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

HA Convention 2010
Dr Liz YP Yuen
Department of Chemical Pathology
Prince of Wales Hospital

SUDDEN DEATH AND CARDIAC DISEASES

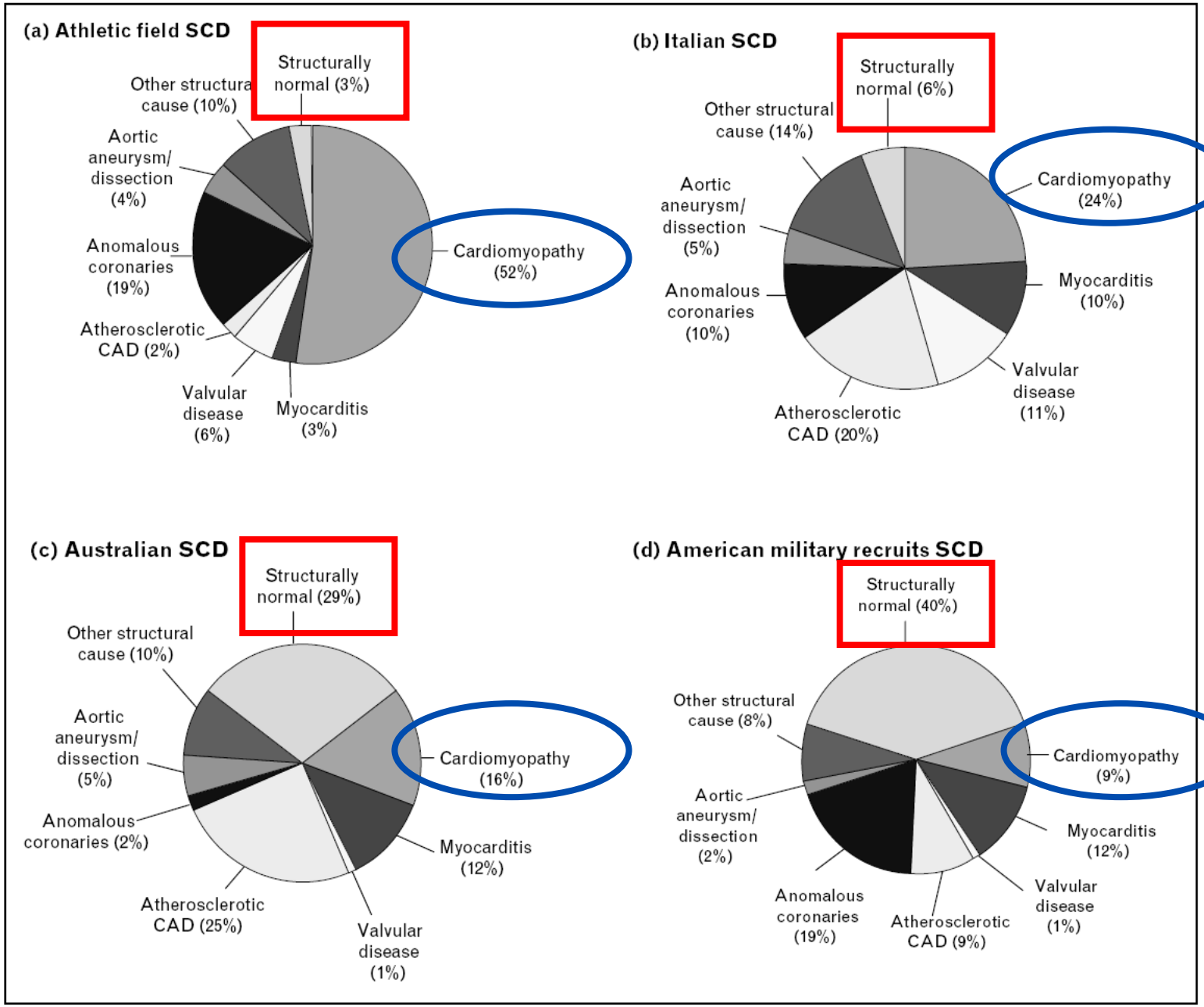
Definition

- 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”.

Sudden Death

- 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



Heritable causes of sudden cardiac death

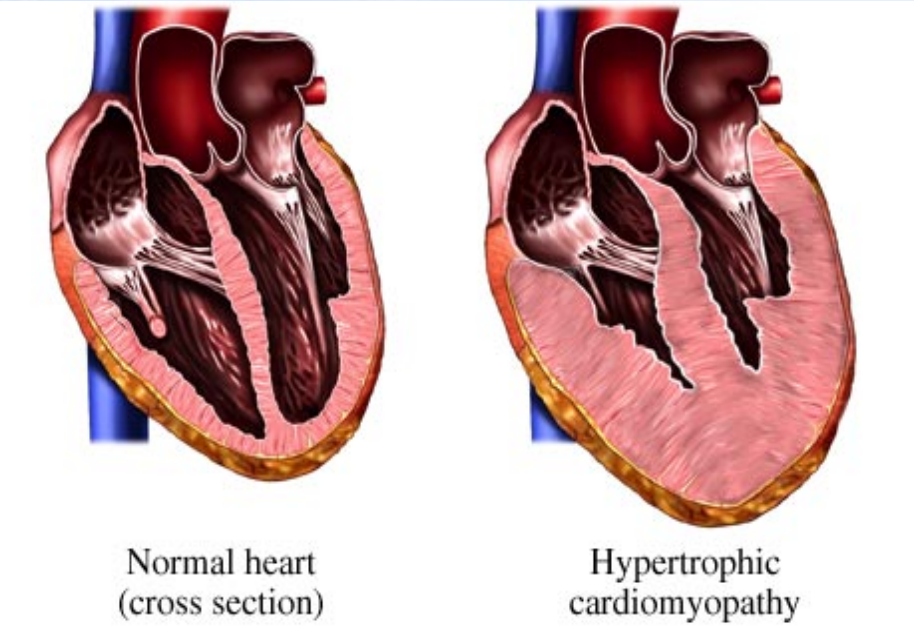
Structural heart defects

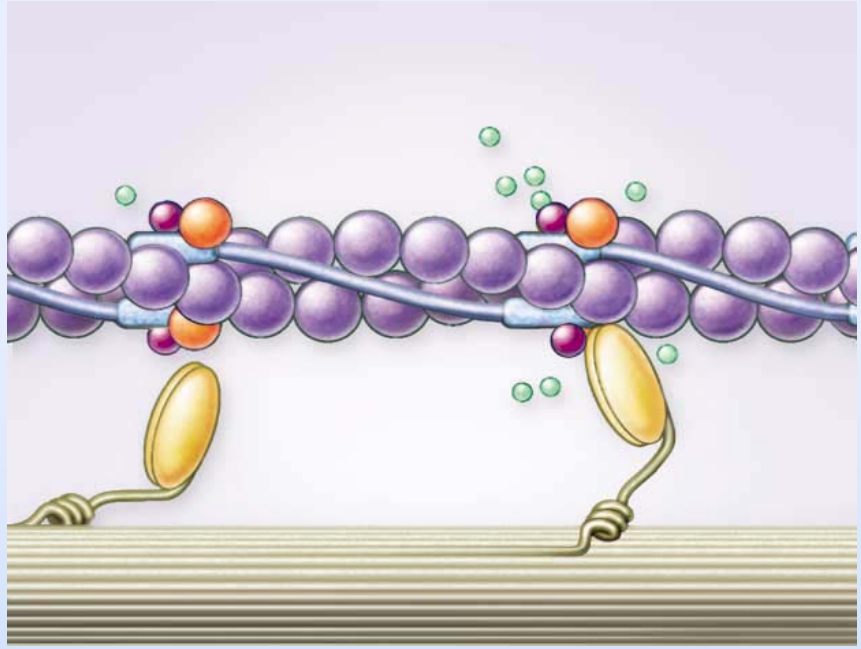
- Hypertrophic cardiomyopathies (HCM)
- Arrhythmogenic right ventricular cardiomyopathy (ARVC)

