



Molecular Autopsy

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**Belgian Society of
CARDIOLOGY**
**Belgian Society of
HUMAN GENETICS**
BELCARGEN



**European
Reference
Network**

for rare or low prevalence
complex diseases

🔗 **Network**
Heart Diseases
(ERN GUARD-HEART)

● **Member**
University Hospital
Leuven — Belgium

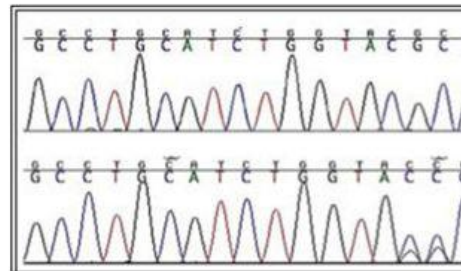
What is a molecular autopsy?



Postmortem blood

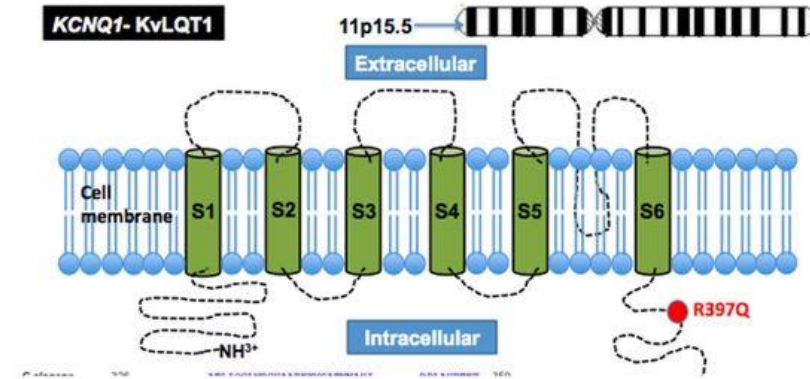


DNA extraction

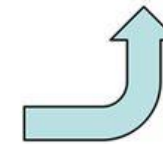


Genetic analysis

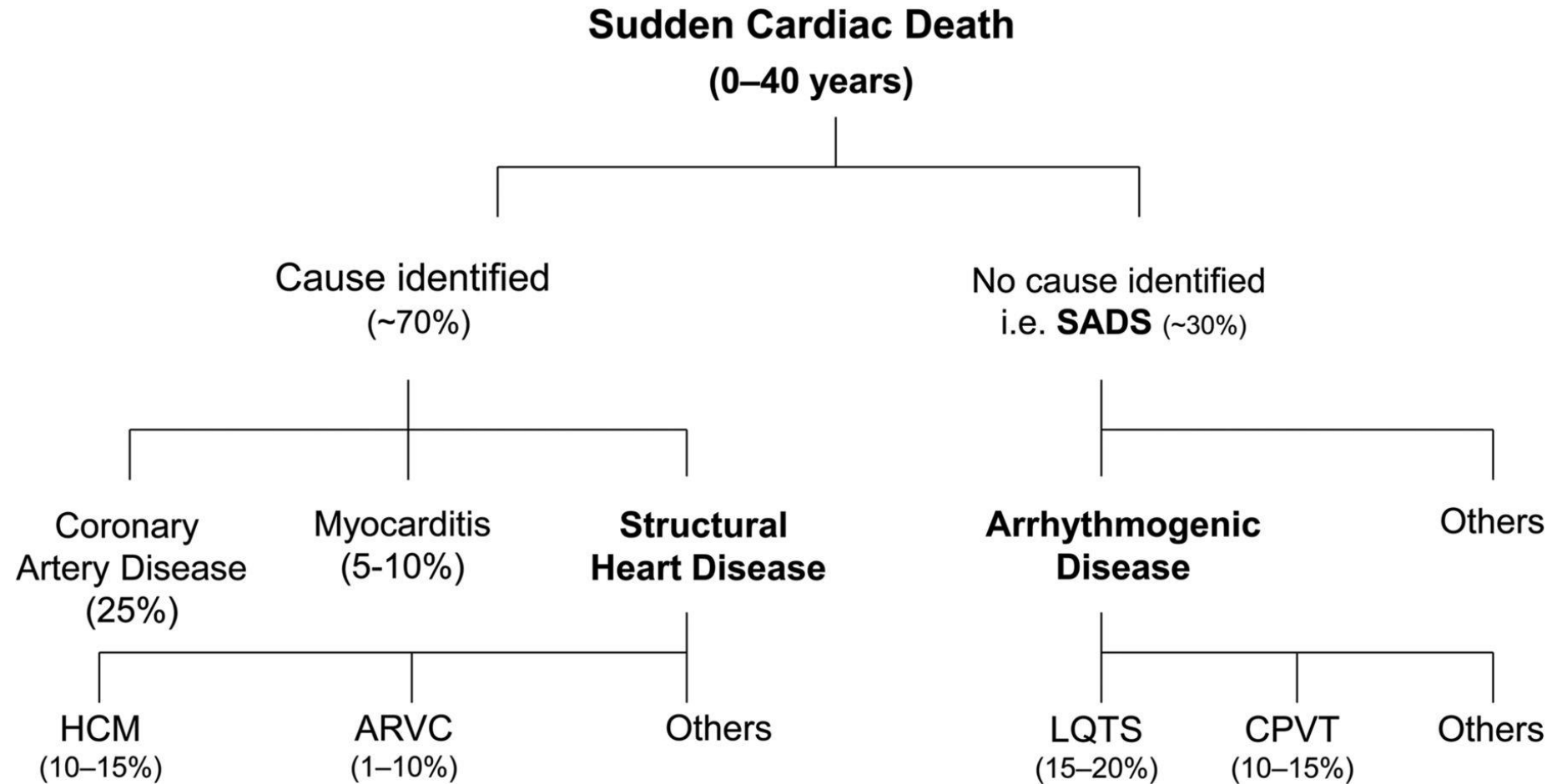
- Sanger sequencing
- Cardiac gene panels
- Whole exome / genomes



