The Application of Molecular Genetics in Sudden Death and Near Death – the Social and Medico-legal Impacts

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SUDDEN DEATH AND CARDIAC DISEASES
Sudden death -

“an unexpected natural death within a short time period, generally 1 hour or less from the onset of symptoms” or

“a non-witnessed death discovered within 24 hours in someone without prior symptoms or any prior condition that would appear fatal”.

Definition
Incidence and causes are age-dependent.
Coronary artery disease in adults > 35y.
Accounts for 5% of all deaths in children and adolescents with an incidence of 1.5 – 8 per 100,000 patient-year.
Figure 1 Causes of sudden cardiac death in young people

(a) Athletic field SCD
- Structurally normal (3%)
- Aortic aneurysm/dissection (4%)
- Anomalous coronaries (19%)
- Atherosclerotic CAD (2%)
- Valvular disease (6%)
- Myocarditis (3%)
- Cardiomyopathy (52%)

(b) Italian SCD
- Structurally normal (6%)
- Aortic aneurysm/dissection (5%)
- Anomalous coronaries (10%)
- Atherosclerotic CAD (20%)
- Valvular disease (11%)
- Myocarditis (10%)
- Cardiomyopathy (24%)

(c) Australian SCD
- Structurally normal (29%)
- Aortic aneurysm/dissection (5%)
- Anomalous coronaries (2%)
- Atherosclerotic CAD (25%)
- Valvular disease (1%)
- Myocarditis (12%)
- Cardiomyopathy (16%)

(d) American military recruits SCD
- Structurally normal (40%)
- Aortic aneurysm/dissection (2%)
- Anomalous coronaries (19%)
- Atherosclerotic CAD (9%)
- Valvular disease (12%)
- Myocarditis (12%)
- Cardiomyopathy (9%)
Heritable causes of sudden cardiac death

Structural heart defects
- Hypertrophic cardiomyopathies (HCM)
- Arrhythmogenic right ventricular cardiomyopathy (ARVC)

Cardiac electrical disorders
- Long QT syndromes (LQTS)
- Catecholaminergic polymorphic ventricular tachycardia (CPVT)
- Brugada syndrome
Hypertrophic Cardiomyopathy

Normal heart (cross section)  Hypertrophic cardiomyopathy

Sarcomere

http://hk.image.search.yahoo.com/
HCM

- Account for ~1/3 of sudden deaths in competitive athletes in the US.
- 55%-70% attributable to one of the 12 sarcomere genes.
- Autosomal dominant with age-related penetrance
- Existing evidence: diagnosis and family screening
- Little prognostic value, cannot predict age of onset and disease severity.
<table>
<thead>
<tr>
<th>Locus Name</th>
<th>Gene Symbol</th>
<th>Protein Name</th>
<th>% of HCM Caused by Mutations in This Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMH1</td>
<td><strong>MYH7</strong></td>
<td>Myosin heavy chain, cardiac muscle beta isoform</td>
<td>40%</td>
</tr>
<tr>
<td>CMH4</td>
<td><strong>MYBPC3</strong></td>
<td>Myosin-binding protein C, cardiac-type</td>
<td>40%</td>
</tr>
<tr>
<td>CMH2</td>
<td><strong>TNNT2</strong></td>
<td>Troponin T, cardiac muscle</td>
<td>5%</td>
</tr>
<tr>
<td>CMH7</td>
<td><strong>TNNI3</strong></td>
<td>Troponin I, cardiac muscle</td>
<td>5%</td>
</tr>
<tr>
<td>CMH3</td>
<td><strong>TPM1</strong></td>
<td>Tropomyosin 1 alpha chain</td>
<td>2%</td>
</tr>
<tr>
<td>CMH10</td>
<td><strong>MYL2</strong></td>
<td>Myosin regulatory light chain 2, ventricular/cardiac muscle isoform</td>
<td>Unknown</td>
</tr>
<tr>
<td>CMH8</td>
<td><strong>MYL3</strong></td>
<td>Myosin light polypeptide 3</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td><strong>ACTC1</strong></td>
<td>Actin, alpha cardiac muscle 1</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td><strong>CSRP3</strong></td>
<td>Cysteine and glycine-rich protein 3, muscle LIM protein</td>
<td>Unknown</td>
</tr>
<tr>
<td>CMH9</td>
<td><strong>TTN</strong></td>
<td>Titin</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>MYH6</strong></td>
<td>Myosin heavy chain, cardiac muscle alpha isoform</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>TCAP</strong></td>
<td>Telothonin</td>
<td></td>
</tr>
<tr>
<td>Other genes implicated in HCM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>TNNC1</strong></td>
<td>Troponin C, slow skeletal and cardiac muscles</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
Sudden death @54y