Cardiac electrical disorders

- Long QT syndromes (LQTS)
- Catecholaminergic polymorphic ventricular tachycardia (CPVT)
- Brugada syndrome

Hypertrophic Cardiomyopathy





Sarcomere

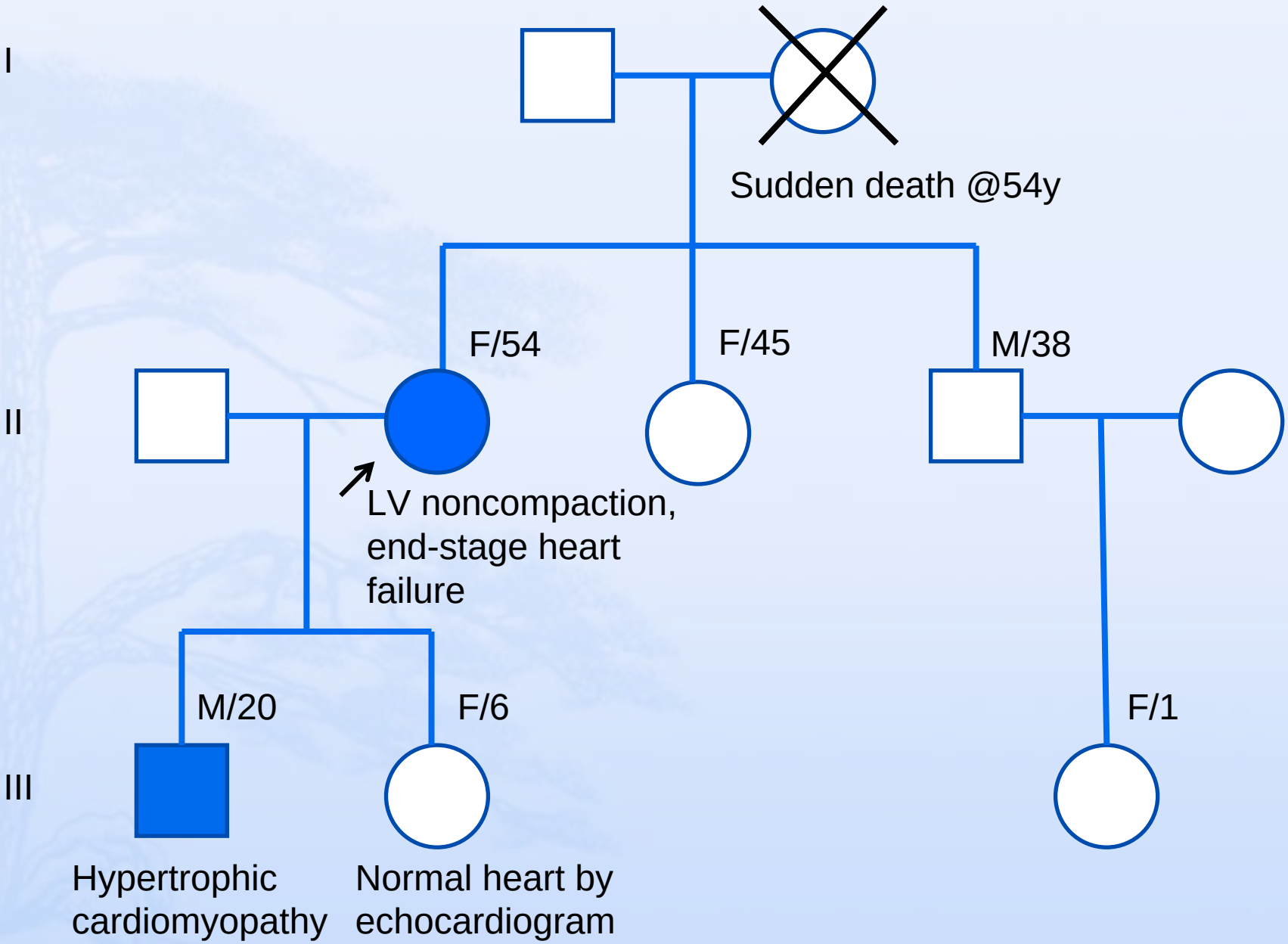
http://hk.image.search.yahoo.com/

Kamisago et al. 2000

HCM

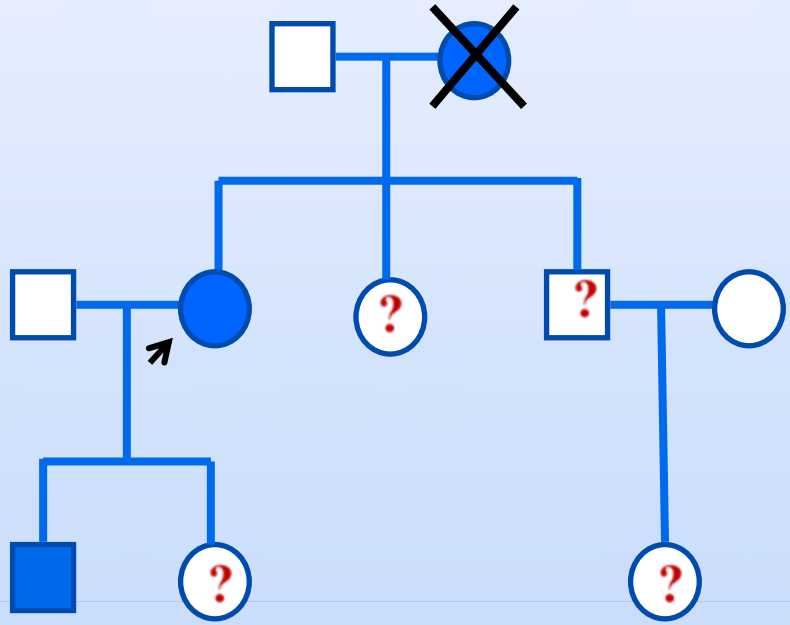
- Account for ~1/3 of sudden deaths in competitive athletes in the US .
- 55%-70% attributable to one of the 12 sarcomere genes.
- Autosomal dominant with age-related penetrance
- Existing evidence: diagnosis and family screening
- Little prognostic value, cannot predict age of onset and disease severity.

