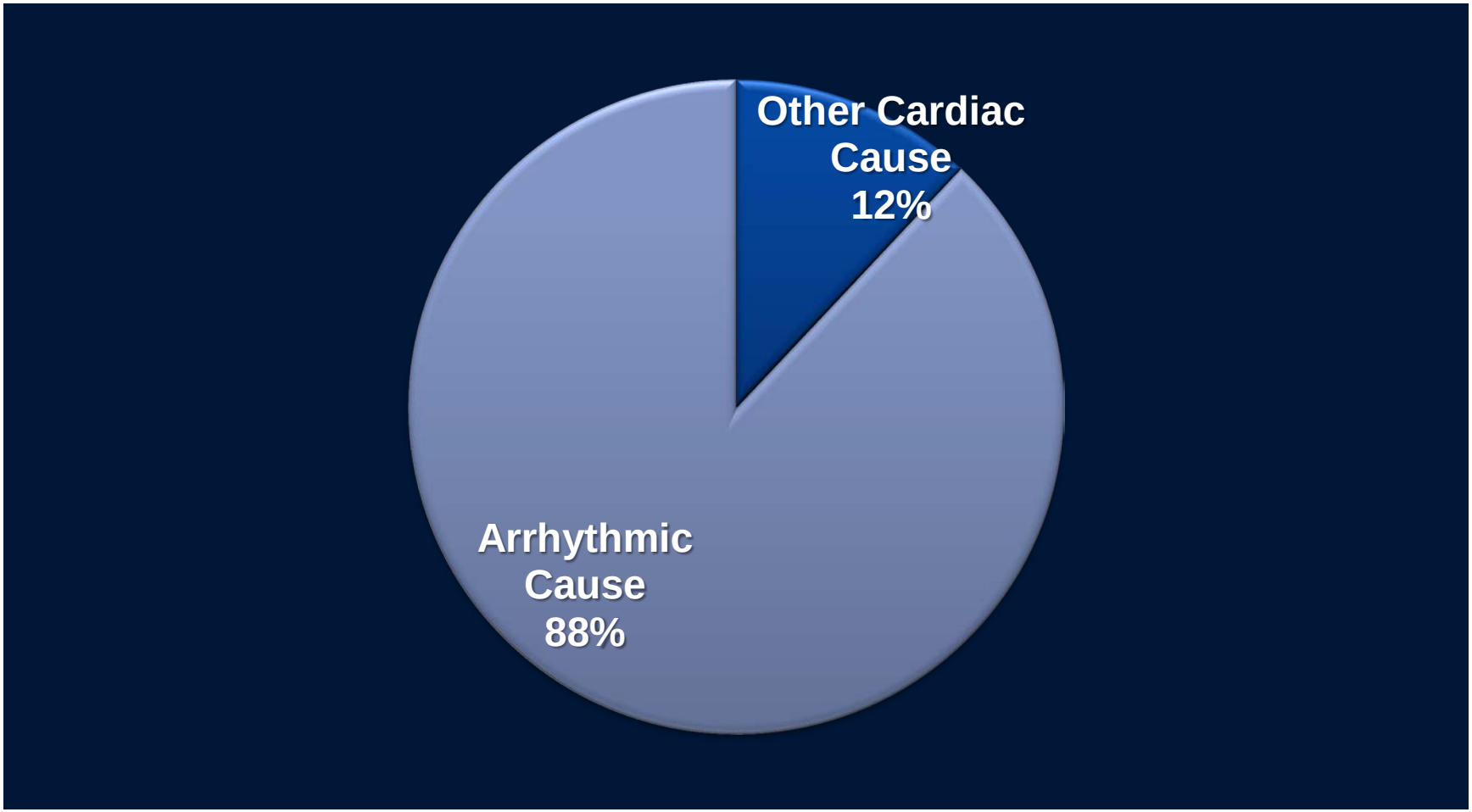
Ventricular fibrillation



Disorganized electrical pulses causes the ventricles to quiver instead of pumping blood.

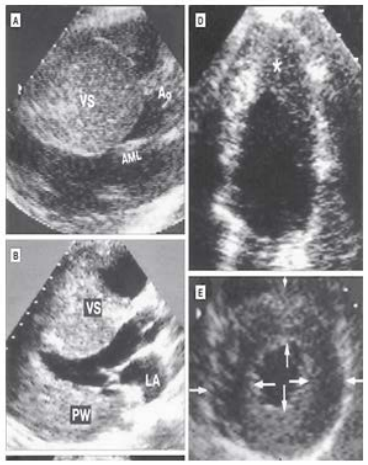
If untreated, causes sudden cardiac arrest and death!

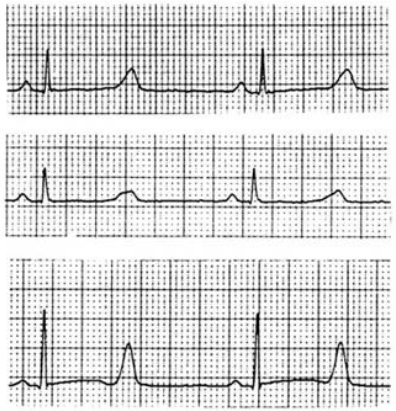
Arrhythmia and SCD



SCD Current Diagnosis

- Clinical/Family History
- ECG
- 2-D Echocardiography
- Tissue Doppler/MRI
- Genetic Testing?



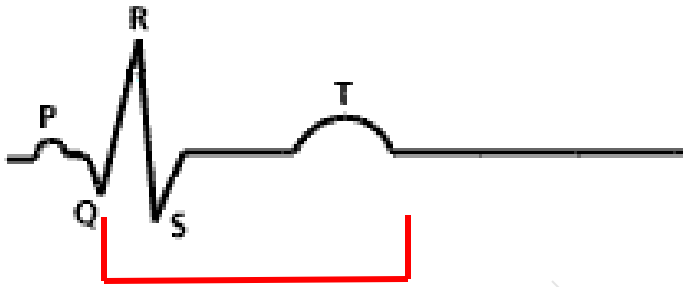


Genetics in Arrhythmias

- For 1st time - DNA analysis should be a fundamental component of postmortem assessment in SCD victims, especially in the young.

Long QT Syndrome

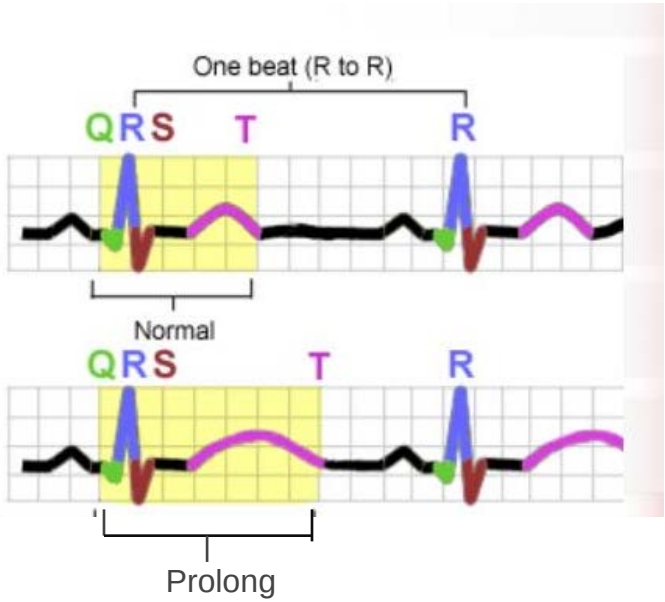
- Common cause of sudden death in children and young adults
- 1:7,000 births
- In the U.S. it causes ~5% of SCD/year
- Symptoms include syncope or SCD usually with physical activity or emotional stress



QT interval

Pathophysiology of Long QT syndrome

- Abnormalities of ion channels that result in long QT intervals (prolongation of phase III-time for repolarization) and predispose to polymorphous ventricular tachycardia (“Torsade de Pointe”)
- Consequently, the wave of excitation may pursue a distinctive pathway around a focal point in myocardium (circus reentrant rhythm), leading to polymorphous ventricular tachycardia, syncope, and possibly sudden death.



Genetics in Long QT

- 60% of Long QT syndrome patients are known genetic causes
- LQT1-LQT7
- All encode for K channels except LQT3 which is linked to the Na channel - very good test question….

