The State of the Molecular Autopsy for Sudden Death in the Young

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Learning Objectives to Disclose:
• To **DETAIL** the state of postmortem genetic testing (AKA, “the molecular autopsy”) for autopsy positive and autopsy negative Sudden Unexplained Death Syndrome (SUDS)
• To **EXAMINE** the three Achilles’ heels that threaten to derail the molecular autopsy

Conflicts of Interest to Disclose:
• Consultant – Boston Scientific, Gilead Sciences, Medtronic, St. Jude Medical, and Transgenomic/FAMILION
• Royalties – Transgenomic/FAMILION
Sudden Death in the Young (SDY)

TELLTALE HEARTS

Nature Medicine 2013
Sudden Death in the Young (SDY)
Sudden Cardiac Death in the Young

Autopsy Positive

Autopsy Negative
Sudden Cardiac Death in the Young
**Clinical Markers for Positive Genetic Test**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Pts</th>
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<tbody>
<tr>
<td>Age Dx &lt; 45 yrs</td>
<td>1</td>
</tr>
<tr>
<td>MLVWT ≥ 20 mm</td>
<td>1</td>
</tr>
<tr>
<td>FH of HCM</td>
<td>1</td>
</tr>
<tr>
<td>FH SCD</td>
<td>1</td>
</tr>
<tr>
<td>Reverse-curve HCM</td>
<td>1</td>
</tr>
<tr>
<td>Hx of Hypertension</td>
<td>-1</td>
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</table>

Scoring range: -1 – 5 pts

Yield of Genetic Testing (%)

<table>
<thead>
<tr>
<th>Score</th>
<th>Yield</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>5%</td>
</tr>
<tr>
<td>2</td>
<td>14%</td>
</tr>
<tr>
<td>3</td>
<td>19%</td>
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<tr>
<td>4</td>
<td>41%</td>
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<tr>
<td>5</td>
<td>67%</td>
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<tr>
<td></td>
<td>81%</td>
</tr>
</tbody>
</table>

Yield of Genetic Testing

- <5% (~80%)

L185R-TPM1

Normal

Van Driest … Ackerman. AJC 90:1123-1127, 2002
HCM: A Disease of the Myofilament

Genetic Testing for HCM

Van Driest ... Ackerman. AJC 90:1123-1127, 2002
Mutation-specific genetic testing is recommended for family members and other appropriate relatives subsequently following the identification of the disease-causative mutation in an index case.

Ackerman, Priori, et al. *Heart Rhythm* 8:1308-1339, 2011 (HRS/EHRA)
State of Postmortem Genetic Testing for Autopsy Negative SUD

A Mother’s Question

Why did my 17-year-old son die?
March 1999 – previously well 17-year-old white male found dead in bed

- Autopsy negative
- Local newspaper editorial positive – “Parents talk to your kids”
- Clue in the family history – mom “fell” from 3-meter diving board at age 9
- Negative standard evaluation – ECG, Echo
“WITHOUT THE MOLECULAR AUTOPSY, OUR FAMILY WOULD HAVE HAD TO LIVE WITH FALSE RUMORS.”
Genetic Diagnosis of LQTS

SUD 3/26/99
In Autopsy Negative SUD, How Often Would a Molecular Autopsy Be Positive?

1. Don’t know what a molecular autopsy is.
2. 5-10%
3. 15-20%
4. 25-30%
5. > 50%
State of Postmortem Genetic Testing

- N = 173 cases of SUD (106 males)
- Average age = 18 ± 13 years (1 - 69 yrs)
- Personal or FHx of Cardiac Events = 70 (40%)

Autopsy Negative Sudden Unexplained Death

~ 25 - 30%

Age- and Sex-Specific Effect on the Yield of a Cardiac Channel Molecular Autopsy

LQTS Mutations - Pathogenic Basis for ~10% of SIDS or SUID

Ackerman et al. JAMA 286:2264-69, 2001
1. In the setting of autopsy negative SUDS, comprehensive or targeted (RYR2, KCNQ1, KCNH2, and SCN5A) ion channel genetic testing may be considered in an attempt to establish probable cause and manner of death and to facilitate the identification of potentially at-risk relatives and is recommended if circumstantial evidence points towards a clinical diagnosis of LQTS or CPVT specifically (such as emotional stress, acoustic trigger, drowning as the trigger of death).
Whole exome sequencing (WES) allows for simultaneous mutational analysis of a patient’s entire library of genes!

