Sudden Cardiac Death Prevention with Molecular Diagnosis

Rong Mao
Professor of Pathology, University of Utah
Medical Director, Molecular Genetics and Genomics
ARUP Laboratories

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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
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’): multifactored, genetics and environmental, lifestyle

• 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

- 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
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

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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

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

If untreated, causes sudden cardiac arrest and death!
Arrhythmia and SCD

- Arrhythmic Cause: 88%
- Other Cardiac Cause: 12%
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
SureSelect target enrichment

Genomic DNA...want just aortopathy genes!

Coverage

MYBPC3 exons

Genomic Sample (Prepped) + SureSelect Hyb Buffer + Biotinylated RNA Library “Baits”

Hybridization

Streptavidin Coated Magnetic Beads

Unbound Fraction Discarded

Wash Beads and Digest RNA

Bead Capture

Amplify

Sequencing

Barcode Index

Courtesy to Dr. Whitney Donahue
Illumina Sequencing

4 reversible dye terminator NTPs
Incorporate one nt, Image
Terminator & dye cleaved

Add 4 Fl-NTP’s + Polymerase
Incorporated Fl-NTP is imaged
Terminator and fluorescent dye are cleaved from the Fl-NTP

X 36 - 50
Illumina Sequencing

Image of clusters during sequencing.
Gene Coverage

coverage

reads

gene

MYBPC3
Pathogenic *KCNQ1* Mutation

NGS data

Sanger Confirmed

c.642C>A, p.Cys314Ter
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

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

c.2864_2865delCT, p.Pro995fs
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

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<th>Variant Type</th>
<th>Location</th>
<th>Nuc. Change</th>
<th>Protein Change</th>
<th>dbSNP Id</th>
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</table>
**TTN** Gene Variant: In Frame deletion/Insertion

NGS data

- c.28287_28288insG
- c.28283_28286delCAGC

Sanger confirmation

- c.28287_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 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**

c.826C>A, p.Leu276Ile

*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
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.
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