LQTS: Genetics

- LQT1; *KCNQ1 (KvLQT1); chr11; Trigger: Stress*
- LQT2; *KCNH2 (hERG); chr7; Trigger: Startle (e.g. sudden noise)*

*LQTS1 and 2 = **87**% of known LQTS*

- LQT3 ; *SCN5A* ; *chr3*; Trigger: Sleep, rest. Beta blocker therapy seems to be the less effective; Ina…***8% of known LQTS***
- LQT4 ????? Gene unknown

LQTS: Genetics

- LQT5 ; *KCNE1* ; *chr21* ; Associated to the Jervell and Lange-Nielsen (JLN) syndrome (congenital deafness)
- LQT6 ; *KCNE2*; *Chr21* ; Triggers: certain drugs, exercise
- LQT7 ; *KCNJ2* ; *Chr17* ; Associated with the **Andersen syndrome** (periodic paralysis and skeletal developmental abnormalities)

Long QT Syndrome (LQTS) Management

- Identify the disease carriers in the family (DNA test familiar mutations)
- For patient with DX of LQTS
  - Avoidance of QT-prolonging drugs
  - Correction of electrolyte abnormalities (hypokalemia, hypomagnesemia, hypocalcemia)
  - Avoidance of genotype-specific triggers for arrhythmias (strenuous swimming in LQT1 patients)
- All patients with a diagnosis with LQTS were recommended a beta-blocker
- ICD implantation with the use of beta-blockers is recommended in LQTS patients with previous cardiac arrest

Identify the individuals at risk

Too Young to Die

Need for Cardiac Disease Panel

- Challenges to identify individual at risk for SCD
  - Genetic heterogeneity: e.g. Long QT syndrome has at least 11 known genes
  - Mutation identification facilitates proper treatment
- Next generation sequencing (NGS) offers a potential solution for the molecular diagnosis of complex inherited disorders
  - Cost effective: $$ *TTN* Sanger Sequencing ≈ $$ 85 gene NGS panel including TTN!

Cardiomyopathy and Arrhythmias Panel (85 Genes)

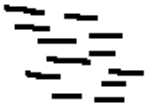
- Hypertrophic Cardiomyopathy: 24 genes, MYH7+MYBPC3=40%
- Dilated Cardiomyopathy: 32 genes, TTN=20%
- Long QT syndrome: 11 genes
- Short QT syndrome: 3 genes
- Brugada syndrome: 8 genes, SCN5A=15-30%
- Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT): 3 genes
- Left Ventricular Non-Compaction (LVNC): 8 genes
- Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC): 8 genes

NGS workflow

Blood

DNA

Shear DNA



Illumina library prep

Genome

Target enrichment

Panel genes
or exome…

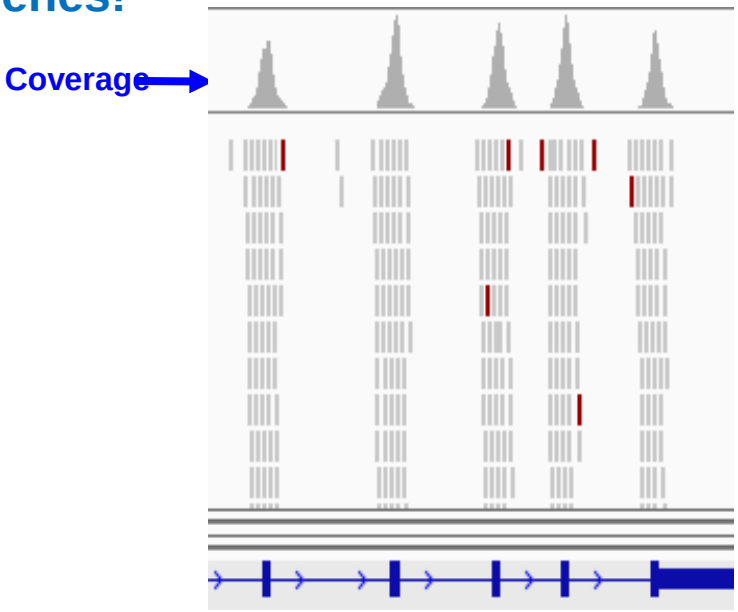
Sequencing & Data analysis

Bravo Platform

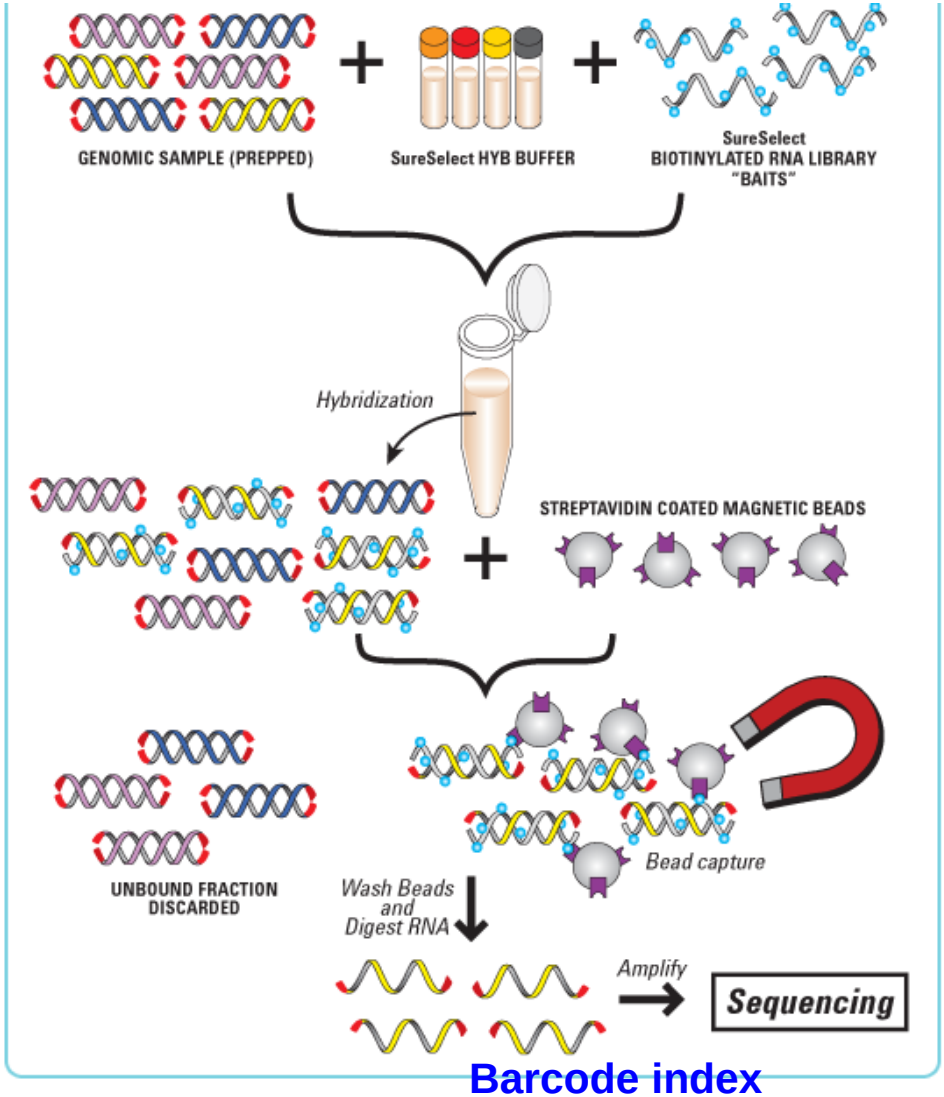
Bravo automation

SureSelect target enrichment

Genomic DNA…want just aortopathy genes!

