Using the Molecular Autopsy to Understand Sudden Cardiac Death

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Chief Medical Examiner
City of New York

Objectives

- 1.Define sudden cardiac death and describe which deaths are most appropriate for molecular testing.
- 2.Explain the current techniques and analysis available for the molecular autopsy, including their limitations.
- 3. Integrate the results of the molecular autopsy into the final determination of cause and manner of death and family counseling.

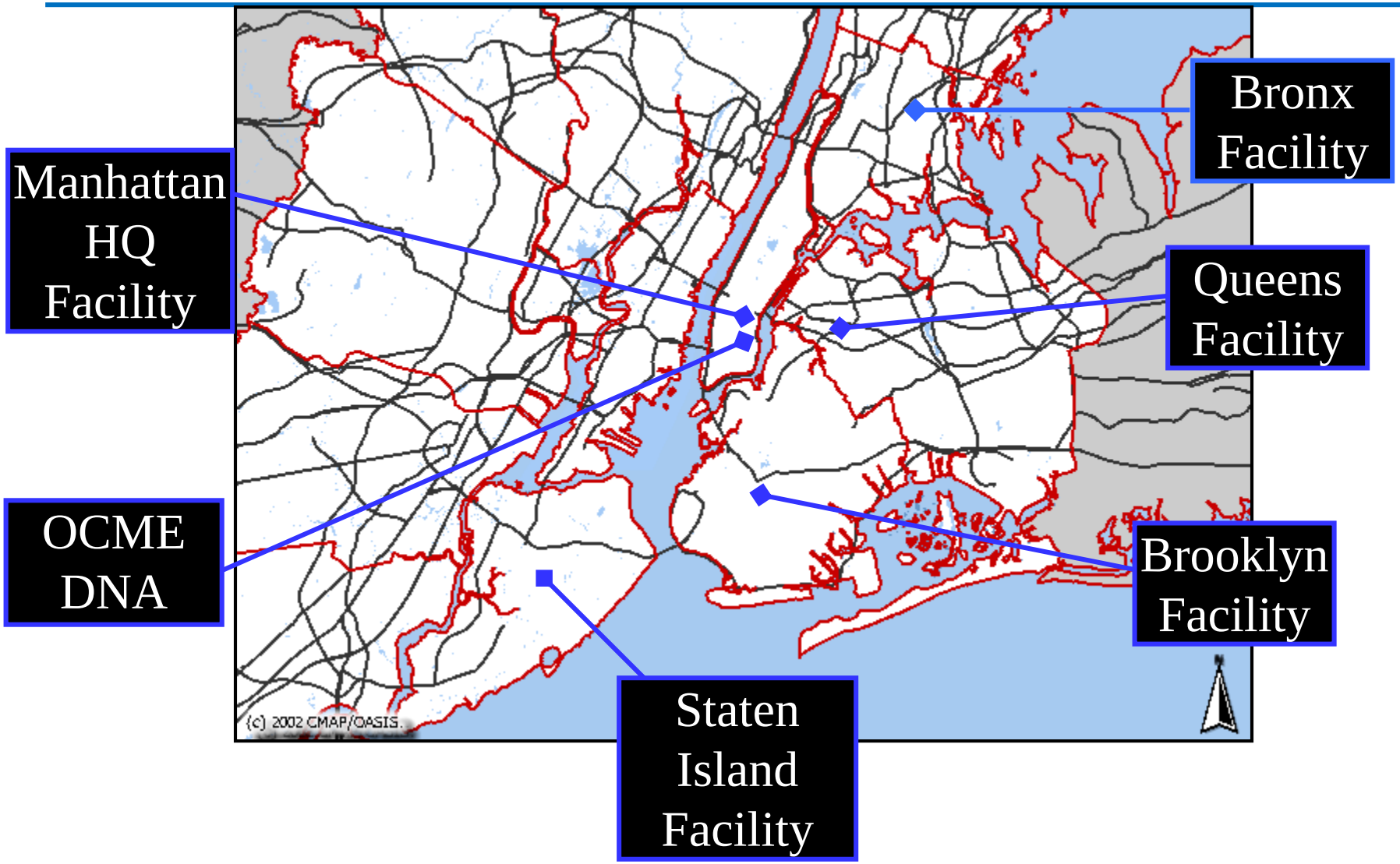
  – I have no disclosures.

Mission NYC OCME

Responsible for investigating deaths resulting from:

- Criminal violence
- Accident or suicide

- **or when death is:**
  - Unattended by a physician
  - Sudden and decedent is in apparently good health
  - Suspicious, or occurs in an unusual manner
- **or when death occurs:**
  - In a correctional facility or in custody
- **the OCME also investigates:**
  - Case that may present a threat to public health
  - Applications to perform cremation


Bronx Facility
Manhattan HQ Facility
Queens Facility
OCME DNA
Brooklyn Facility
Staten Island Facility
(c) 2002 CMAP/OASIS.

Hirsch Center for Forensic Sciences

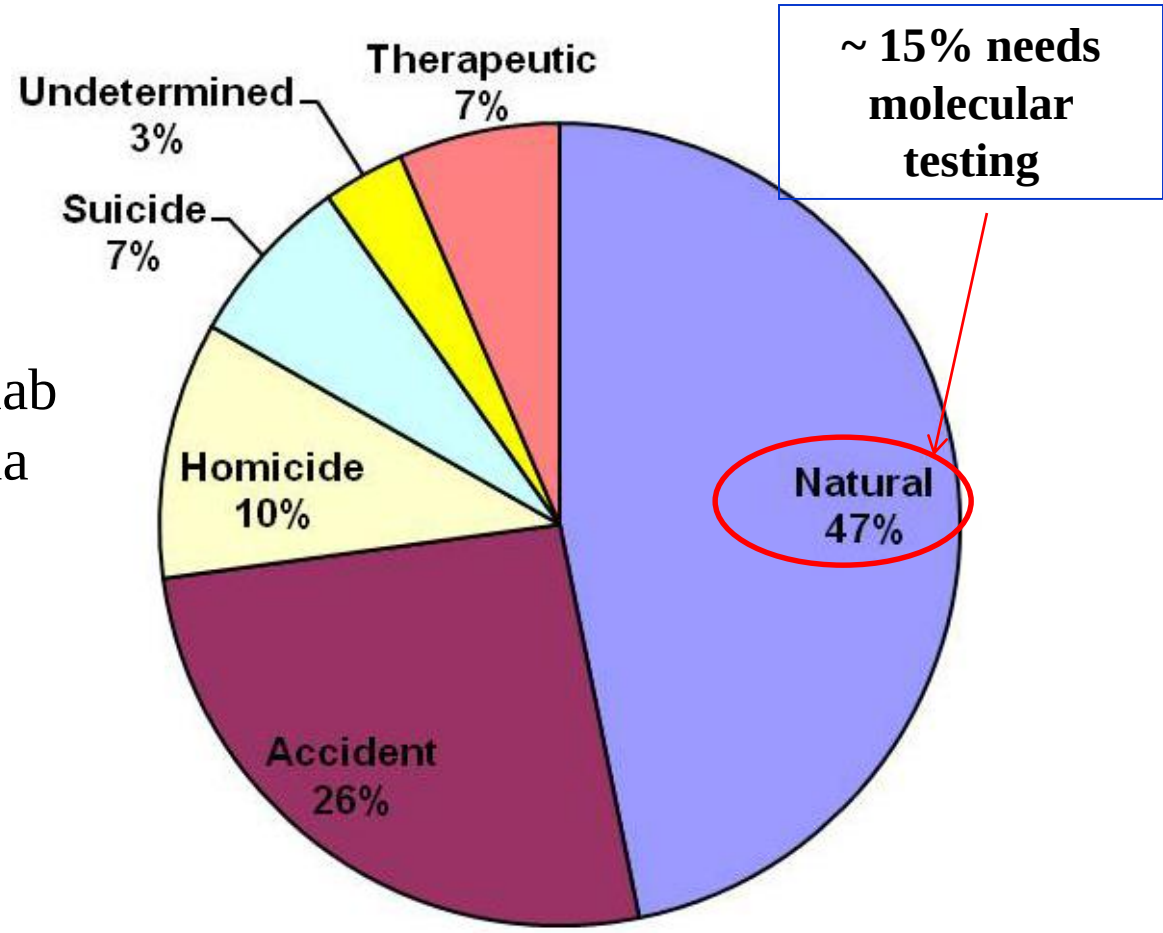
- Dept. of Forensic Biology
- OCME Admin Offices
- Molecular Genetics Laboratory

Services Currently Provided by Molecular Genetics Lab

- Mission - Provides high **quality, timely, and cost-effective** molecular diagnostic services to assist the NYC medical examiners in the determination of the cause of death; Provides on-site professional genetic counseling to families of decedents (since July 2016)
- Molecular diagnostic tests:
  - *Inherited Cardiac Arrhythmias and Cardiomyopathies Molecular Analysis*
  - *Thrombophila Molecular Analysis (FVL and FII)*
  - *Sickle Cell* (SC) *Disease Molecular Analysis*
- Archiving the autopsy specimens for future testing
- Sending out specimen to external testing labo

NYC OCME Cases by Manner of Death

5500 - 6000 autopsies/year
~ 450 case received by MG lab
~ 200 cases for thrombophilia
~ 120 cases for sudden unexpected and unexplained death (SUD)
~ 130 miscellaneous