F/54  M/38

M/20  F/6

LV noncompaction, end-stage heart failure

Normal heart by echocardiogram

Hypertrophic cardiomyopathy @12y
This pedigree

- Familial disease
- Dominant inheritance
- Phenotypes: childhood-onset HCM, end-stage heart failure, (sudden death)
- At risk asymptomatic family members
Clinical vs Genetics Dx

Clinical Screening

- History, physical exam, echocardiography, ECG, etc.
- Always indicated in symptomatic individuals regardless of age.
- A normal baseline echocardiogram and ECG does not rule out HCM in asymptomatic relatives, particularly in children or young adults.
- Require screening and longitudinal FU throughout life.
Genetic Diagnosis

- Testing of at-risk asymptomatic relatives is possible if the disease-causing mutation in the proband is known.
- Genetically heterogeneous
- Mutation detection rate 55-70%
- A negative test result can provide reassurance that the person is not at risk of developing HCM and thus obviate unnecessary screening.
Sudden death @54y
LV noncompaction, end-stage heart failure
Hypertrophic cardiomyopathy @12y

F/54

F/45

M/38

M/20

F/6

F/1

At risk asymptomatic relatives

I

II

III

Hypertrophic cardiomyopathy @12y
Normal heart by echocardiogram
Clinical screening
• III:1 – symptomatic
• II:3 and II:4 – 50% risk; require life-long follow-up and avoidance of risk
• III:2 – 50% risk; require life-long follow-up and avoidance of risk
• III:3 – 25% risk; require life-long follow-up and avoidance of risk
Target 1: **MYH7**
- The beta heavy chain subunit of human muscle myosin
- Chromosome 14q12
- 38 coding exons
- ~300 known mutations throughout the gene, majority being missense / nonsense mutations.
- Account for ~40% familial HCM in Chinese
Sample collection

DNA Extraction

PCR

Sequencing Reaction

Sequencing

Modified from Dr Chole Mak, PMH
- c.2155C>T p.Arg719Trp in exon 19
- First reported in 1994
Clinical screening
• III:1 – symptomatic
• II:3 – 50% risk
• II:4 – 50% risk
• III:2 – 50% risk
• III:3 – 25% risk

Genetic screening
• III:1 – same
• II:3 not at risk
• II:4 Dx confirmed
• III:2 – 50% risk
• III:3 – 50% risk
Cardiac Electrical Disorders
Cardiac electrical disorders

- Cardiac channelopathies
- No detectable structural abnormalities
- Long QT syndromes (LQTS)
- Catecholaminergic polymorphic ventricular tachycardia (CPVT)
- Brugada syndrome (BrS)
Autosomal dominant with reduced penetrance
Genetically heterogeneous
75% attributable to
- KCNQ1 (LQT1)
- KCNH2 (LQT2)
- SCN5A (LQT3)
- KCNE1 (LQT5)
- KCNE2 (LQT6)
## LQTs