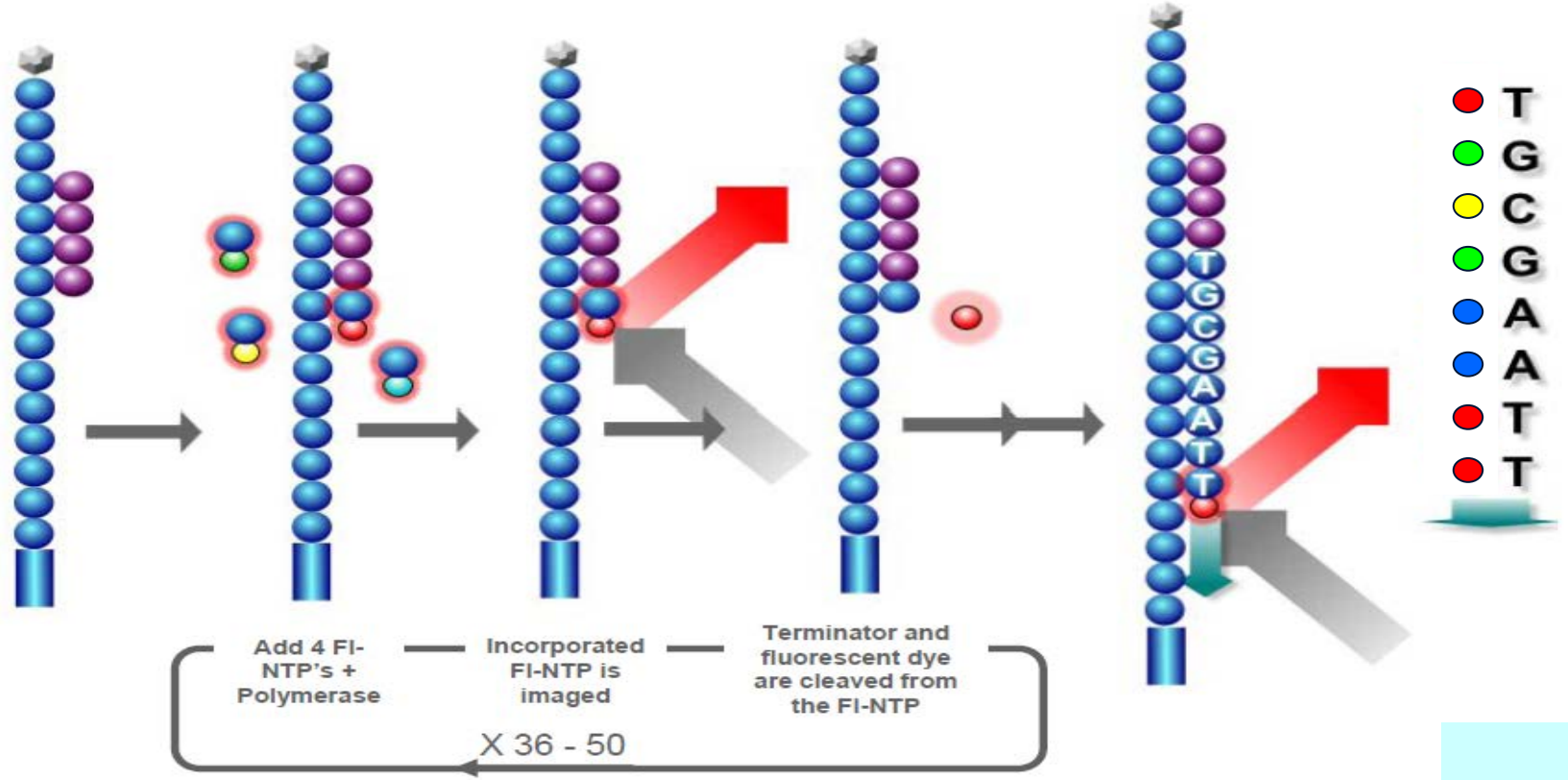


***MYBPC3* exons**



Courtesy to Dr. Whitney Donahue

Illumina Sequencing



4 reversible dye terminator NTPs

Incorporate one nt, Image

Terminator & dye cleaved

illumina

Illumina Sequencing

A
C
G
T

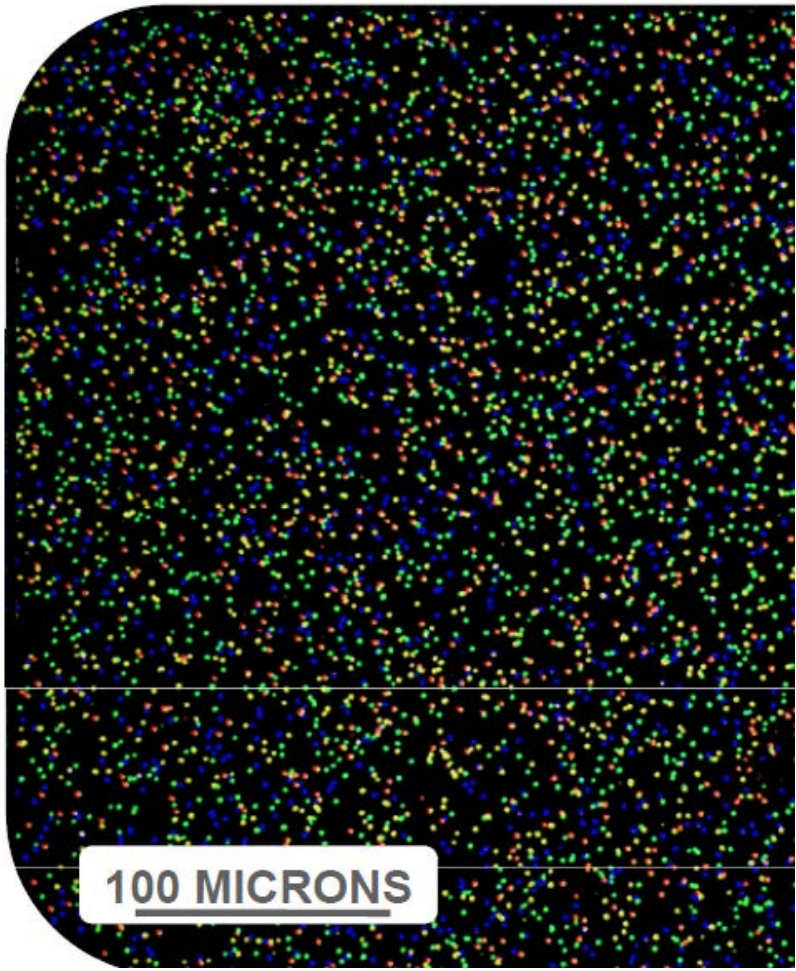
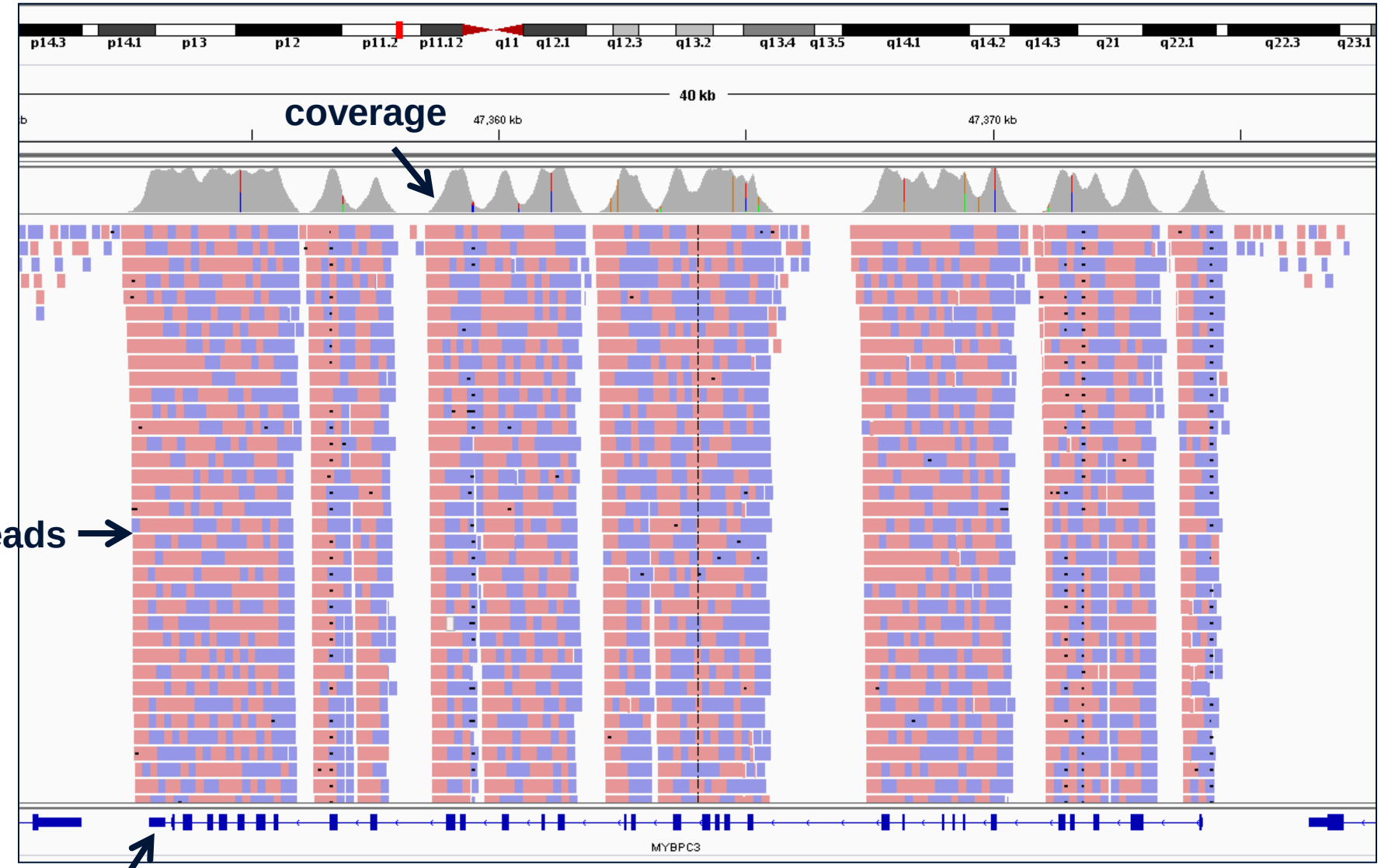