Locus Name	Gene Symbol	Protein Name	% of HCM Caused by Mutations in This Gene
CMH1	***MYH7***	Myosin heavy chain, cardiac muscle beta isoform	40%
CMH4	***MYBPC3***	Myosin-binding protein C, cardiac-type	40%
CMH2	***TNNT2***	Troponin T, cardiac muscle	5%
CMH7	***TNNI3***	Troponin I, cardiac muscle	5%
CMH3	***TPM1***	Tropomyosin 1 alpha chain	2%
CMH10	***MYL2***	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform	Unknown
CMH8	***MYL3***	Myosin light polypeptide 3	1%
	ACTC1	Actin, alpha cardiac muscle 1	Unknown
	CSRP3	Cysteine and glycine-rich protein 3, muscle LIM protein	Unknown
CMH9	***TTN***	Titin	
	MYH6	Myosin heavy chain, cardiac muscle alpha isoform	
	TCAP	Telothonin	
Other genes implicated in HCM			
	TNNC1	Troponin C, slow skeletal and cardiac muscles	Unknown


I
II
III
Sudden death @54y
F/54
F/45
M/38
LV noncompaction, end-stage heart failure
M/20
F/6
F/1
Hypertrophic cardiomyopathy @12y
Normal heart by echocardiogram

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

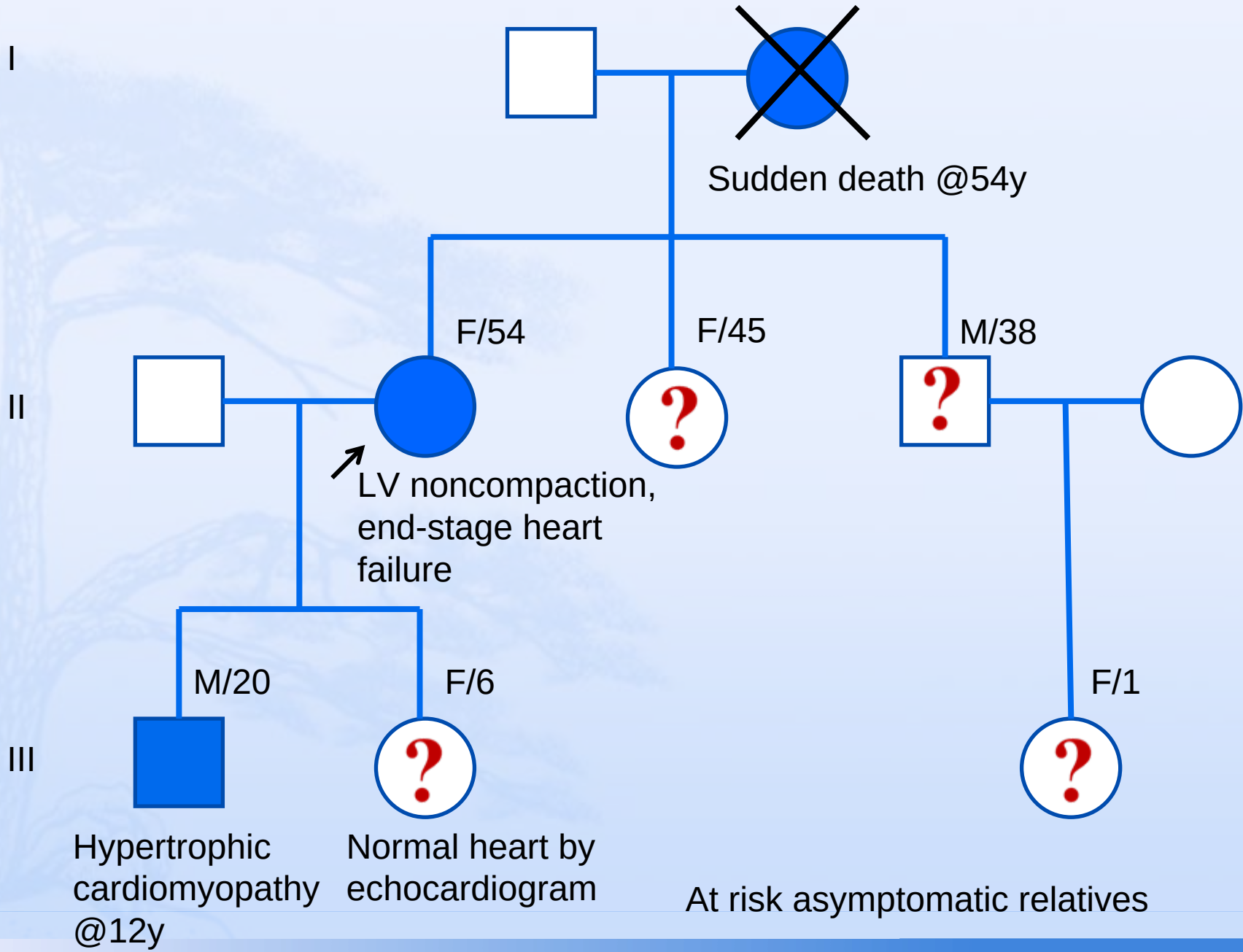
Clinical Screening

- 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.**

Genetics vs Clinical Dx

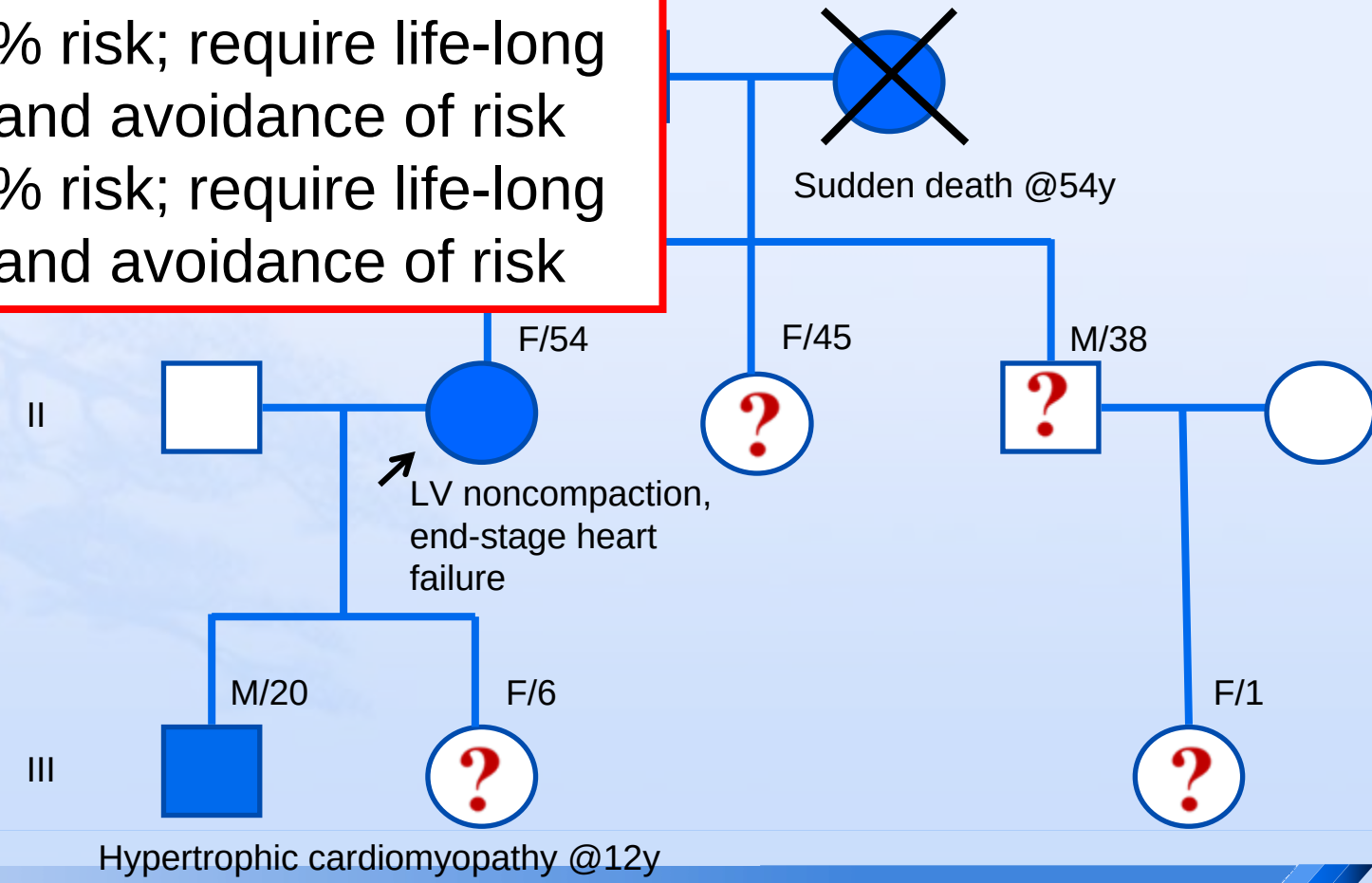
Genetic Diagnosis

- 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.**


I
II
III
Sudden death @54y
F/54
F/45
M/38
LV noncompaction, end-stage heart failure
M/20
F/6
F/1
Hypertrophic cardiomyopathy @12y
Normal heart by echocardiogram
At risk asymptomatic relatives