<table>
<thead>
<tr>
<th></th>
<th>LQT1</th>
<th>LQT2</th>
<th>LQT3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene / Locus</strong></td>
<td>KCNQ1</td>
<td>KCNH2</td>
<td>SCN5A</td>
</tr>
<tr>
<td></td>
<td>11p15.5</td>
<td>7q35-q36</td>
<td>3p21</td>
</tr>
<tr>
<td><strong>Coding exons</strong></td>
<td>16</td>
<td>15</td>
<td>27</td>
</tr>
</tbody>
</table>
| **Arrhythmogenic triggers** | • Physical exertion  
  • Swimming  
  • Auditory stimuli (e.g. alarm clock)  
  • Post-partum period  
  • Sleeping | | |
| **Response to β-blocker** | ++++ | +++ | +/− |
Clinical criteria are insensitive

- A Schwartz score of $\geq 4$ (i.e. a strong probability of LQTS) had a sensitivity of 38%.
- Clinical assessment failed to identify 40% of the genetically affected family members of 310 genotyped probands.

Patients with “concealed” LQTS have a 10% risk of cardiac events by age 40 years if left untreated.

Early identification

- Preventive life-style modifications (e.g. avoidance of strenuous exercise and extreme emotional stress)
- Prophylactic treatment.

Genetic test $\Rightarrow$ gold standard in family screening
Catecholaminergic polymorphic ventricular tachycardia

Syncope and sudden death during physical exertion or emotion (Catecholamine-induced bidirectional VT ⇒ polymorphic VT and VF).

Mean age of onset 7-9yrs

Adrenaline provocation test / Exercise stress test

*Ryr2* – autosomal dominant with high penetrance, 50-55%

*Casq2* – autosomal recessive, 1-2%
Management

- Avoid strenuous exercise / acute emotion
- β-blockers
- Implantable cardioverter defibrillator
- FU by cardiologist
- Screening of family members at risk
F/14
Good past health
Sudden collapse after boarding a bus. Regained consciousness shortly and collapsed again. A similar episode 9 months earlier when quarrelling with a friend.

Baseline ECG – NAD
Echocardiogram – no structural heart disease
MR coronary angiogram – no anomalies of coronary arteries
CT brain – hypoxic brain damage
PVCs, polymorphic VT and non-sustained bidirectional VT when the patient was agitated.
Adrenaline provocation test
- Family history –ve
- RYR2 mutation analysis
  - 104 exons
  - Critical regions
- Heterozygous p.G3946S in exon 88
- Both parents were negative
- De novo mutation
- Spastic paraplegia
- Non-communicable
- Bed-ridden
Postmortem genetic analysis
First report in 1999
*KCNQ1* mutation (LQT1) identified in a 19-yr-old woman who died after a near-drowning.

- Definitive Dx for at least 60 relatives
- Prophylactic therapy in mutation carriers
Figure 2: Summary of the Yield of Postmortem Cardiac Channel Genetic Testing in Cases of Autopsy-Negative SUD

Depicted is a pie chart summarizing the frequency and distribution of cardiac channel genotypes detected after a molecular autopsy of 49 medical examiner/coroner-referred cases of sudden unexplained death (SUD).
Cost-effectiveness analysis

- Commercial LQTS genetic testing available in the US (US$5400).
- Symptomatic index cases who received a definite or inconclusive clinical diagnostic scores for LQTS.

<table>
<thead>
<tr>
<th></th>
<th>LQTS genetic testing</th>
<th>BRCA1/2 screening and oophorectomy</th>
<th>Mammography screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per year of life saved</td>
<td>$2,500</td>
<td>$3,900-$1,600,000 for high- and average-risk women</td>
<td>$21,400-$117,680 for women ages 50-69yrs and 70-79yrs respectively.</td>
</tr>
</tbody>
</table>
“More accurately diagnose and treat affected individuals.”

“Clinicians can make more informed judgement about treatment.”