Image of clusters during sequencing.

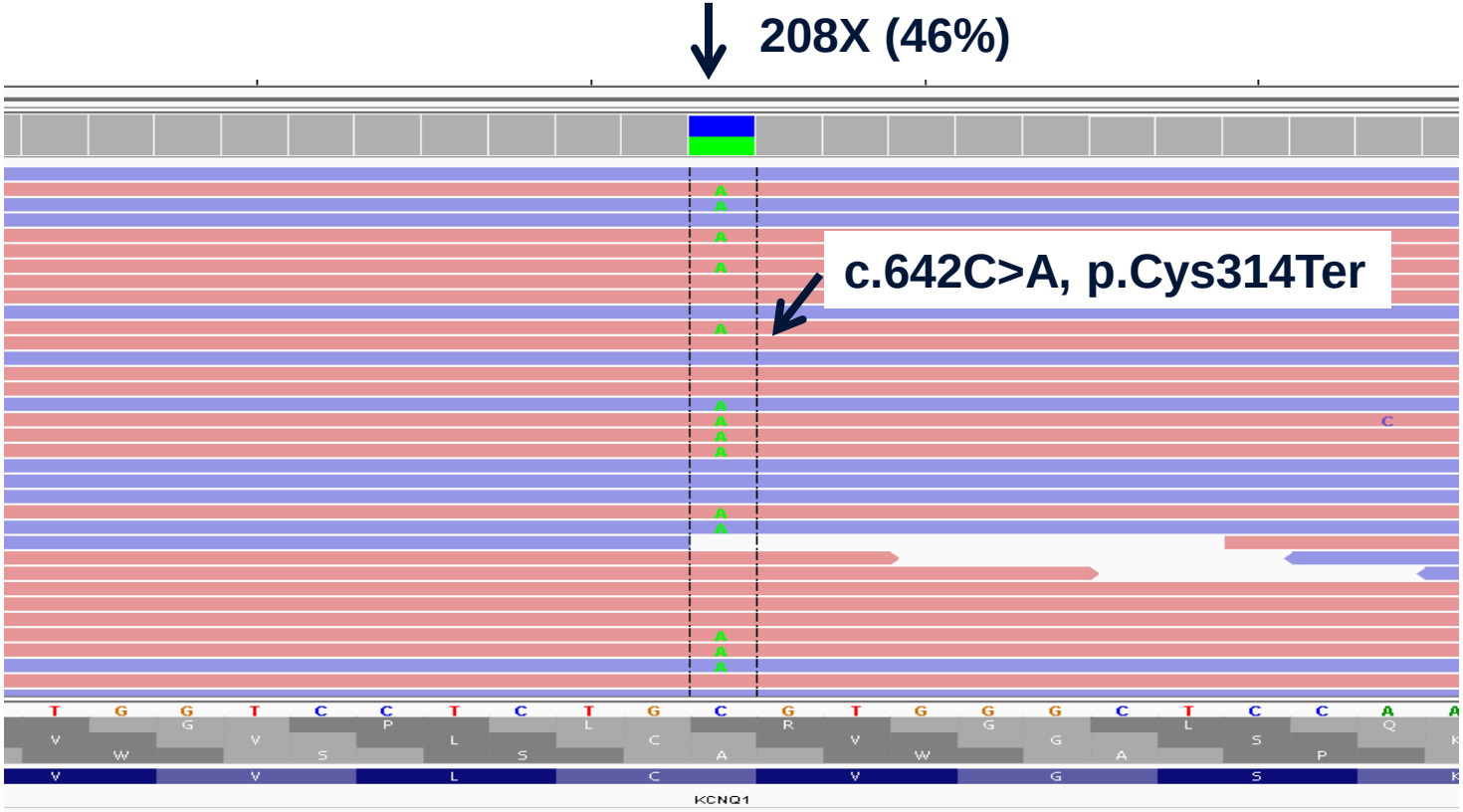
Gene Coverage



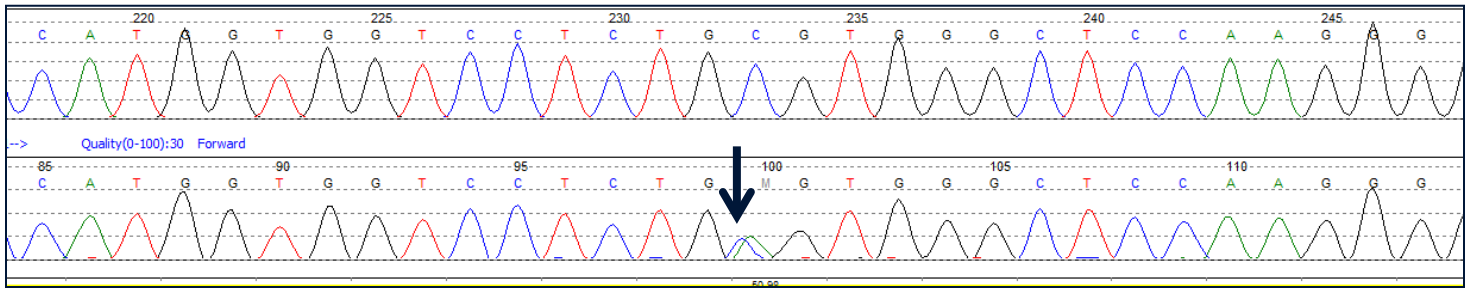
MYBPC3

Pathogenic *KCNQ1* Mutation

NGS data



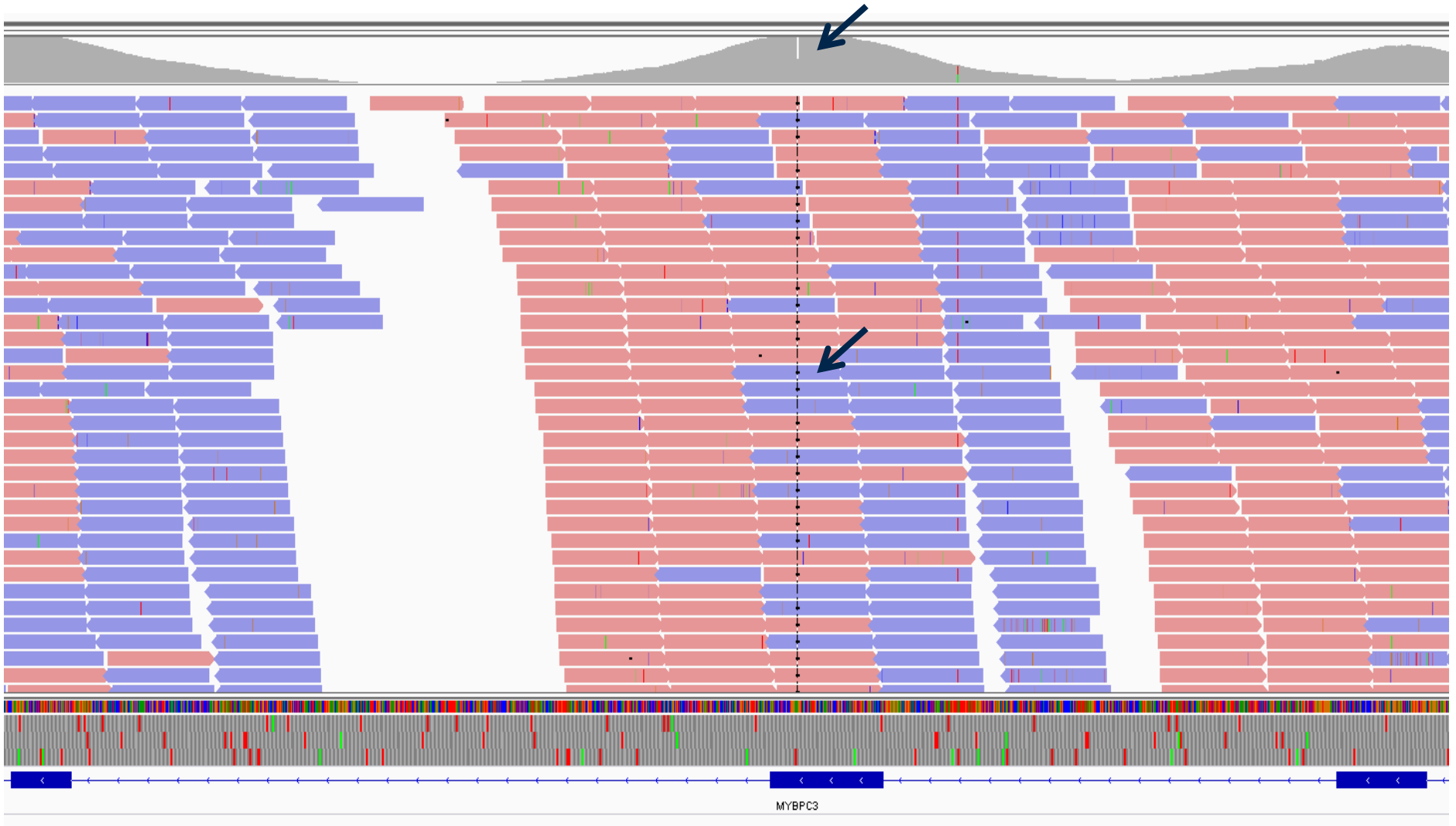
Sanger Confirmed



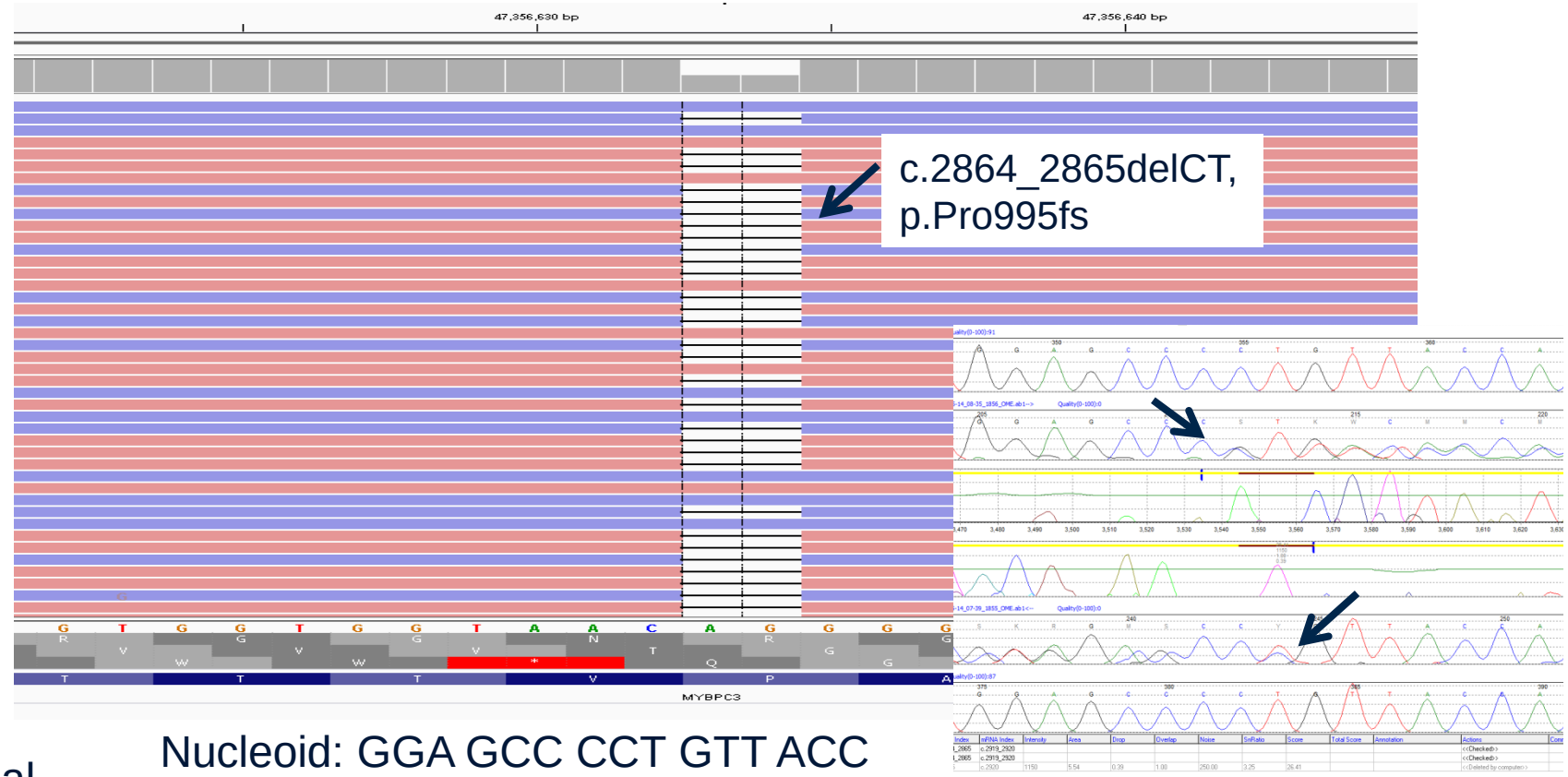
Interesting Case

- 65 yrs. Caucasian male
- no symptoms
- Cardiac findings: abnormal ECG, abnormal ECHO
  - Atrial fibrillation
  - Implanted pacemaker
- No previous DNA testing
- No family history of cardiomyopathy or arrhythmias
- Tested with Cardiac panel 85 genes