Gene specific targeted analysis
29 consecutive sudden death cases (21 males, 26.7 ± 5.9 years) collected at the Office of the Medical Examiner, Cook County, Illinois from January 2012 to December 2013 were referred to Mayo Clinic for molecular autopsy.

Will … Ackerman. HRS 2015
"Clinically Actionable" Variants

RyR2

Y462S

28 yo white male found in bed, equivocal autopsy.

FKBP12.6 Binding Domain

N4782S

14 yo white male, found unresponsive on floor after playing/exertion, negative autopsy.

SR lumen

Cytosol

C4967

27 yo black female, sudden collapse, equivocal autopsy noting cardiomyopathy.

MYH7

R783H

R783H

Four MYH7 (β-myosin heavy chain) Subunits

ATP binding

Actin binding domains

Colied-Coil region

IQ domain

Head Neck
Molecular Autopsy’s 3 Achilles’ Heels

1. Cost
   Insurance companies do NOT like to pay for things when you have died!

2. Medical Examiner’s SOP
   Paraffin-embedded tissue is NOT DNA friendly!

3. Interpreting the Molecular Autopsy
   “X” does NOT always mark the spot!
Molecular Autopsy of SCD

- Need blood spot card, blood in EDTA, or frozen tissue

Tissue is the Issue!!

1. For all SUDS and SIDS cases, collection of a tissue sample is recommended for subsequent DNA analysis/genetic testing.

Ackerman, Priori, et al. *Heart Rhythm* 8:1308-1339, 2011
Is the “X” that marks the spot truly THE disease-causing mutation?

What’s the “Background Noise Rate”? What’s the Signal-to-Noise Ratio?
Autopsy Negative SUD is NOT a Good Phenotype!

- LQTS – 15%
- CPVT – 10%
- BrS – 3%

KCNQ1, KCNH2, SCN5A, RYR2
Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: Implications for arrhythmogenic susceptibility

Journal of the American College of Cardiology

Distinguishing Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia–Associated Mutations From Background Genetic Noise

KCNQ1, KCNH2, SCN5A, RYR2 = ~5%

13.5%

2003
- “Maybe” Test Result

“Possible Deleterious”
“Variant of Uncertain Significance (VUS)”

“Genetic Purgatory is a Real Place and its Scary!”

Postmortem Genetic Testing

MAYO CLINIC
Case Presentation

Ackerman. *Heart Rhythm* 2015
Case Presentation

Ackerman. *Heart Rhythm* 2015
Case Presentation

+ = L537P-SCN5A = BrS1
Genetic Purgatory is Real

Kapplinger ... Ackerman. Circ CV Genet 2015 (in press)
Case Presentation

L537P

WILDTYPE
Case Presentation

+ = L537P-SCN5A = “One Hell of Situation”! (PJS)
The State of the Molecular Autopsy

1. ~25% of autopsy negative SUD and 10% of SIDS - Channelopathic!
2. Should postmortem genetic testing become a standard part of the conventional autopsy for “autopsy negative” SUD?
3. Formalin-fixed paraffin embedded tissue is the enemy of a “comprehensive” molecular autopsy.
4. “X” does NOT always mark the spot!
MAYO CLINIC
WINDLAND SMITH RICE
SUDDEN DEATH
GENOMICS LABORATORY

Dr. Scholl Foundation, CJ Foundation for SIDS
Hannah Wernke Memorial Foundation
Sheikh Zayed Saif Mohammed Al Nahyan Fund
National Institutes of Health
“To heal the sick and advance the science”
Dr. Charles W. Mayo