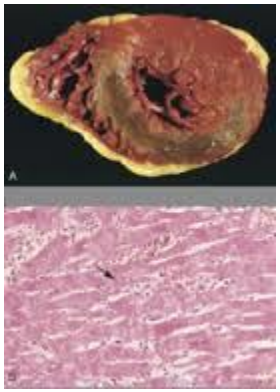


OCME Death Investigation Protocol of SUD

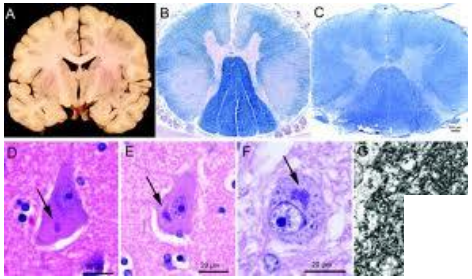
Scene

Toxicology/chemistry

Autopsy

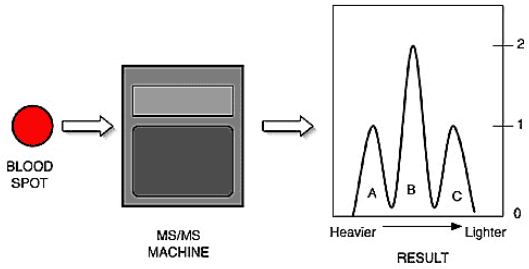


Pathological Exam



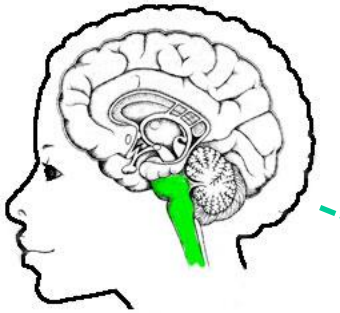
Microbiology

Cause of Death Unexplained



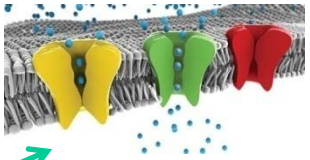
Metabolic Screening

Etiologies of Cardiac Arrhythmia

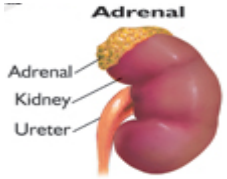


Epilepsy, serotonin (brain stem)

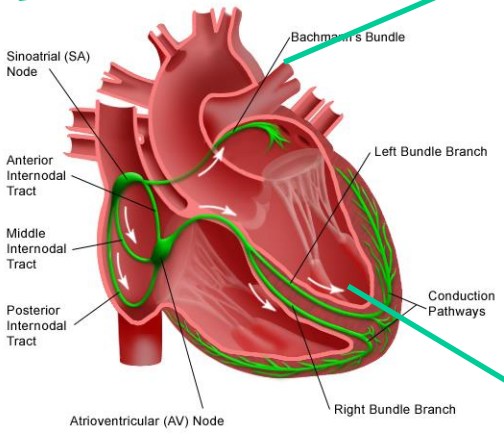
Gene Testing!!

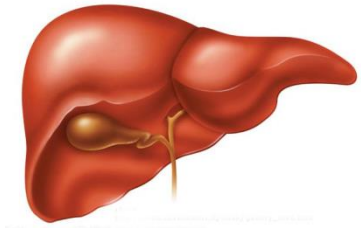


conduction disorders (e.g. Channelopathies)

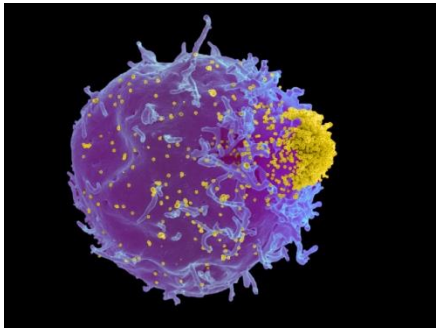


Kidney or Endocrine disorders

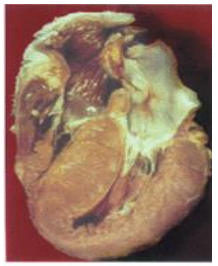


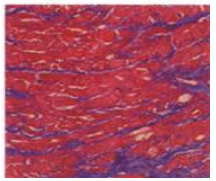


Metabolic disorders



Immune system disorders





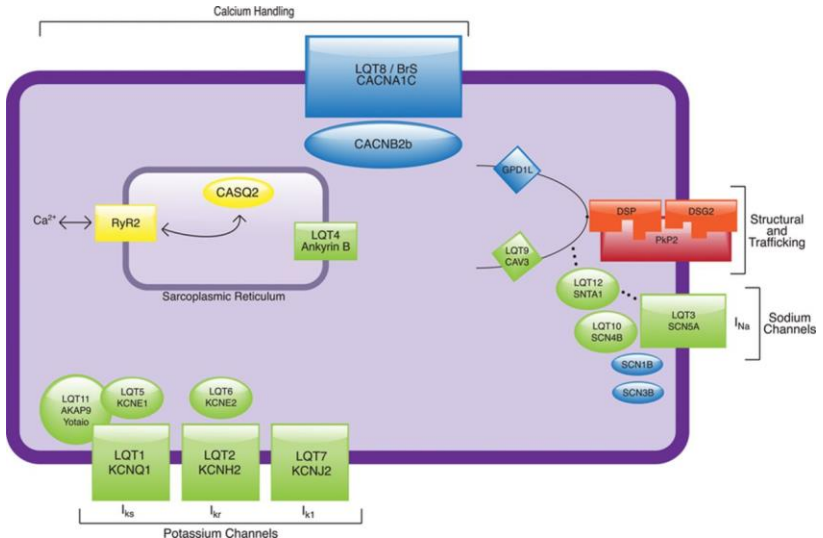
Cardiomyopathies in children & athletes*
(HCM, DCM, LVNC, RCM)

Background on 95-Cardiac-Gene Panel

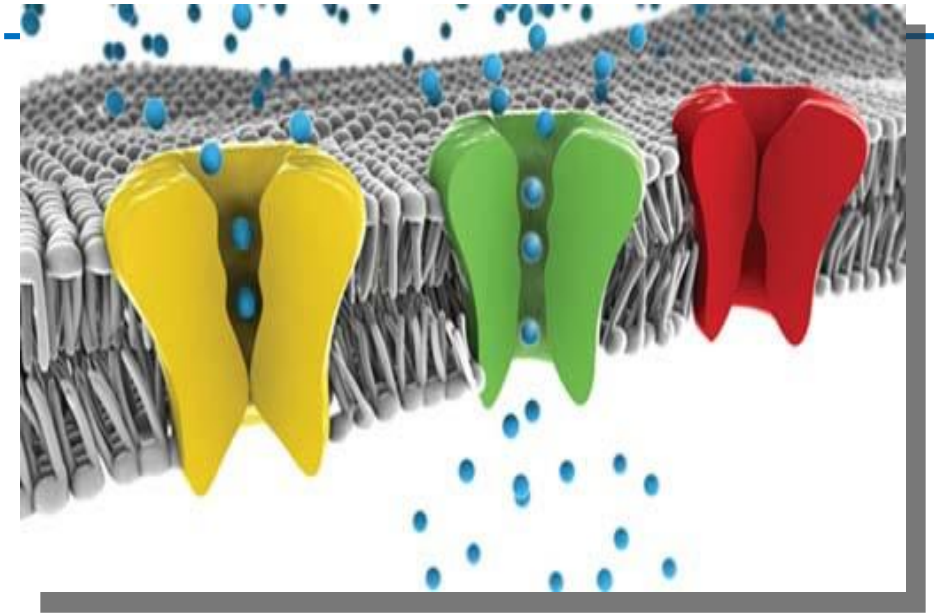
Diseases	Gene Names
Cardiac Channelopathy/Conduction System Disorders (LQTS, Brugada syndrome, SQTS, CPVT, VF)	ABCC9,AKAP10,AKAP9,ANK2,ARHGAP24,CACNA1C,CACNA2D1,CACNB2,CALM1,CALM2,CASQ2,CAV1,CAV3,DPP6,GJA1,GJA5,GPD1L,HCN4,KCNA5,KCND2,KCND3,**KCNE1**,KCNE1L**,** **KCNE2,**KCNE3,KCNE4,**KCNH2**,KCNJ2,KCNJ5,KCNJ8,**KCNQ1**,NPPA,PRKAG2,RANGRF,SCN10A,SCN1B,SCN2B,SCN3B,SCN4B,**SCN5A**,SLMAP,SNTA1,TRDN,TRPM4
Cardiomyopathy (HCM,DCM, LVNC, ARVC)	ACTC1,ACTN2,ANKRD1,BAG3,CALR3,CRYAB,CSRP3,CTF1,DES,DSC2,DSG2,DSP,DTNA,EMD,FHL2,GATAD1,GLA,JPH2,JUP,LAMA4,LAMP2,LDB3,LMNA,MYBPC3,MYH6,MYH7,MYL2,MYL3,MYLK2,MYOZ2,MYPN,NEBL,NEXN,PKP2,PLN,PRDM16,PTPN11,RBM20**,RyR2**,SGCD,TAZ,TCAP,TGFB3,TMEM43,TMPO,TNNC1,TNNI3,TNNT2,TPM1,TTN,VCL