MYBPC3 Gene



MYBPC3 Gene Frameshift Mutation



Normal

Nucleoid: GGA GCC CCT GTT ACC
Protein: Gly Ala Pro Val Thr

Frameshift

Arg Tyr His

Nucleoid: GGA GCC C**XX** GTT ACC ACC
Protein: Gly Ala Pro Val Thr Thr

MYBPC3 causes HCM

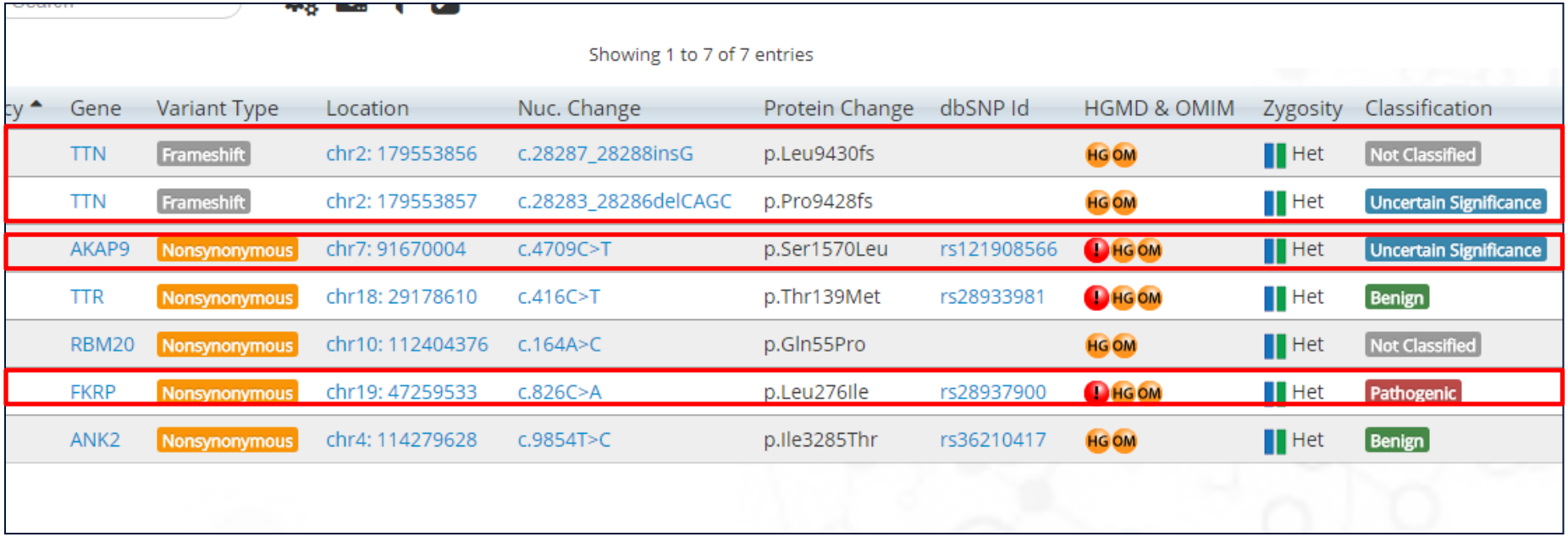
- *MYBPC3:* cardiac myosin-binding protein, assist the structure and function of sarcomere
- *MYBPC3* mutations: hypertrophic cardiomyopathy (HCM) or dilated cardiomyopathy (DCM)
- Autosomal dominant
- Age of onset: teenage to adult
- Arrhythmias, angina, sudden death

Molecular diagnostics dilemma: which variant(s) is/are disease causing?

Interesting Case

- 77 yrs. Caucasian female
- Symptoms: hypertension
- Cardiac findings: abnormal ECG and ECHO
  - normal wall thickness
  - ejection fraction reduced - 28%
  - atrial fibrillation and atrial flutter
- No previous DNA testing for pt
- Family history of cardiomyopathy/arrhythmia: YES-previous DNA testing, but reports are not available

Variants Found

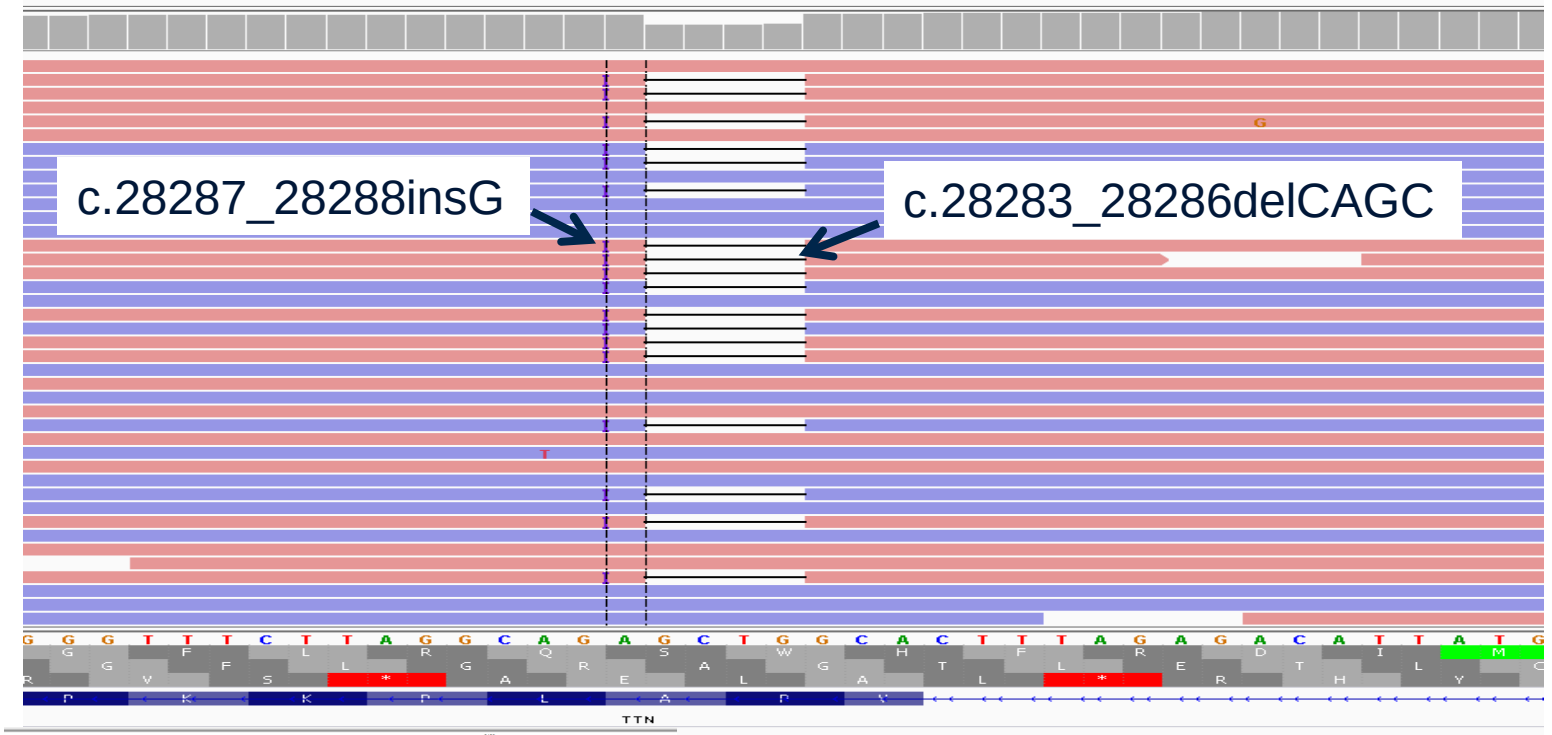


Showing 1 to 7 of 7 entries

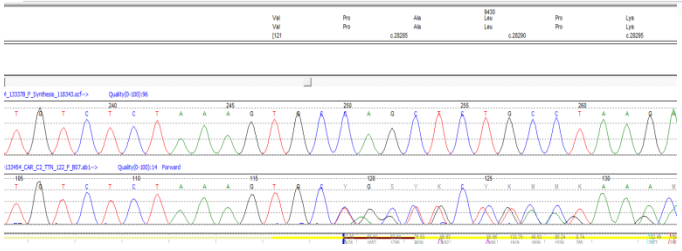
cy	Gene	Variant Type	Location	Nuc. Change	Protein Change	dbSNP Id	HGMD & OMIM	Zygosity	Classification
	TTN	Frameshift	chr2: 179553856	c.28287_28288insG	p.Leu9430fs		HG OM	Het	Not Classified
	TTN	Frameshift	chr2: 179553857	c.28283_28286delCAGC	p.Pro9428fs		HG OM	Het	Uncertain Significance
	AKAP9	Nonsynonymous	chr7: 91670004	c.4709C>T	p.Ser1570Leu	rs121908566	! HG OM	Het	Uncertain Significance
	TTR	Nonsynonymous	chr18: 29178610	c.416C>T	p.Thr139Met	rs28933981	! HG OM	Het	Benign
	RBM20	Nonsynonymous	chr10: 112404376	c.164A>C	p.Gln55Pro		HG OM	Het	Not Classified
	FKRP	Nonsynonymous	chr19: 47259533	c.826C>A	p.Leu276Ile	rs28937900	! HG OM	Het	Pathogenic
	ANK2	Nonsynonymous	chr4: 114279628	c.9854T>C	p.Ile3285Thr	rs36210417	HG OM	Het	Benign