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



Genetic Diagnosis

- 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

1

2

3

Sample collection

DNA Extraction

PCR

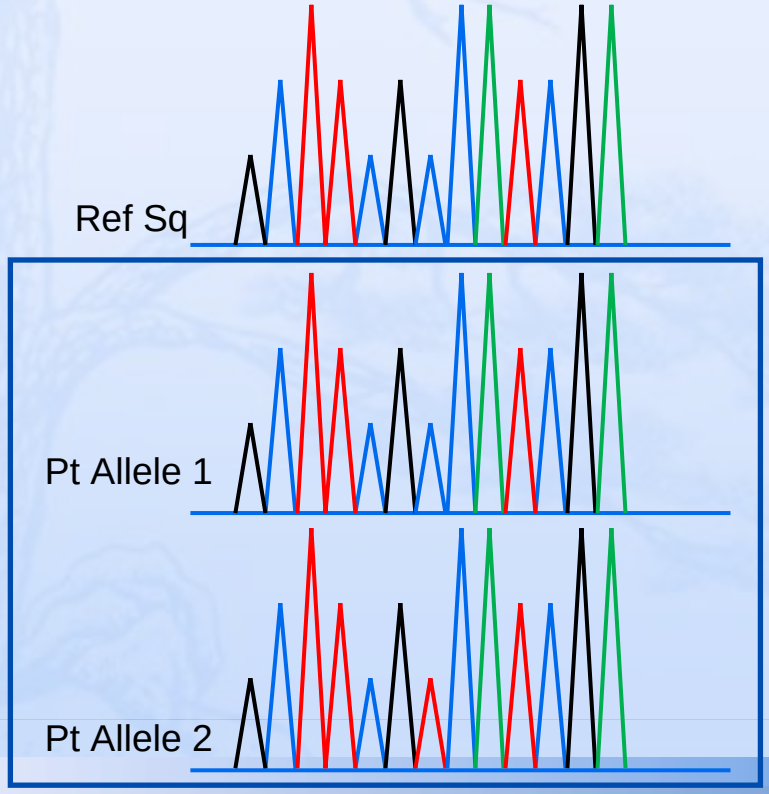
4

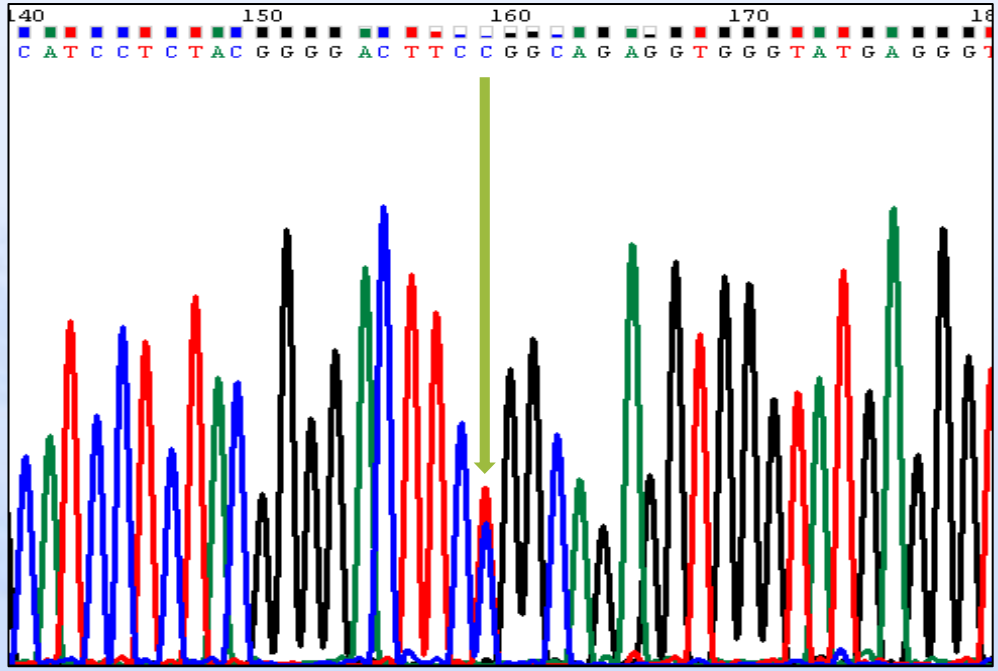
Sequencing Reaction

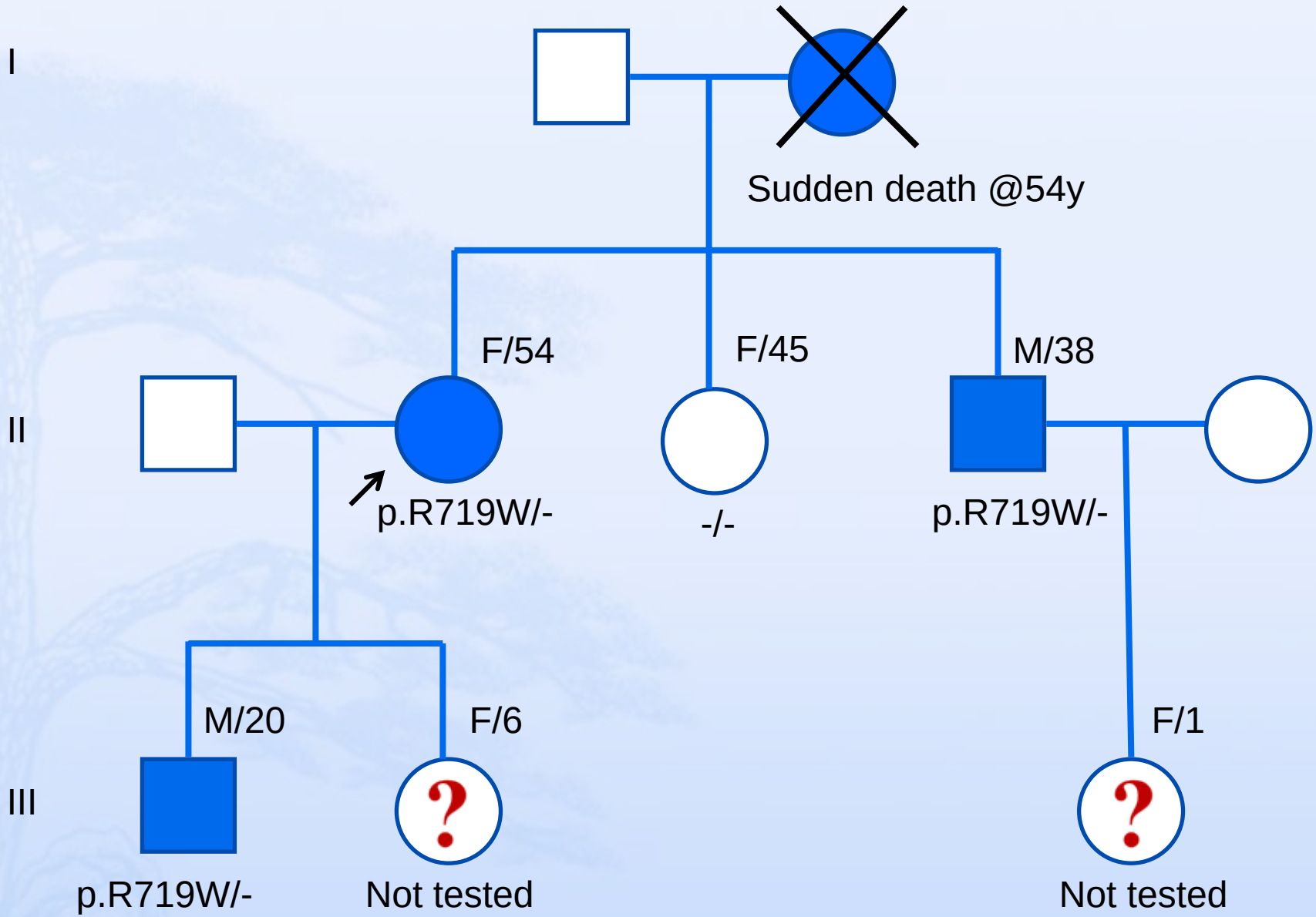
Sequencing

Sequencing results

- c.2155C>T p.Arg719Trp in exon 19
- First reported in 1994





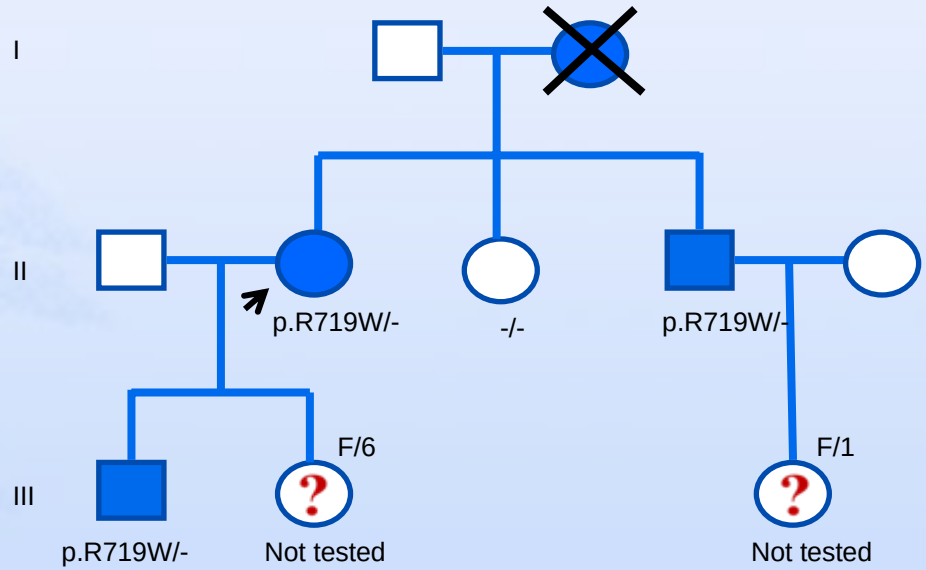

I
II
III
Sudden death @54y
F/54
F/45
M/38