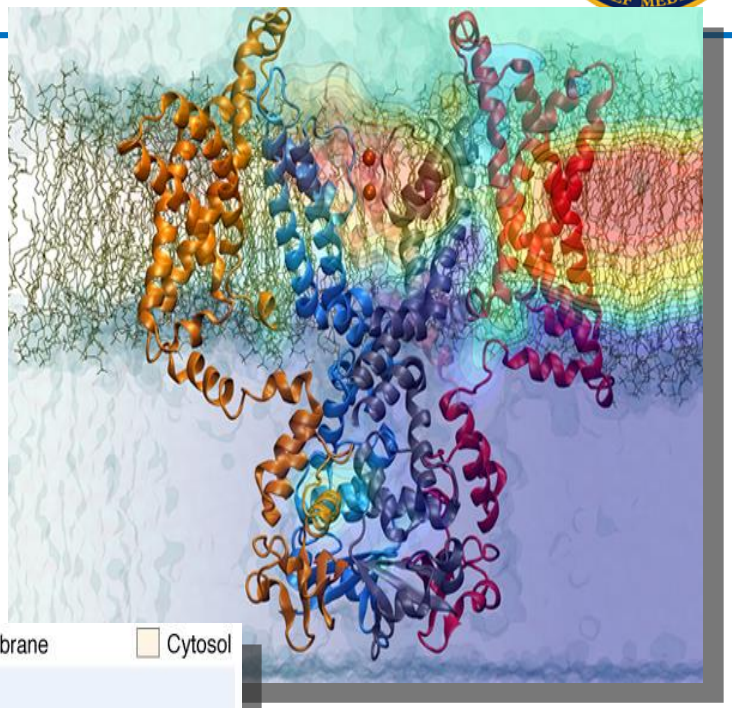
Cardiac Channelopathy Genes

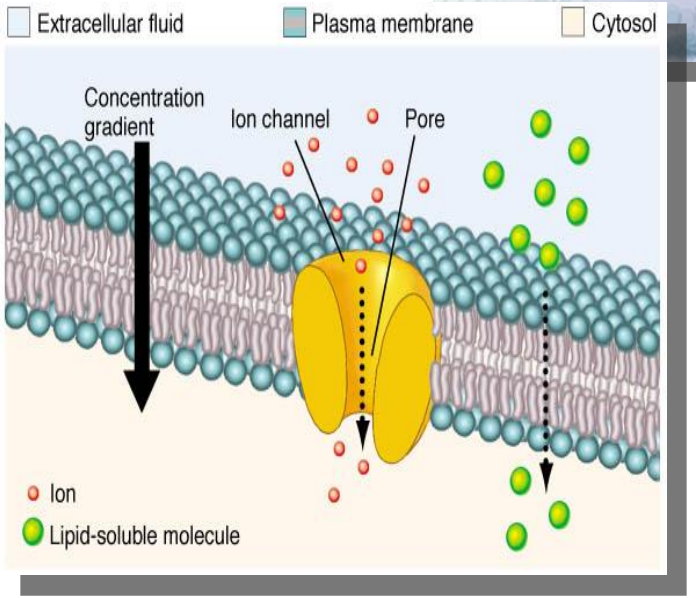
Disease	# Gene	Gene Name
LQT	15	AKAP9, ANK2, CACNA1C, CALM1, CALM2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, KCNQ1, SCN4B, SCN5A, SNTA1
SQT	3	KCNH2, KCNQ1, KCNJ2
Brugada	15	ABCC9, CACNA1C, CACNB2, GPD1L, KCND3, KCNE3, HCN4, KCNJ8, RANGRF, SCN10A, SCN1B, SCN3B, SCN5A, SLMAP, TRPM4
CPVT	4	CASQ2, KCNJ2, RyR2, TRDN
AF	14	ABCC9, GJA5, KCNA5, KCNE1L, KCNE2, KCNE4, KCNJ2, KCNQ1, NPPA, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A
Conduction, Other	9	AKAP10, ARHGAP24, CACNA2D1, CAV1, DPP6, GJA1, KCND2, TRPM4, PRKAG2



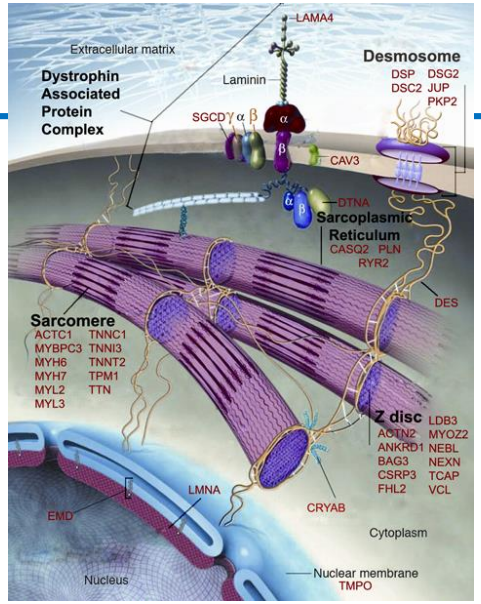
Voltage Gated Ion Channels





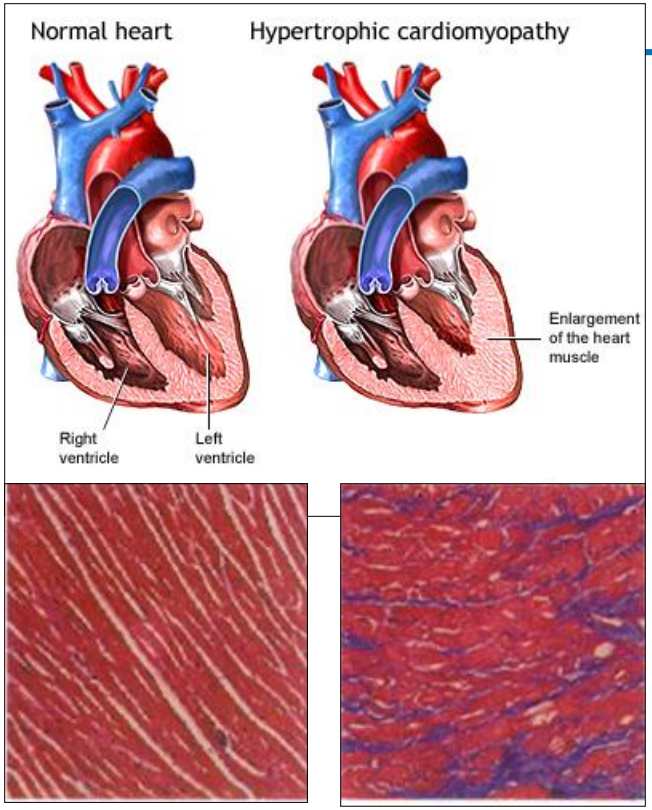


Cardiomyopathy Genes

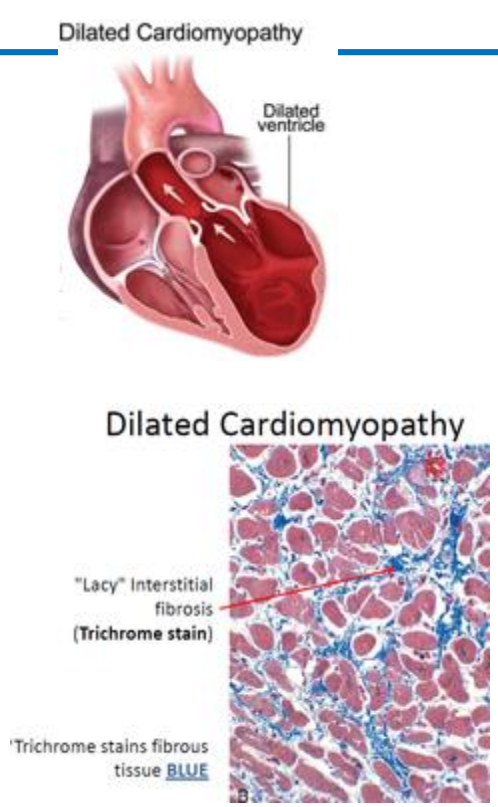


Disease	# Gene	Gene Name
HCM	29	ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CAV3, CSRP3, JPH2, LAMP2, LDB3, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, MYPN, NEXN, PLN, PRKAG2, RyR2, TCAP, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL
DCM	39	ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CRYAB, CSRP3, CTF1, DES, DSC2, DSG2, DSP, EMD, FHL2, GATAD1, LAMA4, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, MYPN, NEBL, NEXN, PKP2, PLN, RBM20, SCN5A, SGCD, TAZ, TCAP, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL
ARVC	10	DES, DSC2, DSG2, DSP, JUP, PKP2, RyR2, TGFB3, TMEM43, TTN
LVNC	11	ACTC1, DTNA, LDB3, LMNA, MYBPC3, MYH7, PRDM16, TAZ, TNNT2, TPM1, VCL
RCM	6	ACTC1, BAG3, DES, MYH7, TNNI3, TNNT2
Other	2	GLA, PTPN11

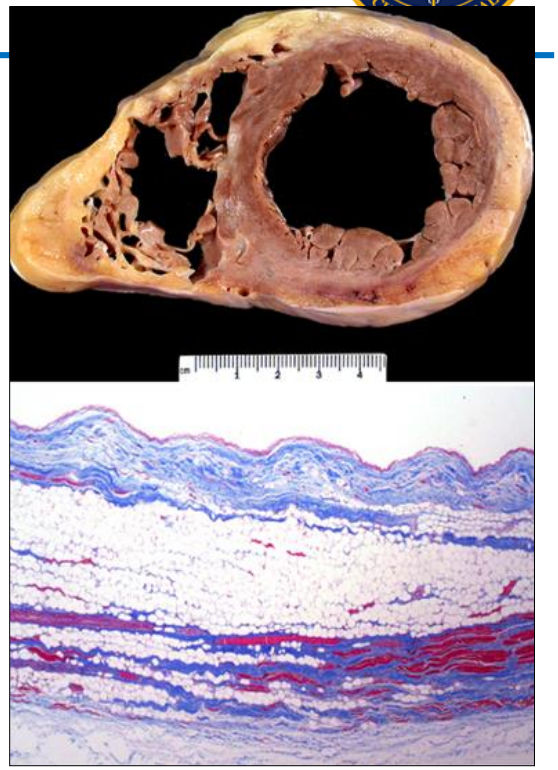
Cardiomyopathies



Hypertrophic Cardiomyopathy (HCM)



Dilated Cardiomyopathy (DCM)



Arrhythmogenic Cardiomyopathy (AC)

David Zieve, MD et al;
Allen Patrick Burke, MD, et al.