TTN Gene Variant: In Frame deletion/Insertion

NGS data



Sanger confirmation

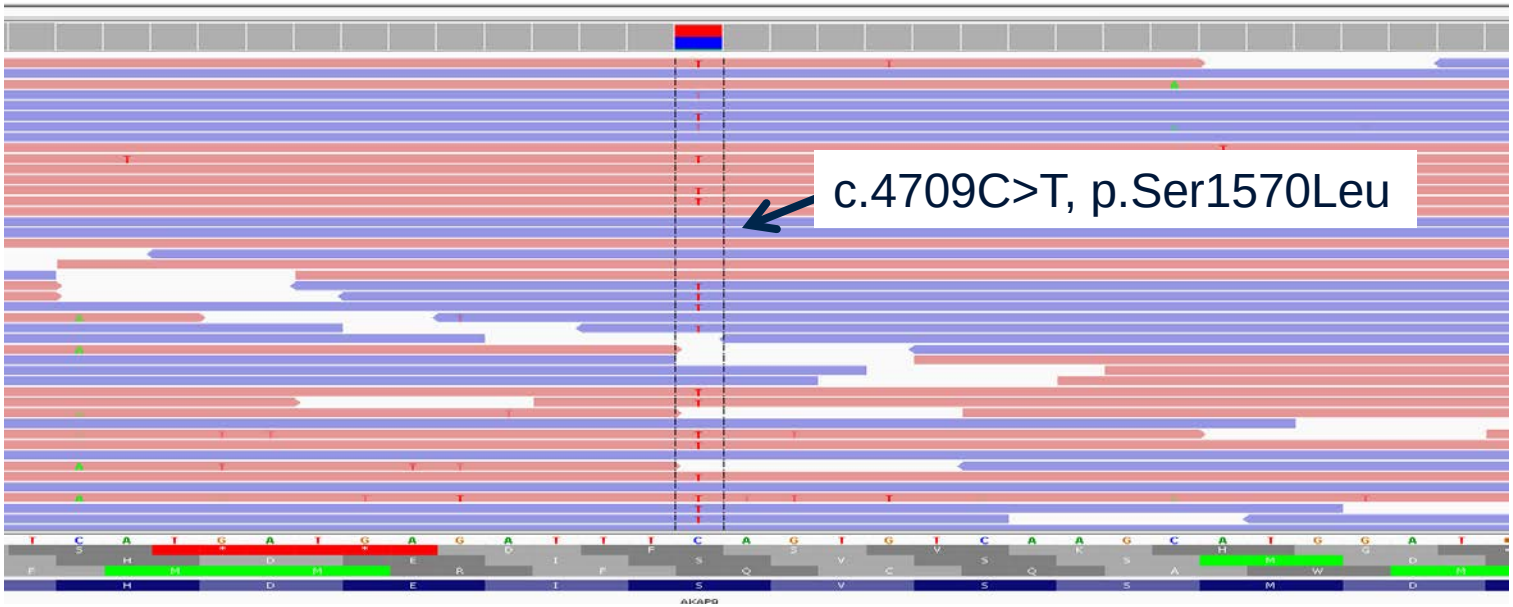


c.28283_28287delinsTG, -5bp,+TG;
p. Pro9428_ala9429delinsLeu, -proline and alanine, +leucine.

Disease causing? Not sure

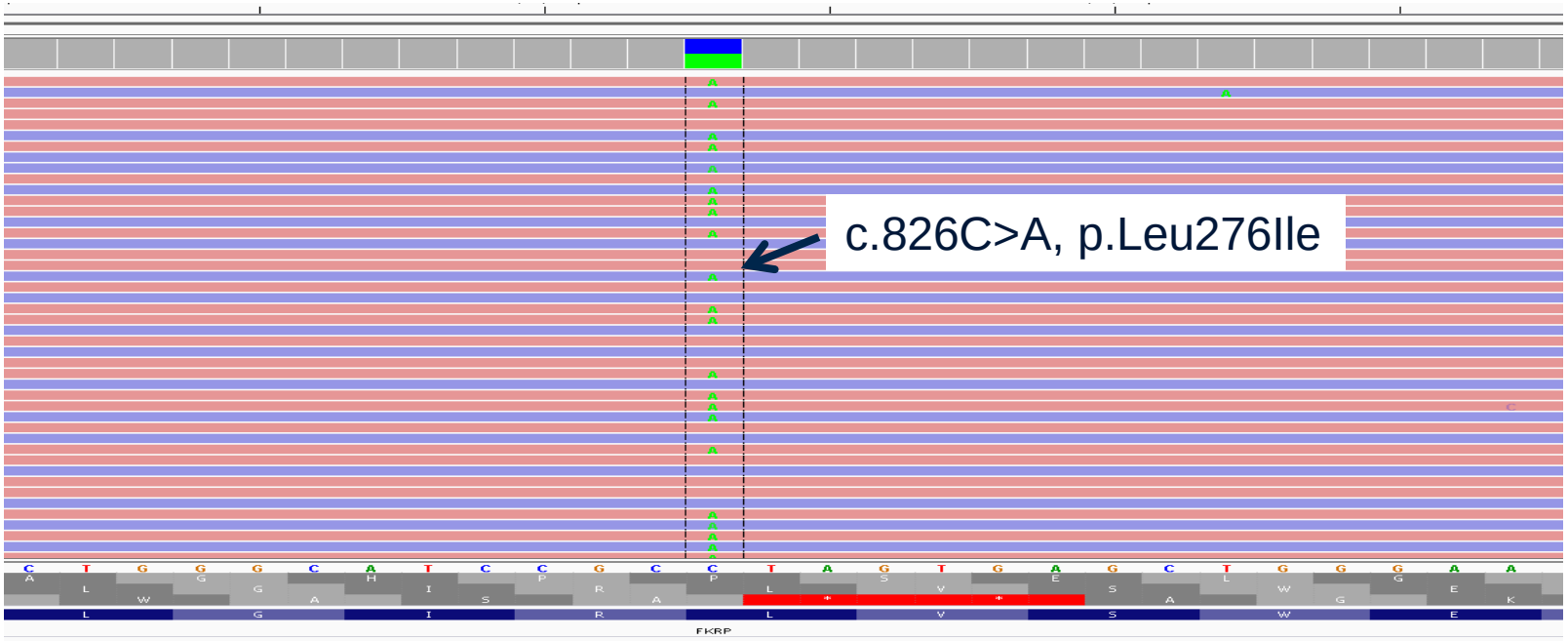
TTN mutations have variable phenotype; can causes either HCM, DCM, early onset with fetal cardiomyopathy

AKAP9 Gene Variant: Missense



AKAP9 variant p.Ser1570Leu has been reported in a LQT family, however, limited information, the clinical significance is uncertain (VUS). *AKAP9* mutations can cause dominant LQT-11.

FKRP Gene Variant: Missense



FKRP mutations are causing autosomal recessive Muscular dystrophy-dystroglycanopathy, left ventricular hypertrophy. Leu276Ile is known disease causing mutation, un-detected second mutation, however, not test deep intron and promoter regions.

Question?

- Which gene/mutation response to patient's disease?
- One of them? All of them? Or none of them?

Uncertain!

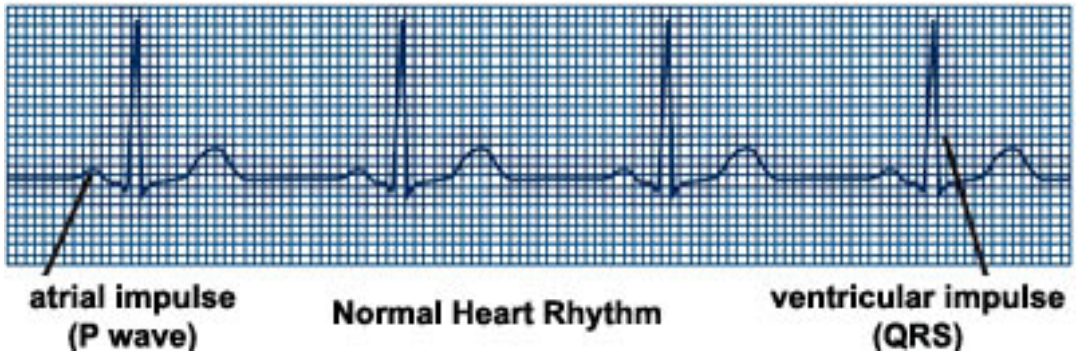
Who should have genetic testing?