p.R719W/-
-/-
p.R719W/-
M/20
F/6
F/1
p.R719W/-
Not tested
Not tested

Clinical screening

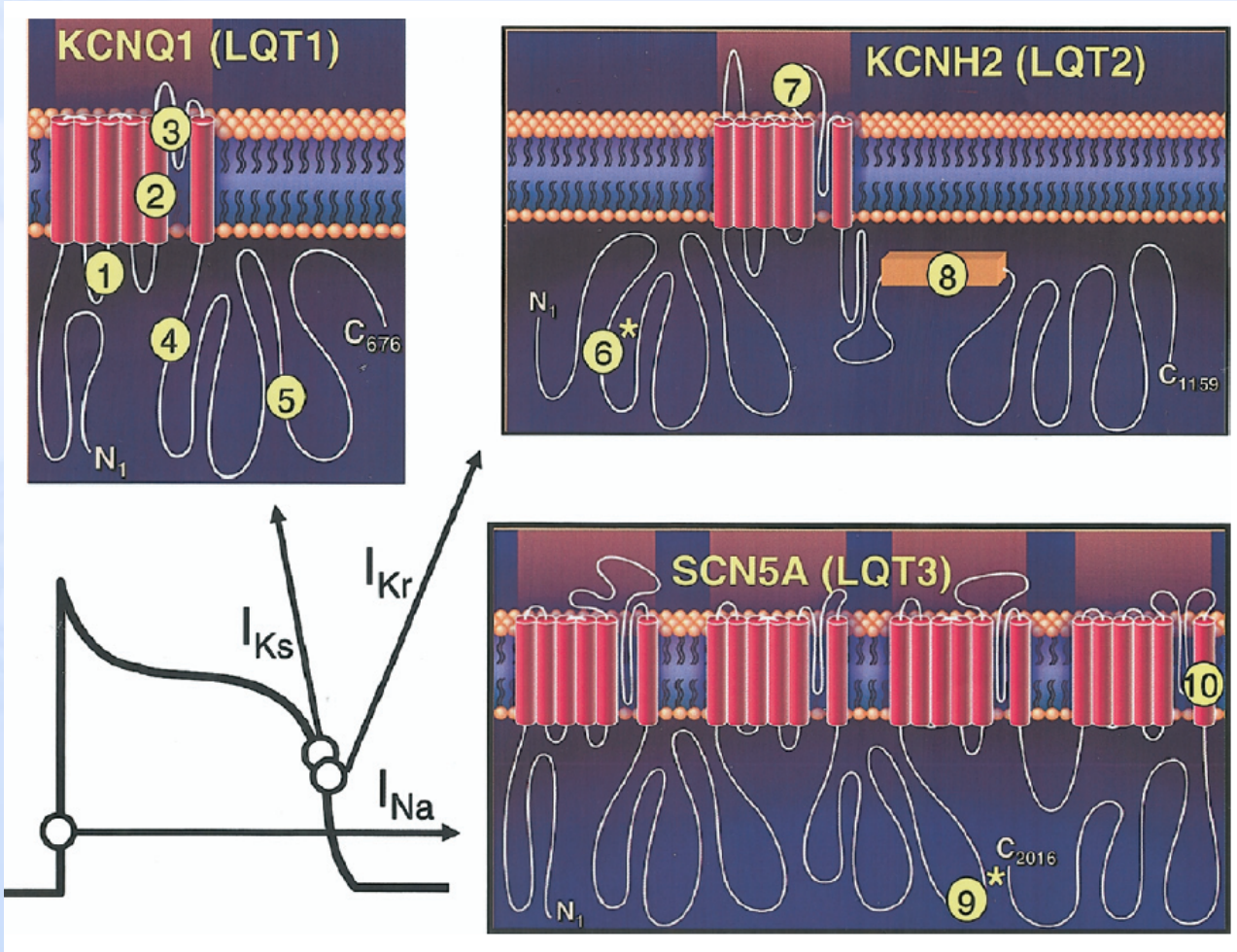
- III:1 – symptomatic
- II:3 – 50% risk
- II:4 – 50% risk
- III:2 – 50% risk
- III:3 – 25% risk

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



Tester et al. 2007

Cardiac electrical disorders

- Cardiac channelopathies
- No detectable structural abnormalities

- Long QT syndromes (LQTS)
- Catecholaminergic polymorphic ventricular tachycardia (CPVT)
- Brugada syndrome (BrS)