Testing Methodology

- Postmortem tissues preserved in RNA*later*®; bloodstain cards for DNA extraction
- Target gene enrichment (Haloplex) and Sequencing by Illumina Miseq
- SOFTGENETICS software (NextGENe, Geneticist Asistant)
- Sanger Sequencing low coverage regions and variant confirmation

Next-Generation Sequencing/Sanger confirmed Variant Interpretation

- Variant Annotation
- ACMG Guidelines

Clinical databases: ClinVar, HGMD, ARVD
Publications: pubmed search
MAF in 1000 genome, ESP6500, ExAC, gnomAD
In silico prediction: PP2, SIFT/proven, MutationTaster
Cardiac pathological findings

Benign/likely benign Clinical Databases classified
Or, MAF >0.5%, and predicted as "benign" by multiple in silico analyses, >3 internal cases

VUS (Variant of Uncertain Significance)
•Neither benign nor pathogenic

Pathogenic/likely Pathogenic
• Clinical Databases classified with strong evidence
• loss of function variants
• novel, or ultra rare MAF <0.04%, and predicted as "deleterious", and correlating phenotype (long QT, cardiac findings)

Case 1 - a *de novo pathogenic* variant in the LQT2 gene

Case 1 – Case Info and Testing Results

- 18y, Asian, female college student was found dead in her bedroom
- Negative autopsy, tox, microbiological tests, etc.
- Panel of Molecular Analysis of 95 Cardiac Genes

Results:

- Total # Benign variants: 182
- Total # VUS variants: 1
- Total # Likely Pathogenic: **1**

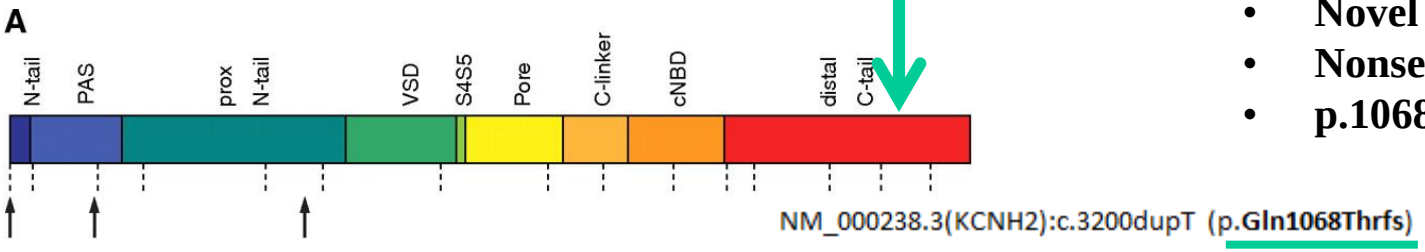
Results Table				
Gene	**Variant (cDNA, genome on Assembly GRCh37)**	**Variant (protein)**	**Zygosity**	**Classification**
KCNH2	NM_000238.3:c.3202C>T g.7:150644093G>A	NP_000229.1:**p.Gln1068Ter** (nonsense variant)	Heterozygous	Likely Pathogenic
CACNA2D1	NM_000722.2:c.2070T>G g.7:81601164A>C	NP_000713.2:**p.Ile690Met** (missense variant)	Heterozygous	Variant of Uncertain Significance (VUS)

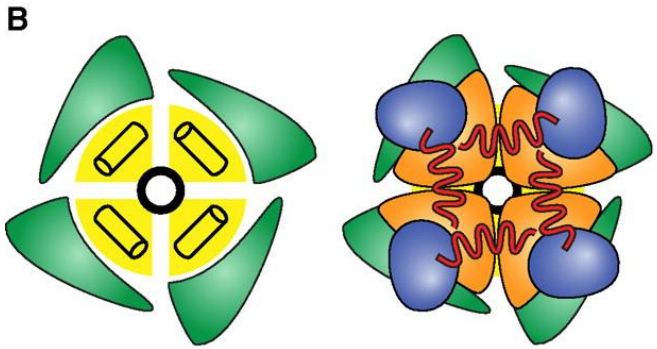
Case 1 - Variant Interpretation

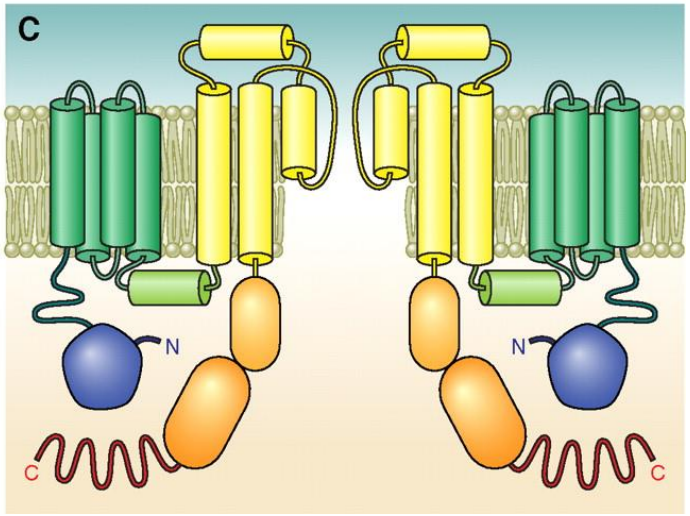
KCNH2 p.**Gln1068Ter**

Likely Pathogenic

- **Novel**
- **Nonsense variant**
- **p.1068 frameshift reported**



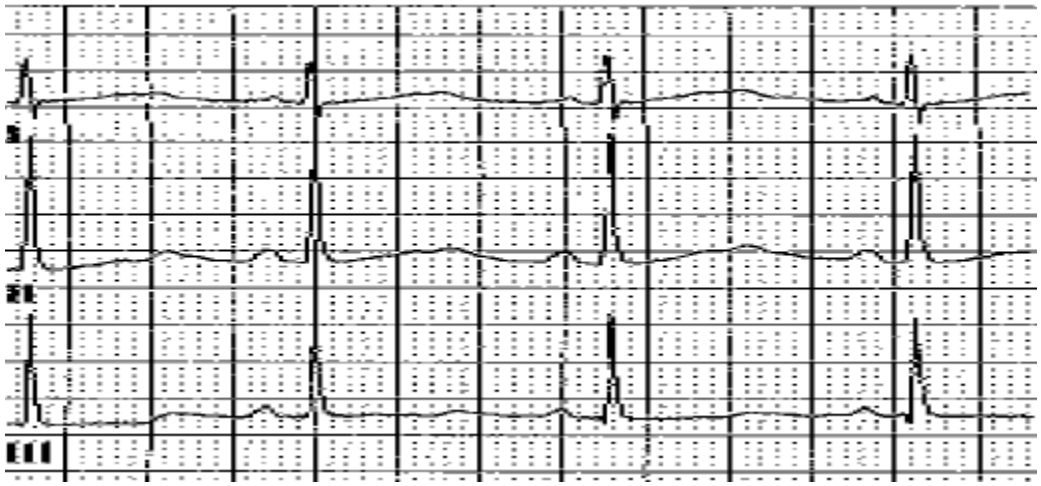




HERG protein encode by KCNH2 gene
Total 1159 amino acids

Case 1 - Medical History Review

Feb 24, 2017: initial ECG
March 24, 2017: stress test
April 14, 2017: missed f/u
May 14, 2017: died



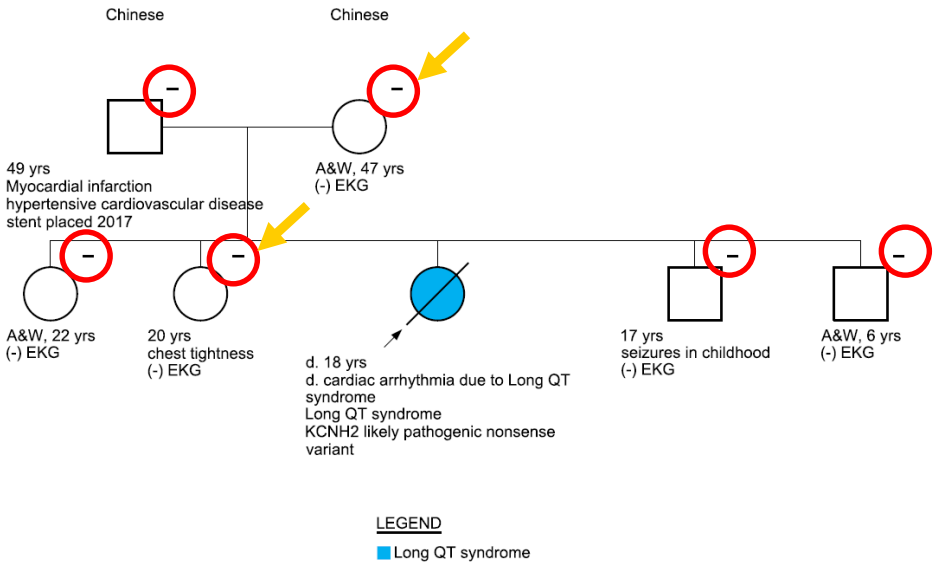
ID:008579218 24-FEB-2017 13:30:59 BETH ISRAEL HOSPITAL-DM ROUTINE RETRIEVAL