Pathogenic
(disease-causing)
mutation

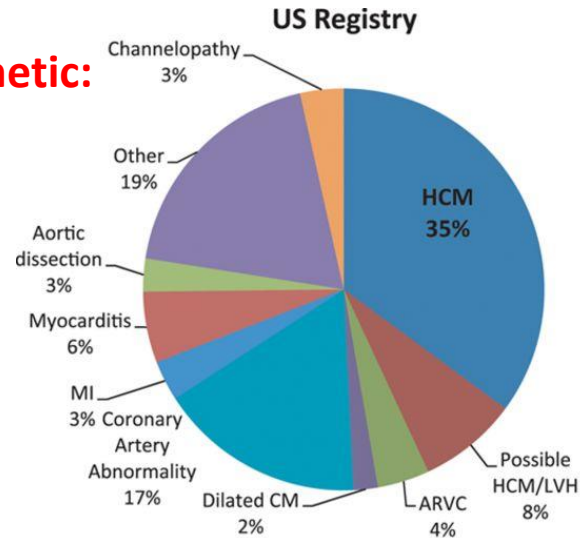


Causes of SCD in the Young

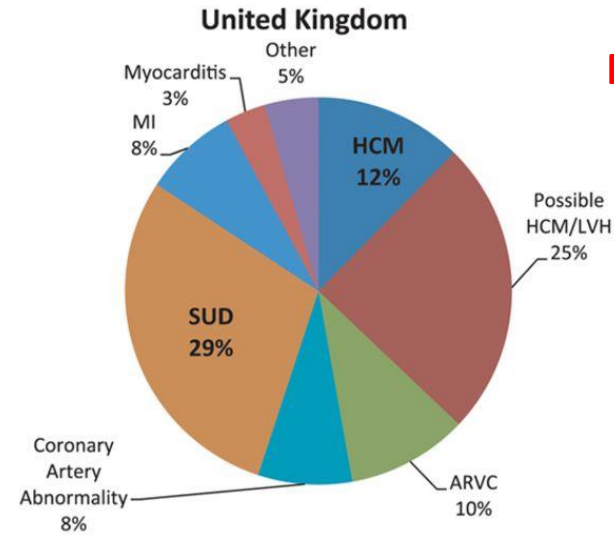


Etiology Sudden Death <35 years

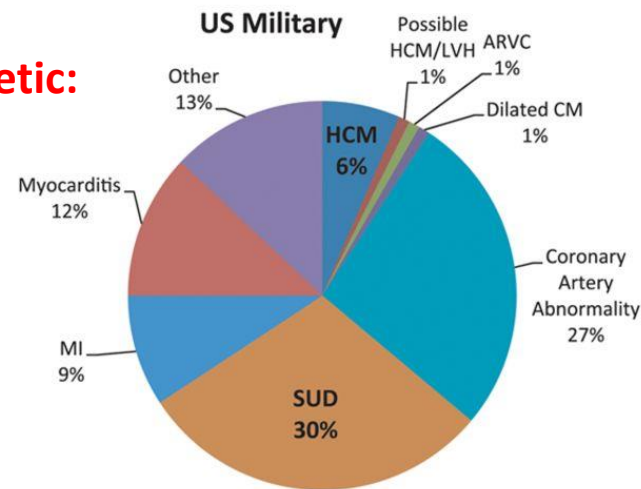
**Possibly genetic:
55%**



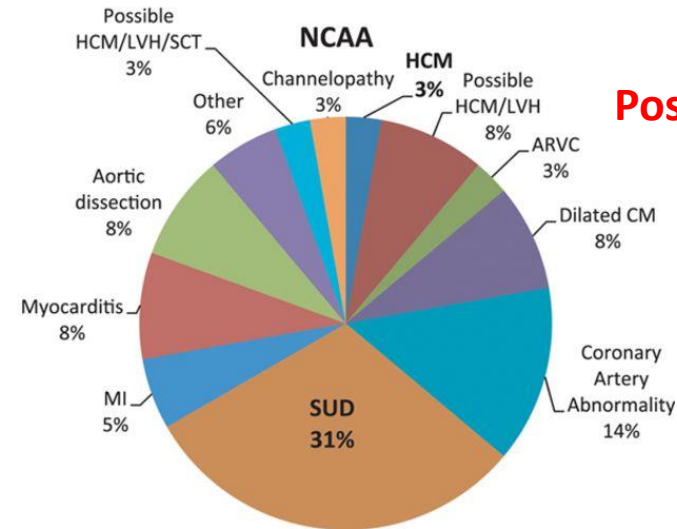
**Possibly genetic:
76%**



**Possibly genetic:
39%**



**Possibly genetic:
67%**



Why focus on age ≤ 35

Causes of SCD in Denmark according to age

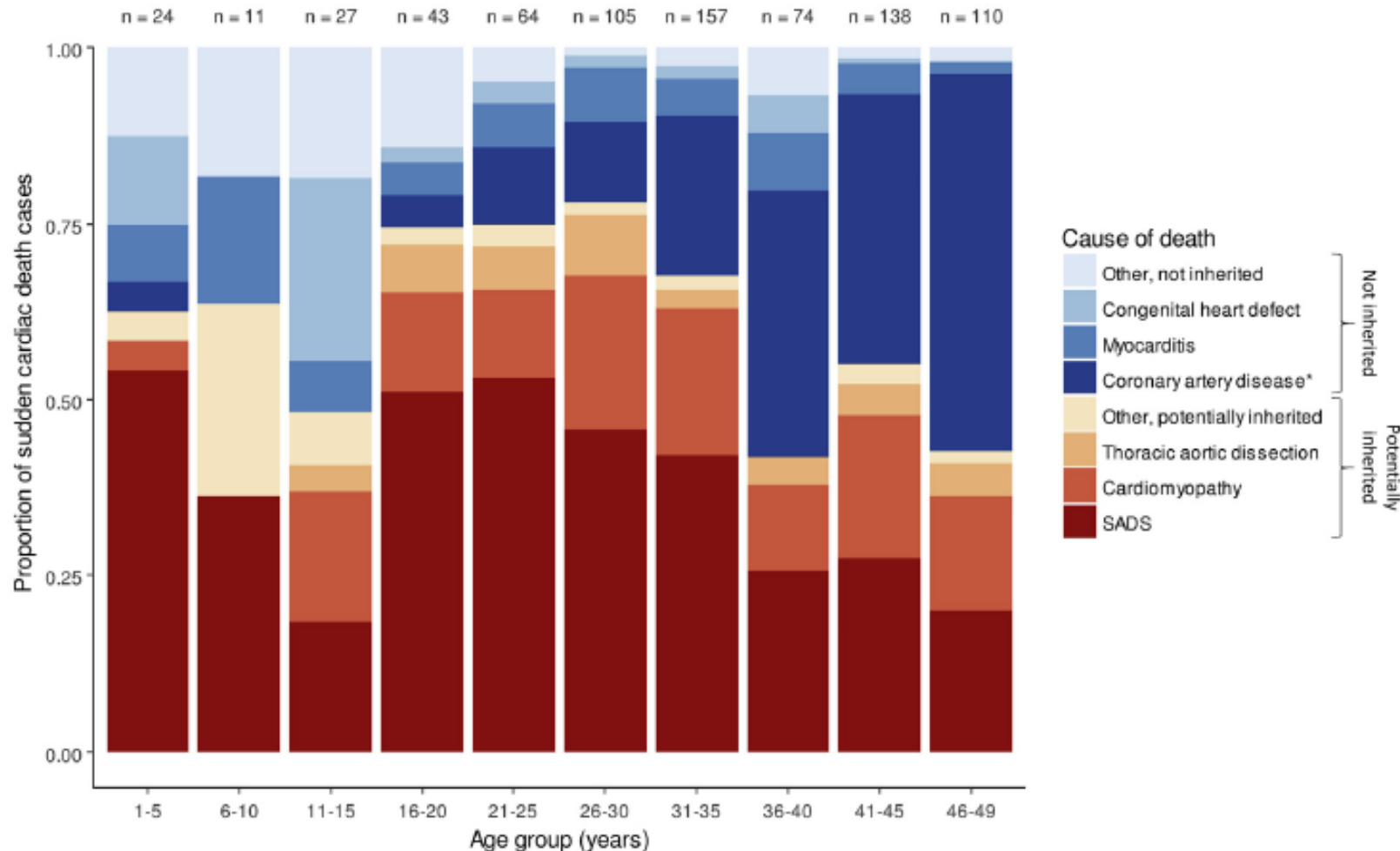


Figure 1 Distribution of causes of death among autopsied cases of sudden cardiac death (n = 753) according to age in persons aged 1–49 years in Denmark (J.T.