Congenital LQTS

- Autosomal dominant with reduced penetrance
- Genetically heterogeneous
- 75% attributable to
  - KCNQ1 (LQT1)
  - KCNH2 (LQT2)
  - SCN5A (LQT3)
  - KCNE1 (LQT5)
  - KCNE2 (LQT6)

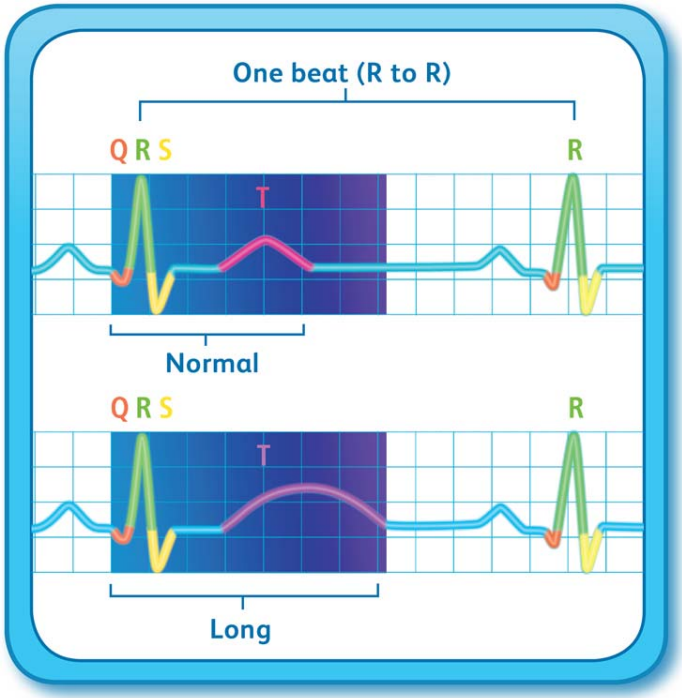


Illustration showing prolonged QT interval on an electrocardiogram (ECG)

LQTS

	LQT1	LQT2	LQT3
Gene / Locus	KCNQ1 11p15.5	KCNH2 7q35-q36	SCN5A 3p21
Coding exons	16	15	27
Arrhythmogenic triggers	• Physical exertion • Swimming	• Auditory stimuli (e.g. alarm clock) • Post-partum period	• Sleeping
Response to β-blocker	++++	+++	+/-

Identification of asymptomatic LQTS carriers

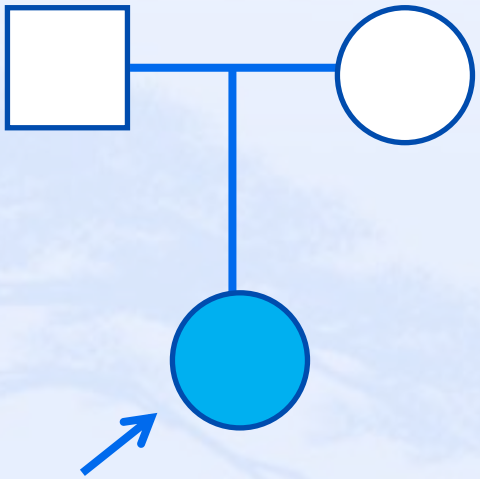
- Clinical criteria are insensitive
  - A Schwartz score of ≥ 4 (i.e. a strong probability of LQTS) had a sensitivity of 38%.
  - Clinical assessment failed to identify 40% of the genetically affected family members of 310 genotyped probands.
- Patients with "concealed" LQTS have a 10% risk of cardiac 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 ⇒ gold standard in family screening

CPVT

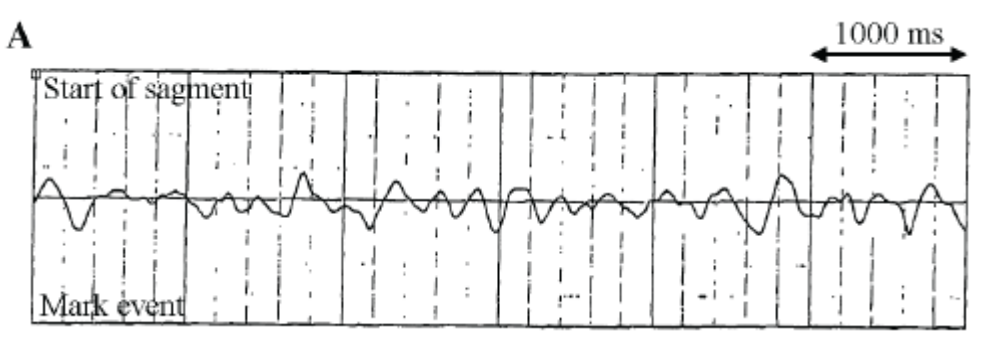
- Catecholaminergic polymorphic ventricular tachycardia
- Syncope and sudden death during physical exertion or emotion (Catecholamine-induced bi-directional VT ⇒ polymorphic VT and VF).
- Mean age of onset 7-9yrs
- Adrenaline provocation test / Exercise stress test
- *RYR2* – autosomal dominant with high penetrance, 50-55%
- *CASQ2* – autosomal recessive, 1-2%

CPVT

- Management
  - Avoid strenuous exercise / acute emotion
  - β-blockers
  - Implantable cardioverter defibrillator
  - FU by cardiologist
  - Screening of family members at risk



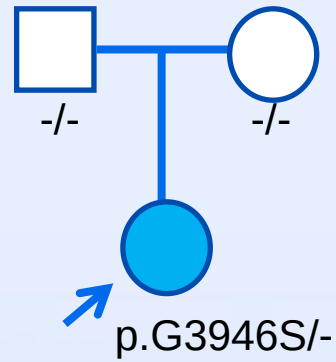
F/14
Good past health
Sudden collapse after boarding a bus. Regained consciousness shortly and collapsed again.
A similar episode 9 months earlier when quarrelling with a friend.



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

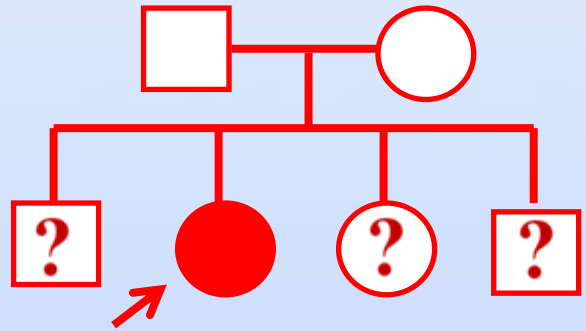
Mok et al. 2006

- 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



Molecular Autopsy

- 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

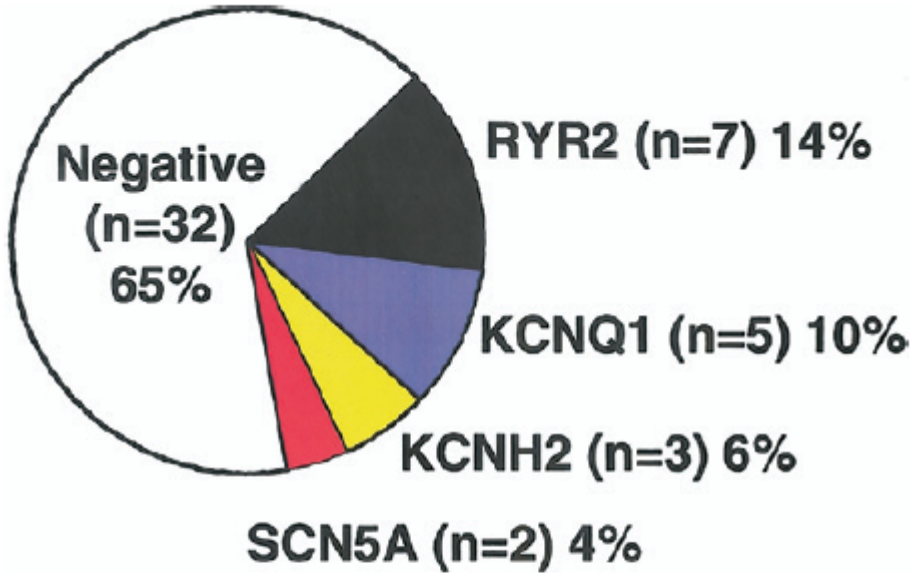


Figure 2 **Summary of the Yield of Postmortem Cardiac Channel Genetic Testing in Cases of Autopsy-Negative SUD**

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.