Vent. rate	85	BPM
PR interval	124	ms
QRS duration	72	ms
QT/QTc	436/518	ms
P-R-T axes	70 78	36

NORMAL SINUS RHYTHM
PROLONGED QT INTERVAL OR TU FUSION, CONSIDER MYOCARDIAL DISEASE, ELECTROLYTE IMBALANCE, OR DRUG EFFECTS
ABNORMAL ECG
NO PREVIOUS ECGS AVAILABLE
Confirmed by MISRA, DEEPIKA (2023) on 2/24/2017 3:56:48 PM

Comment:
The QT-interval was prolonged at baseline and was prolonged in the recovery phase, with a QT of 370 msec and a QTc of 638 msec in minute-4 of recovery; patients with a QTc ≥ 480 msec at minute-4 of recovery have been shown to have a high likelihood of having LQT1 or LQT2.[1]

Case 1 - Additional Studies



- Family Study (Dr. Marina Cerrone at NYU)

De novo

Results Table				
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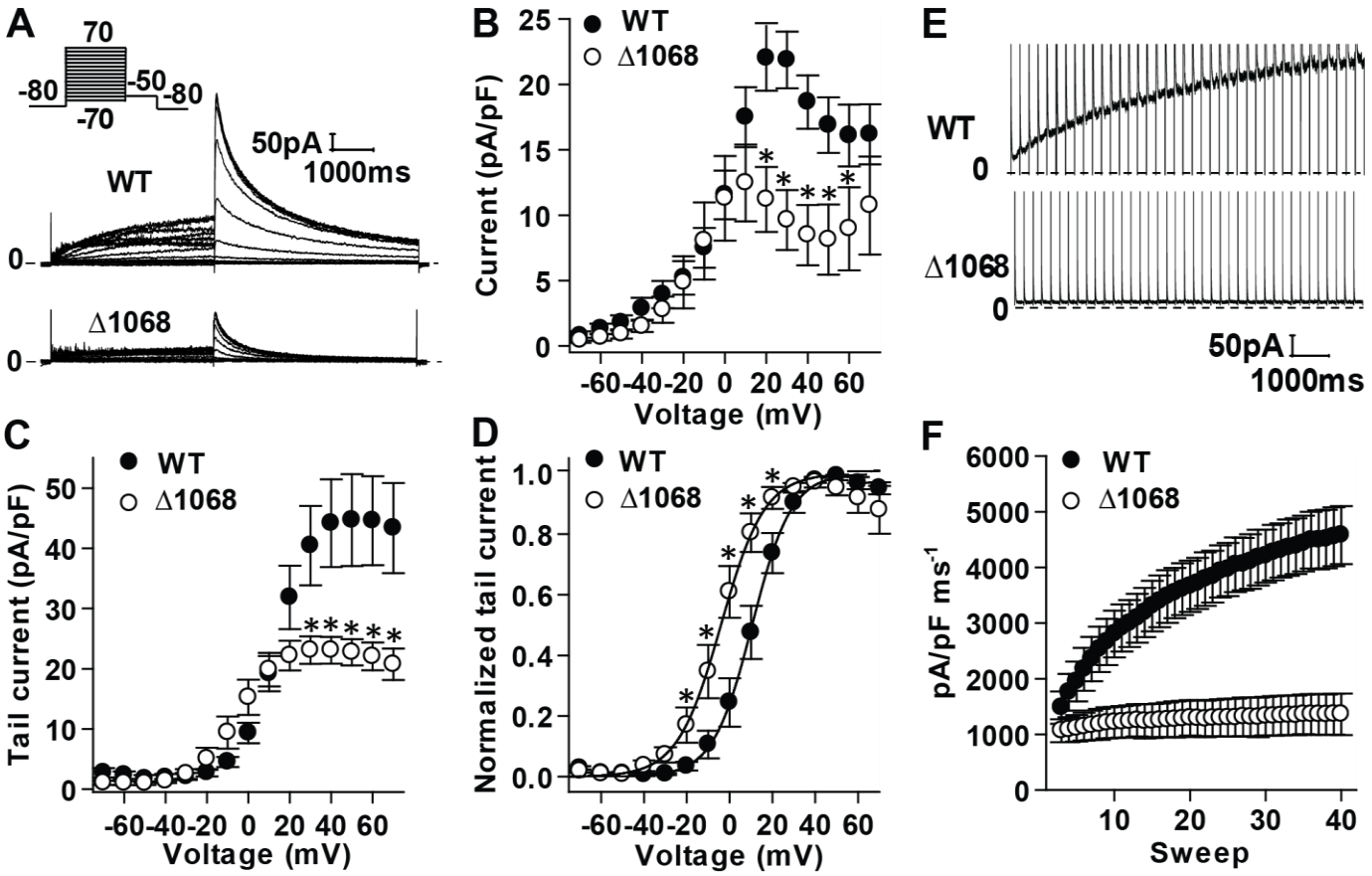
inherited

Case 1 – Functional Studies



Comparison of the Healthcare for Surviving Family Members With and Without Molecular Autopsy

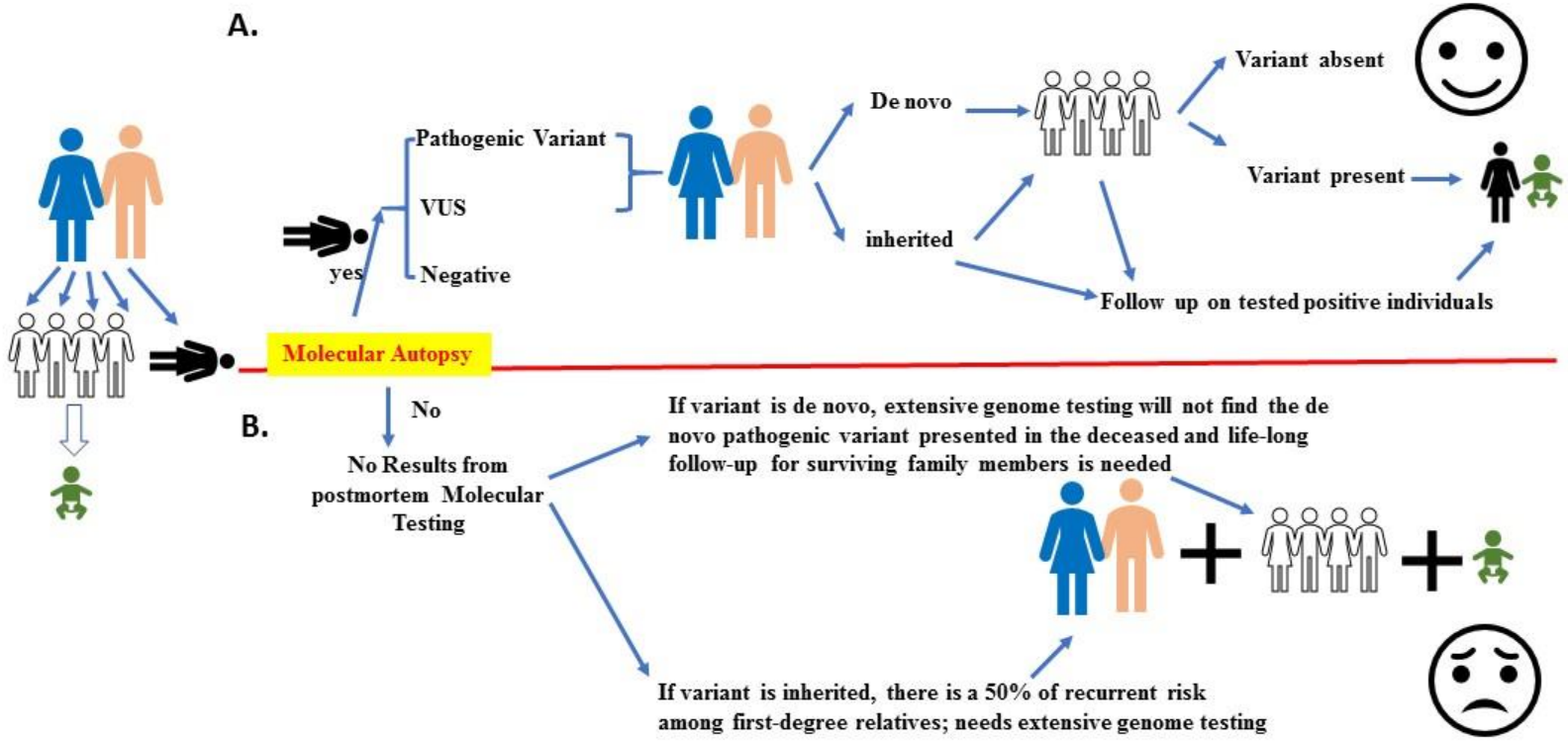


Figure 3: Comparison of the Healthcare for Surviving Family Members With and Without Molecular Autopsy

Myocarditis and a Coexisting LQT variant

Myocarditis and a Coexisting Pathogenic LQT variant

- 5-year-old Hispanic girl with mild asthma found unresponsive in bed.