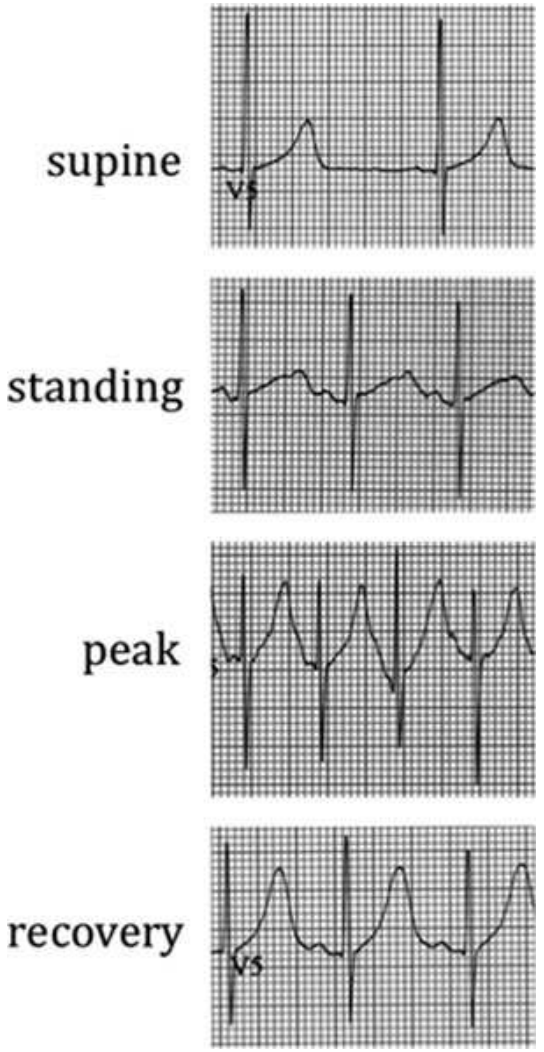
- Patient has symptoms, hard breathe, chest pain, cardiac arrest, SCD
- Abnormal cardiac findings: ECG, Echocardiography
- Family history
- If familial mutation identified, testing should be offer to all family members at risk

Interesting case

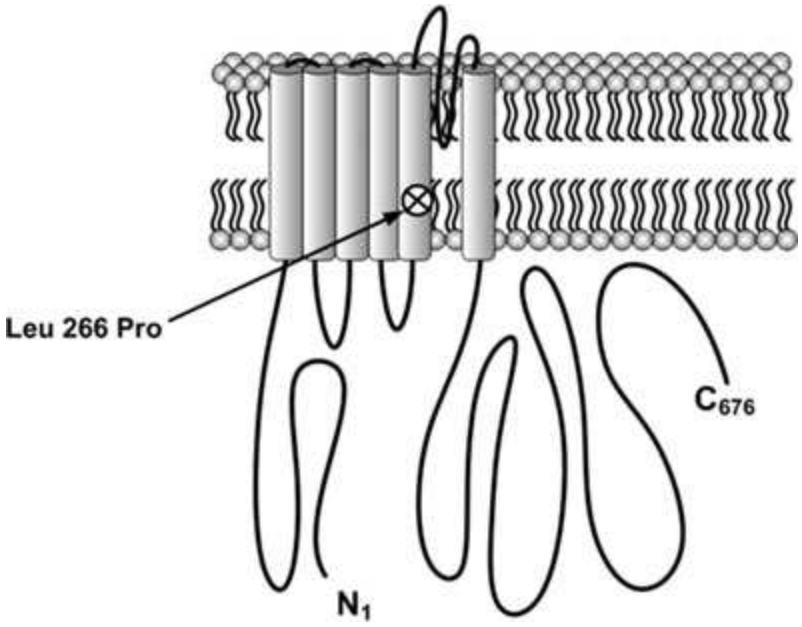
- 51 yo male with cardiac arrest
- 4 of 11 siblings died in their 40s and 50s of “heart attack” (all male) with presumed myocardial infarction
- Coronaries normal, ECHO normal
- 13 yo brother unexplained drowning

Identify asymptomatic carrier



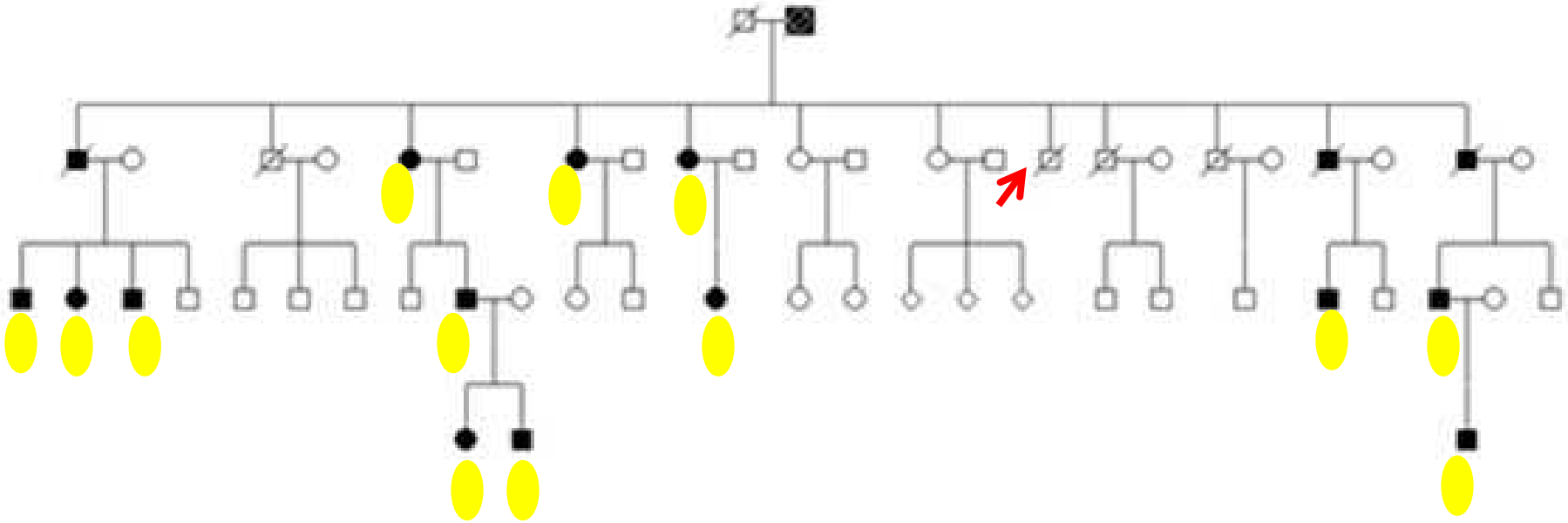


Genetic Testing



Gene: KCNQ1, c.797T>C, p. Leu266Pro

Family Pedigree



Identify the individuals at risk

- Screening the relatives or people with suggestive symptoms
  - Many conditions relatively easy to identify (if you know what you're looking for)
  - Not everyone affected is at risk
  - Varying success rates at accurately identifying at-risk people
  - Some can be treated with medication
  - High-risk people offered implantable defibrillator (ICD or 'shock-box')
  - Future generations at risk

Managing risk

- Avoid competitive sport or very strenuous exertion
- Recreational sport, PE classes, etc. usually safe
- Medications in some (e.g. b-blockers)
- Continued observation in all
- Implantable defibrillators in some
  - Cost implications
  - Complications

Summary

- Sudden cardiac death(SCD) is one of the most common causes of death in U.S.
- Early diagnosis of the condition can maximize optimal medical therapy.
- Genetic testing can discover the disease-causing gene/mutation; therefore, identify at-risk family members. Cure is not possible, but correct management can prevent SCD.

Acknowledgement

ARUP R&D and Bioinformatics

- Shale Dames
- Genevieve Pont-Kingdon
- Pinar Bayrak-Toydemir

ARUP Clinical Variant Scientist

- Alexander Chapin
- Timothy Tidwell

ARUP Institute for Clinical & Experimental Pathology

ARUP Genomics Lab

- Hunter Best
- Mike Graczyk

ARUP Genetics Counselors

- Chris Miller
- Patti Krautscheid
- Sara Brown

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