	LQTS genetic testing	BRCA1/2 screening and oophorectomy	Mammography screening
Cost per year of life saved	$2,500	$3,900-$1,600,000 for high- and average-risk women	$21,400-$117,680 for women ages 50-69yrs and 70-79yrs respectively.

Philips et al. 2005

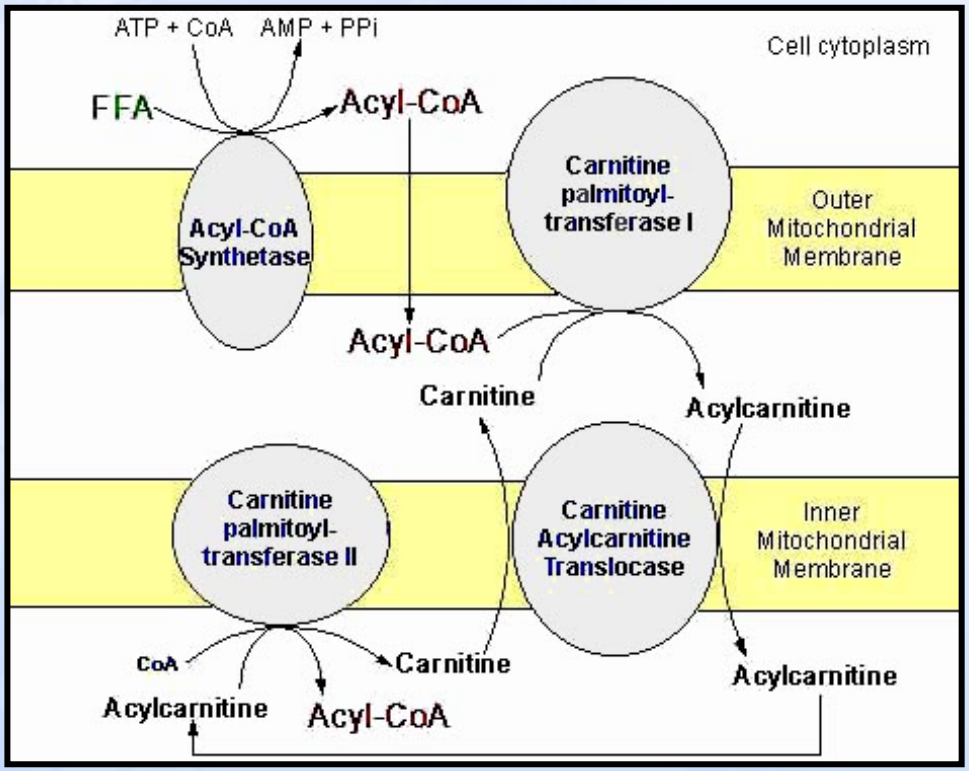
- "*More accurately diagnose and treat affected individuals*."
- "*Clinicians can make more informed judgement about treatment*."
- The cost and benefits of testing at-risk family members not included in the estimation $\Rightarrow$ likely to be more favourable.

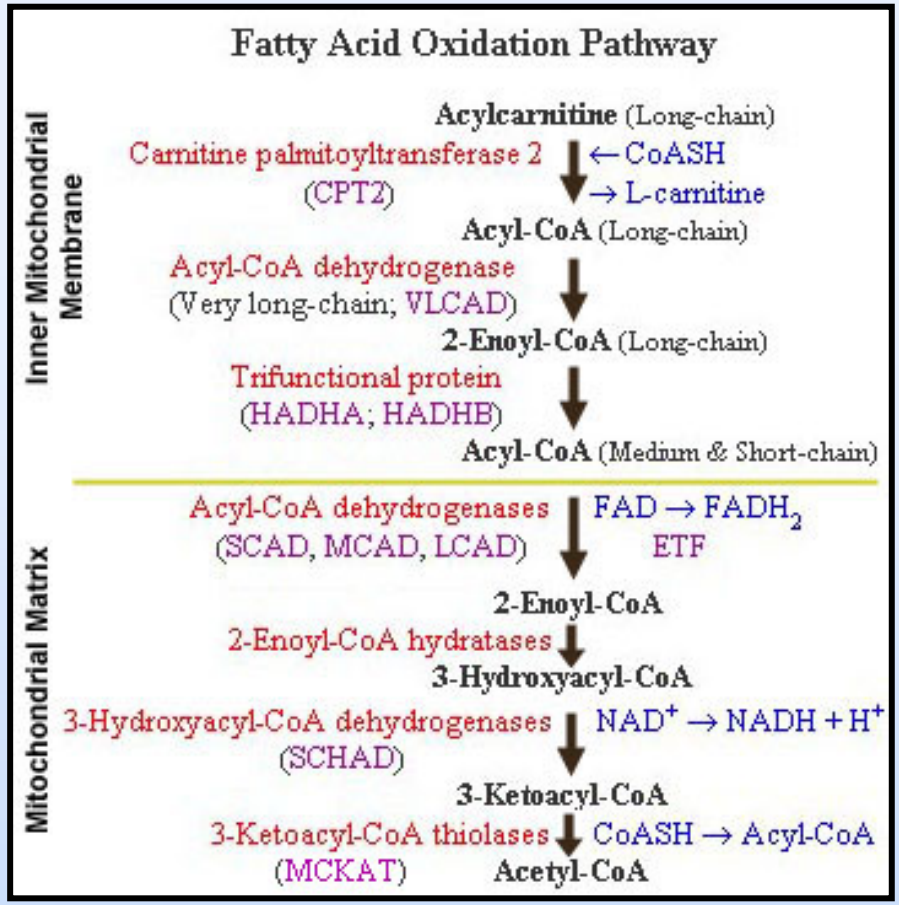
Challenges

- 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





http://images.google.co.uk/imgres

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.

Lam *et al*. 2003

TABLE 1. Clinical features of the three patients diagnosed with carnitine-acylcarnitine translocase deficiency

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

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

Patient No.	Initial plasma glucose (mmol/L)	Maximum ammonia level (μmol/L)	Long-chain acylcarnitine profiles	Dicarboxylic acid in urine	Lactate (mmol/L)	Autopsy
1	Not done	Not done	Not done	Raised	Not done	Steatosis in myocardium and hepatocytes
2	1.5	455	Raised	Raised	5.2	Not applicable
3	1.3	216	Raised	Raised	8.9	Not done

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.