CAUSE OF DEATH: **MYOCARDITIS OF PROBABLE VIRAL ETIOLOGY.**

MANNER OF DEATH: **NATURAL.**

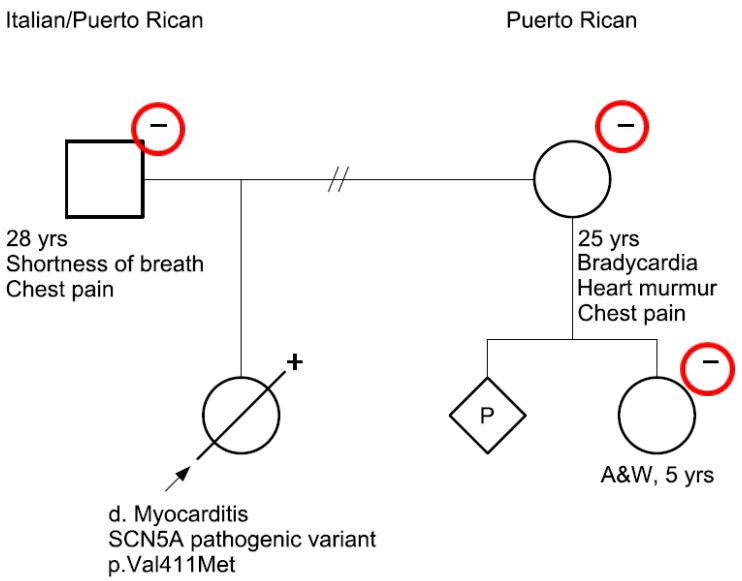
- **We tested 95-cardiac-gene panel**

Results Table				
Gene	**Variant (cDNA, genome on Assembly GRCh37)**	**Variant (protein)**	**Zygosity**	**Classification**
SCN5A	NM_198056.2:c.1231G>A g.3:38647549C>T	NP_932173.1:**p.Val411Met** (missense variant)	Heterozygous	Pathogenic

- The variant has been reported 5 separate times as a pathogenic variant in ClinVar. This variant had been published in literature in several unrelated patients with LQTS. In vitro study supported the function defects of the sodium channel with the p.Val411Met change. The variant is not found in large population databases. Multiple in silico variant effect analyses consistently predict the deleterious effect of this variant.

Sample ID	Receiving date	Sample Type	Adeno	CMV	EBV	Parvo	HHV6	RSV	Entero	Influenza	HCV
FBMG14-0402	9/7/2016	Heart	-	-	-	+	-	-	-	-	-

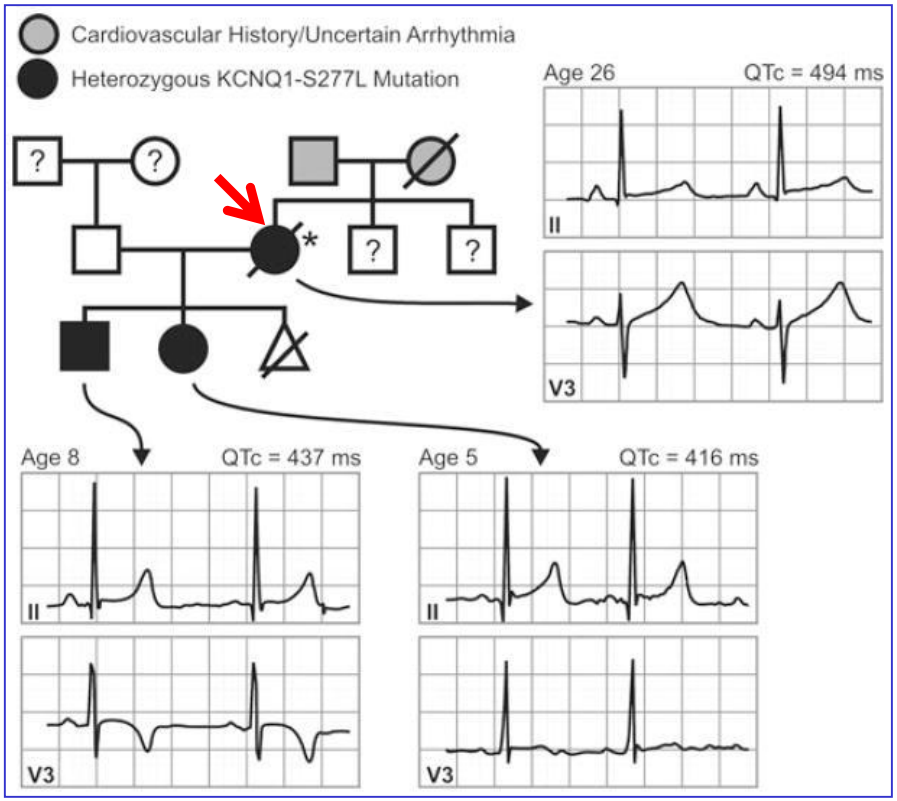
Recurrent, de novo LQT Variant in Myocarditis



- **De novo in this case**
- It is a recurrent, de novo variant : This variant was found to be absent from parental testing and therefore suspected to have occurred de novo in two unrelated individuals with LQTS (Carrasco et al., 2012; Stattin et al., 2012).
- Implication of testing cardiac genes in Myocarditis: triggering or compounding effect?

Cocaine Intoxication and a Coexisting LQT1 Variant

Cocaine Intoxication and a Coexisting LQT1 Variant



Chen (2011) *Pacing Clin Electrophysiol* 34: 1652

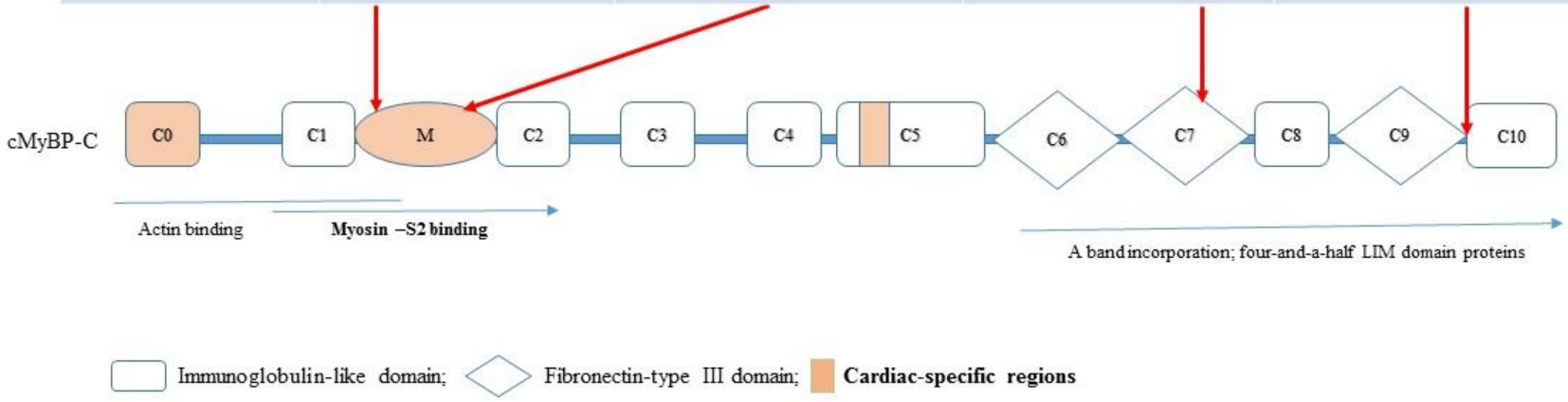
- A 26-year-old Hispanic female sudden collapse at home
- Postmortem toxicology study revealed
  - ethanol 0.01g%
  - cocaine (0.06 mg/L) and cocaine derivatives (ethylbenzoylecgonine 0.16 mg/L benzoylecgonine 1.0 mg/L) in her serum
- springtime of the following year, her husband took the two kids to visit the cardiologist at Montefiore. Her past medical: she referred for cardiac evaluation after a prolonged QT interval was noted on a screening ECG obtained during a preoperative assessment for umbilical hernia repair.
- She reported having a syncopal episode preceded by dizziness at age 10, and another episode several weeks prior to her cardiac evaluation
- ECG revealed normal sinus rhythm with a variable resting QTc duration (maximal recorded resting QTc =494 ms)
- **OCME tested and found a pathogenic LQT1 variant in**
- two young children resting QTc intervals for the children were not prolonged; A treadmill test was performed, both children exhibiting prolonged QTc at 2 min 40 sec recovery
- Both children carry the variant

Four HCM Cases – Genotype/Phenotype Observation

Four HCM Cases (A to D) – Genotype/Phenotype Observation

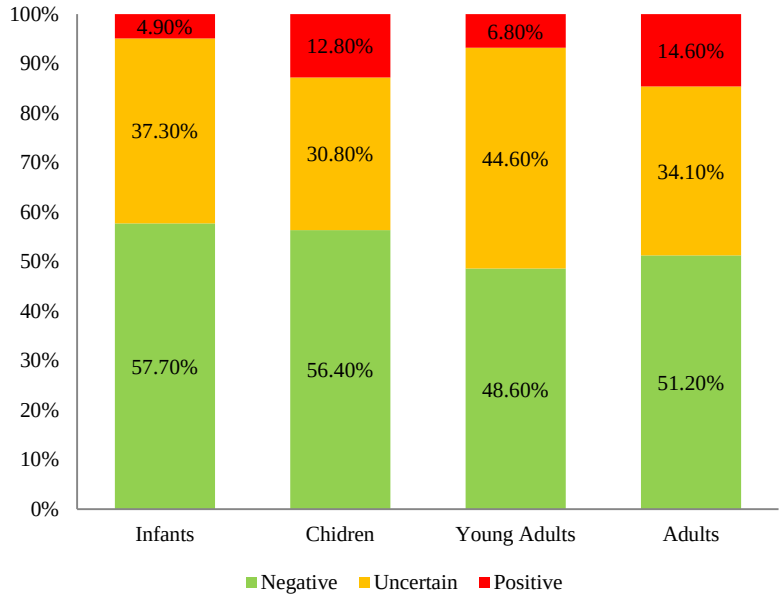
Figure 1. Correlate HCM phenotype with Genotype of Predicated Protein-truncating Variants Located in the cMyBP-C Protein