The cost and benefits of testing at-risk family members not included in the estimation ⇒ likely to be more favourable.
Genetic studies are expensive (time, manpower).
Interpretation of results may not be straight-forward.
How to prioritize patients?
Genetic counselling
SUDDEN INFANT DEATH AND INBORN ERRORS OF METABOLISM
Fatty Acid Oxidation
CACT deficiency

- The 1st case in Hong Kong diagnosed in 2003
- M/3D, sudden death in hospital
- ~20 reported cases – sudden neonatal death is the commonest presentation.
- A lot of difficulties in the investigation process.
### TABLE 1. Clinical features of the three patients diagnosed with carnitine-acylcarnitine translocase deficiency

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Gestation (weeks)</th>
<th>Birth weight (kg)</th>
<th>Consanguinity</th>
<th>Feeding</th>
<th>Time at presentation (hours)</th>
<th>Initial symptoms</th>
<th>Survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>38.4</td>
<td>2.41</td>
<td>No</td>
<td>Formula</td>
<td>41</td>
<td>Sudden cardiac arrest</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>35.6</td>
<td>2.71</td>
<td>No</td>
<td>Breast-feeding</td>
<td>32</td>
<td>Sudden cardiac arrest</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>37.4</td>
<td>2.3</td>
<td>No</td>
<td>Formula</td>
<td>28</td>
<td>Cardiorespiratory failure</td>
<td>No</td>
</tr>
</tbody>
</table>

### TABLE 2. Laboratory findings of patients with carnitine-acylcarnitine translocase deficiency at presentation and autopsy findings

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Initial plasma glucose (mmol/L)</th>
<th>Maximum ammonia level (μmol/L)</th>
<th>Long-chain acylcarnitine profiles</th>
<th>Dicarboxylic acid in urine</th>
<th>Lactate (mmol/L)</th>
<th>Autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
<td>Raised</td>
<td>Not done</td>
<td>Steatosis in myocardium and hepatocytes</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>455</td>
<td>Raised</td>
<td>Raised</td>
<td>5.2</td>
<td>Not applicable</td>
</tr>
<tr>
<td>3</td>
<td>1.3</td>
<td>216</td>
<td>Raised</td>
<td>Raised</td>
<td>8.9</td>
<td>Not done</td>
</tr>
</tbody>
</table>
Founder mutation in CACT

- **CACT IVS2-10T>G**
- Homozygous in all 3 patients.
- Reported previously in 2 Chinese parents of CACT patients (UK and USA).
- Allow quick target mutation analysis.
Challenges

- Slow development in paediatric biochemical tests.
- Turnaround time cannot meet urgent need.
- Peri-mortem and post-mortem sampling protocol for IEM investigation.
- Genetic information alter the investigation protocol.
- Accumulation of expertise and sharing of clinical experience.
UNEXPECTED DEATHS CAUSED BY INBORN ERRORS OF METABOLISM
M/14

Presented to AED twice within 10hrs.

Acute deterioration after 9hrs of observation in AED.

Died 38hrs after admission.

No biological samples saved for metabolic investigation.

Retrospective family history – twin sister died of unknown cause in infancy.
F/18 Hx of epilepsy not on AED

M/14 Unexpected death

Died in infancy of unknown cause

Urine: ↑ glutaric, 2-Ohglutaric, ethylmalonic, isovalerylglycine, hexanoylglycine
Plasma: ↑ C4 to C16 acylcarnitines
⇒ Glutaric aciduria type II

Abnormal plasma acylcarnitine!
3 known genes for GAII
- ETFA
- ETFB
- EFTDH

2 Novel mutations in ETFA
- p.V618F (paternal allele)
- p.Q262R (maternal allele)
M/14 Unexpected death
Died in infancy of unknown cause

F/18 Hx of epilepsy not on AED
p.V168F/-

M/14 Unexpected death
p.V168F/p.Q262R
Challenges

- GAI1 – a relatively common IEM in Hong Kong
- Clinical suspicion
- An inquest was held for this patient
  - TAT of metabolic investigation.
  - More comprehensive newborn screening program.
Conclusion

1. Increasing knowledge of genetic basis of heritable disorders which can cause sudden death / significant mortality and morbidity.

2. Increasing evidence which support of introduction of genetic testing into clinical practice.

3. Current situation in Hong Kong
   a) No lack of clinical requests – lack of clinical guidelines
   b) Piecemeal development – lack of central policy
   c) Better support for up-stream and front-line investigations required.
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References