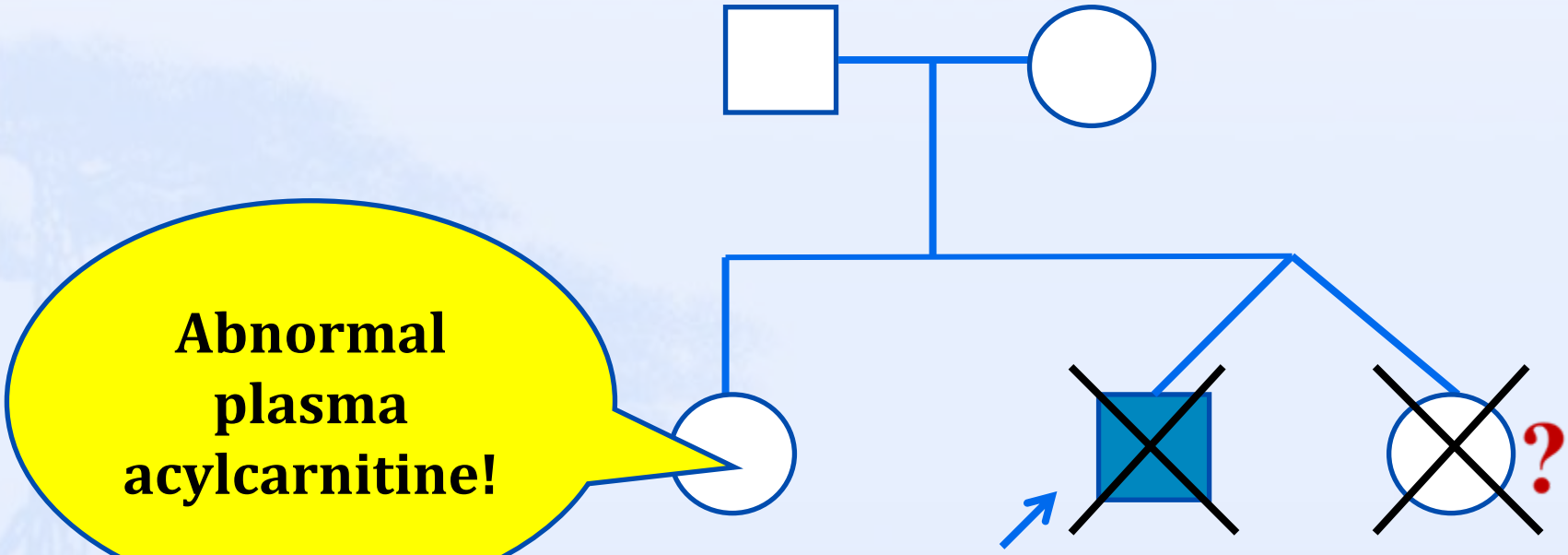
Challenges

- 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

GAII

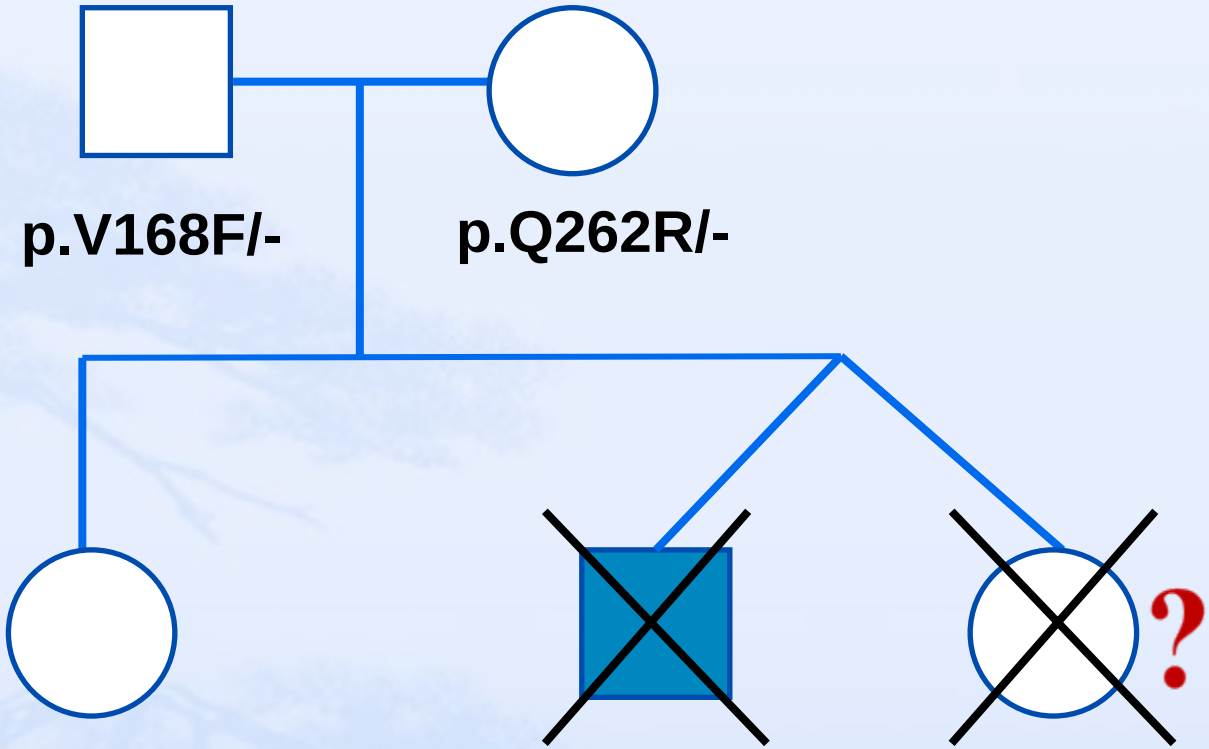
- M/14
- Presented to AED twice within 10hrs.
- Acute deterioration after 9hrs of observation in AED.
- Died 38hrs after admission.
- No biological samples saved for metabolic investigation.
- Retrospective family history – twin sister died of unknown cause in infancy.



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

- 3 known genes for GAII
  - *ETFA*
  - *ETFB*
  - *EFTDH*

- 2 Novel mutations in *ETFA*
  - p.V618F (paternal allele)
  - p.Q262R (maternal allele)



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

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

Died in infancy of unknown cause

Challenges

- GAII – 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.

Acknowledgement

Princess Margaret Hospital

- Dr Albert Chan
- Dr Chloe Mak
- Dr Hencher Lee
- Dr Carol Siu
- Dr NS Mok
- Dr KY Chan
- Dr CC Shek
- Dr SY Lee

HKU Pathology

- Prof CW Lam

Grantham Hospital Cardiac Medicine Unit

- Dr Katherine Fan

THANK YOU

References

1. Tester DJ, Ackerman M. Postmortem long QT syndrome genetic testing for sudden unexplained death in the young. J Am Coll Cardiol 2007;49:240-6.
2. Wedekind H et al. Cardiac arrhythmias and sudden death in infancy: implication for the medicolegal investigation. Int J Legal Med 2007;121:245-257.
3. Tester DJ, Ackerman M. The role of molecular autopsy in unexplained sudden cardiac death. Curr Opin Cardiol 2006;21:166-172.
4. Lam CW et al. Ethnic-specific splicing mutation of the carnitine-acylcanitine translocase gene in a Chinese neonate presenting with sudden unexpected death. Chin Med J (Engl) 2003;116:1110-1112.
5. Lee RSY et al. carnitine-acylcanitine translocase deficiency in three neonates presenting with rapid deterioration and cardiac arrest. Hong Kong Med J 2007;13:66-68.
6. Philips KA et al. Cost-effectiveness analysis of genetic testing for familial long QT syndrome in symptomatic index cases. Heart Rhythm 2005;2:1294-1300.
7. Bai et al. Yield of genetic screening in inherited cardiac channelopathies. How to prioritize access to genetic testing. Circ Arrhythmia Electrophysiol 2009;2:6-15.
8. Lee et al. Role of postmortem genetic testing demonstrated in a case of glutaric aciduria type II. Diag Mol Pathol 2010 (in press).