Case Information	Case A	Case B	Case C	Case D
Ratio (heart weight (g)/BMI (kg/m2))	34.90 (890/25.5)	23.56 (980/41.6)	14.71 (450/30.6)	13.98 (600/42.9)
Age-at-death, gender, ethnicity	38 years, male, African American	38 years, male, Hispanic	57 years, male, Hispanic	67 years, male, African American
PTVs in *MYBPC3*	p.Gly278GlufsTer22 g.11:47369221delC	p.Phe295SerfsTer5 g.11:47368998delA	p.Gln969Ter g.11:47356593G>A	c.3491-2A>T g.11:47354255T>A

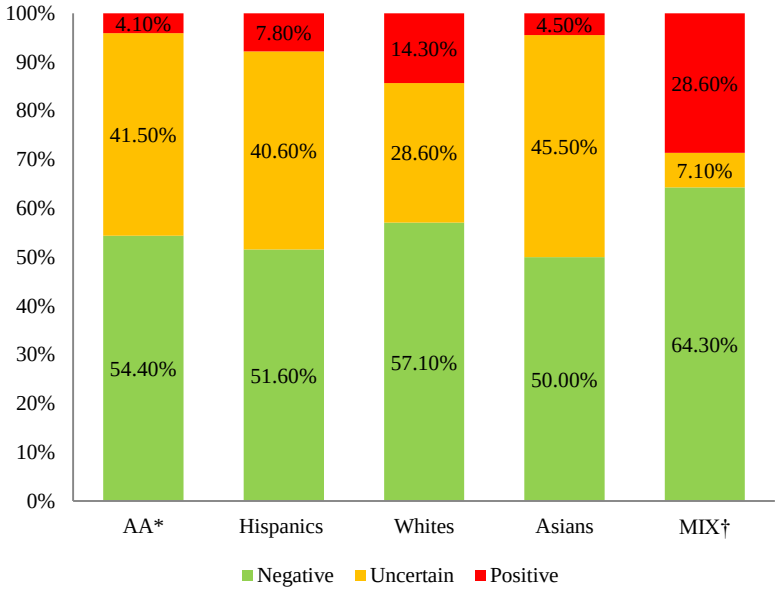


Utility of Testing 95-cardiac genes in Autopsy Negative Sudden Natural Death

Testing Yield in Autopsy Negative Cases (276 Cases)



By Age



By Ethnicity

Circ Cardiovasc Genet. 2017;10:e001839. DOI: 10.1161/CIRCGENETICS.117.001839

>50% cases remained negative after the 95-cardiac-gene testing

Current Test Menu (Since July 2019)

TEST NAME	DESCRIPTION
Cardiac-focused Sudden Death Molecular Analysis (Panel A)	**Total 137 genes: cardiovascular system conditions (cardiomyopathies, cardiac channelopathy, pulmonary arterial hypertension), and non-cardiac channelopathy (Bartter/Gitelman's syndrome, familial hyperinsulinism)**
Epilepsy-focused Sudden Death Molecular Analysis (Panel B)	**Total 132 genes:** associated with epilepsy (12 channel genes are in both Panel A and Panel B)
Cardiac & Epilepsy Sudden Death Molecular Analysis (Panel C)	**Total 257 genes:** Panel A +Panel B
Aortopathy Analysis	**19** genes associated with aortic aneurysms and dissections
Malignant Hyperthermia Susceptibility Analysis	**2** genes associated with malignant Hyperthermia Susceptibility
Thrombophilia Analysis	**3** genes for anticoagulants (*SERPINC1, PROS1, PROC*) and 2 SNPs (*FVL and FII G20210A*)
Sickle Cell Disease Analysis	Hemoglobin S and C

Results of Testing Panel A (August – November 2019)

Cases	# Cases	Percentage of Total
# Cases with P/LP	5	11%
# Cases with VUS	26	59%
# Cases with B/LB	13	30%
Total # Cases Tested	44	100%

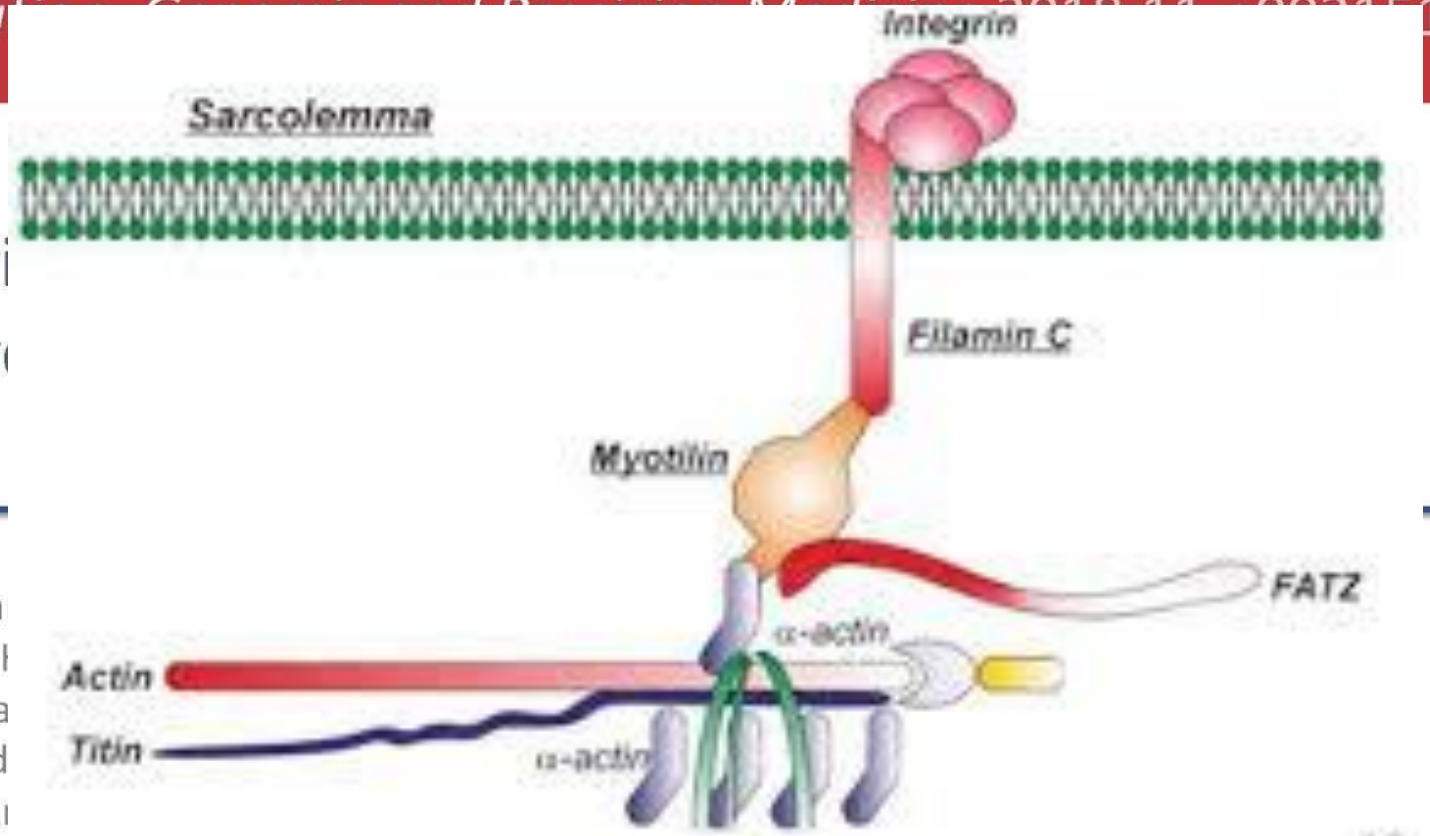
Cases with P/LP Variants

Case	Demographic of Decedent	ME	Molecular Finding	Condition	Comments
19MG0089	23y, Male, White	Hayes	FLNC, LP	Arrhythmogenic Cardiomyopathy (AC)	Not on 95 gene panel
18MG0236	51y, Female, Black	Smiddy; Slone	PKP2, P	AC	On 95 gene panel
19MG0265	33y, Female, White	Drobyshe va; Slone	JUP, LP	AC	On 95 gene panel
19MG0314	13y, Male, Hispanic	Kelly	RYR2, LP RYR2, VUS;	CPVT	On 95 gene panel

FLNC Gene Encodes Gamma Filamin

JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 68, NO. 22, 2016

Circulat

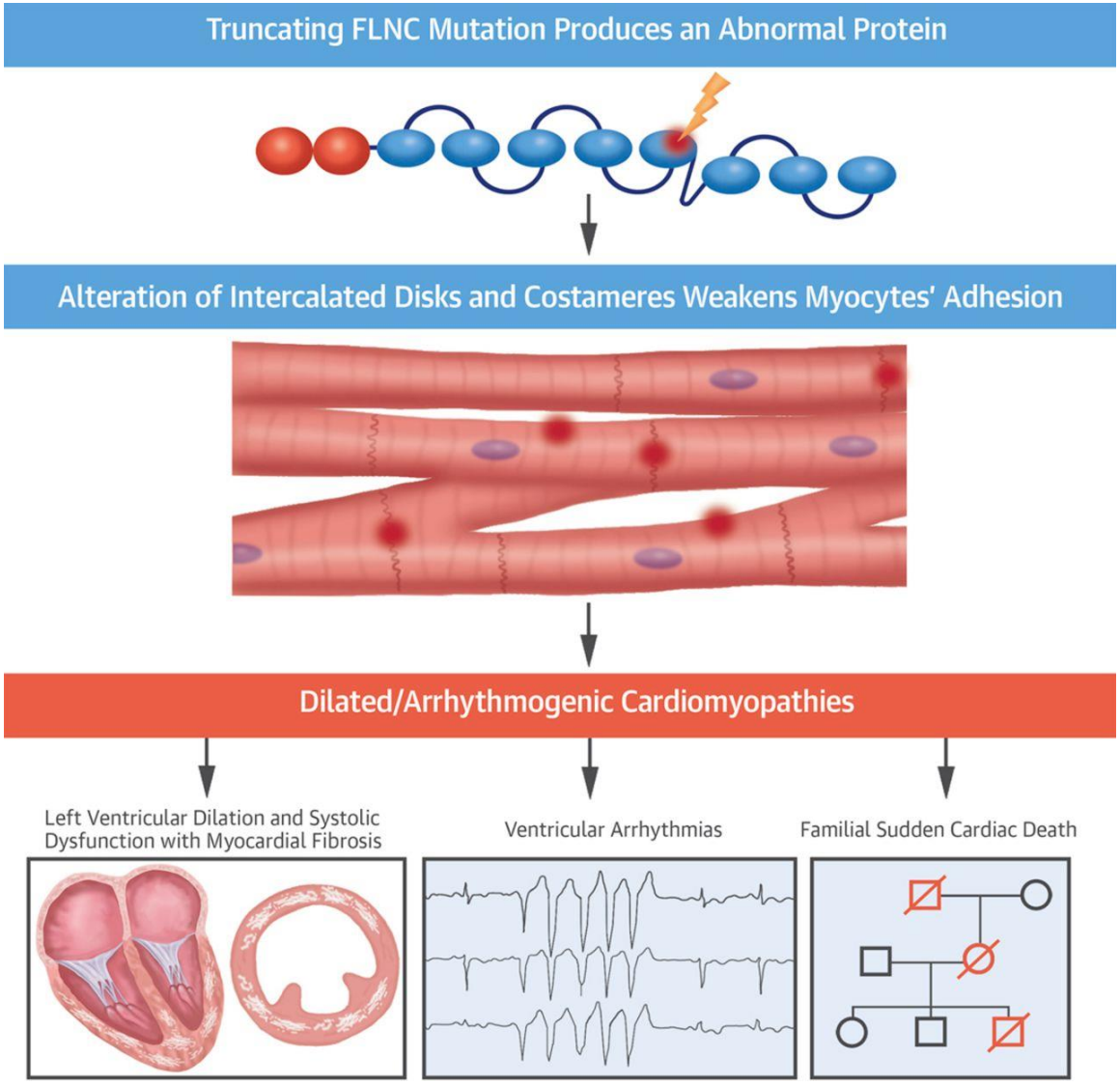


Vari
Car

Garda
Anna I
Brynja
Sigurd
Karl Ar
Unnur Thorsteinsdottir, Patrick Sulem, Gudmundur Thorgeirsson,
Daniel F. Gudbjartsson, Hilma Holm, Kari Stefansson

anchoring of membrane proteins for the actin cytoskeleton

Possible Mechanism



Source: Journal of the American College of Cardiology

Case Example - *FLNC* Variant

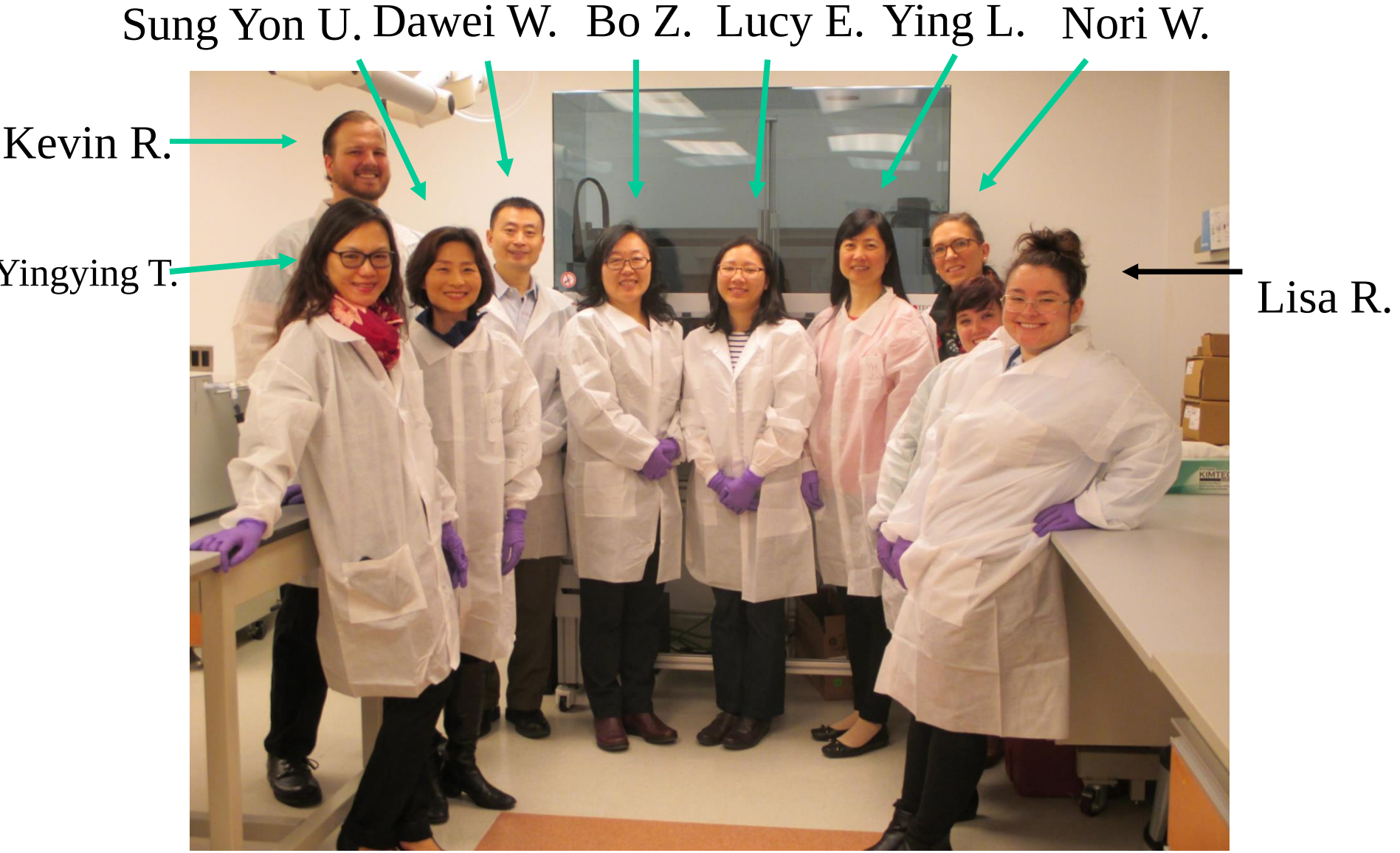
Results Table				
Gene	**Variant (cDNA, genome on Assembly GRCh37)**	**Variant (protein)**	**Zygosity**	**Classification**
FLNC	NM_001458.4:c.1565delC g.7:128480617delC	NP_001449.3:**p.Pro522GlnfsTer2** (frameshift)	Heterozygous	Likely Pathogenic

Novelty: the variant is novel; parental studies can help to determine if this novel variant arose *de novo.*

Variant type, zygosity, or function domain: deletion, frameshift, heterozygous

This variant is a single nucleotide deletion at c.1565delC, leading to amino acid change at position 522 from Proline to Glutamine, and a premature stop codon at codon number 2 into the new reading frame (p.Pro522GlnfsTer2). Compared to the total 2725 amino acids of the wild-type *FLNC* encoded protein, this variant is expected to lead to a truncated protein product.

Current Staff in MG Lab



OCME's Partner Programs

- **Clinical Programs:**
  - Dr. Tom McDonald at Montefiore/Einstein
  - Dr. Wendy Chung at Columbia University
  - Dr. Mark Sherrid at NYU (cardiomyopathy, HCM)
- **Functional Studies**
  - Dr. William Coetzee at NYU
  - Dr. Tom McDonald at Einstein
  - Dr. Marina Cerrone/Mario Delmar at NYU
- **Exome/genome testing (cohort vs. controls burden test)**
  - Dr. David Goldstein at Columbia University

Objectives Review

- 1.Define sudden cardiac death and describe which deaths are most appropriate for molecular testing.
- 2.Explain the current techniques and analysis available for the molecular autopsy, including their limitations.
- 3. Integrate the results of the molecular autopsy into the final determination of cause and manner of death and family counseling.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