-H., unpublished data). SADS = sudden arrhythmic death syndrome. *Coronary artery disease, especially in young persons, may be due to inherited disease (eg, familial hypercholesterolemia).

1st possibility: Autopsy result in SD: negative

- Structurally normal heart
- Normal cardiac histopathology
- No other cause of death identified
- Normal toxicology
- No pre-death clinical features suggestive for other causes of sudden death (eg Epilepsy)

→ SADS

Large Postmortem Case series

Table 2

Proportion of sudden unexplained death in the young in published postmortem case series

Year	Country	SCD autopsies (n)	SADS (%)	Reference
2001	Italy	273	17	Corrado <i>et al.</i> ¹⁷
2004	Australia	193	31	Doolan <i>et al.</i> ¹³
2005	Australia	241	29	Puranik <i>et al.</i> ¹⁸
2009	United Kingdom	118	23	De Noronha <i>et al.</i> ¹⁹
2011	Denmark	469	29	Winkel <i>et al.</i> ⁹
2011	Ireland	342	26	Margey <i>et al.</i> ⁸

SCD, sudden cardiac death; SADS, sudden arrhythmic death syndrome (normal postmortem).

Emerging questions if SADS occurs

- **Why** did my son/daughter/partner/sibling suddenly die?
- How can we **prevent** this happening to another family member?

SADS – Multicenter collaboration

302 SADS Cases

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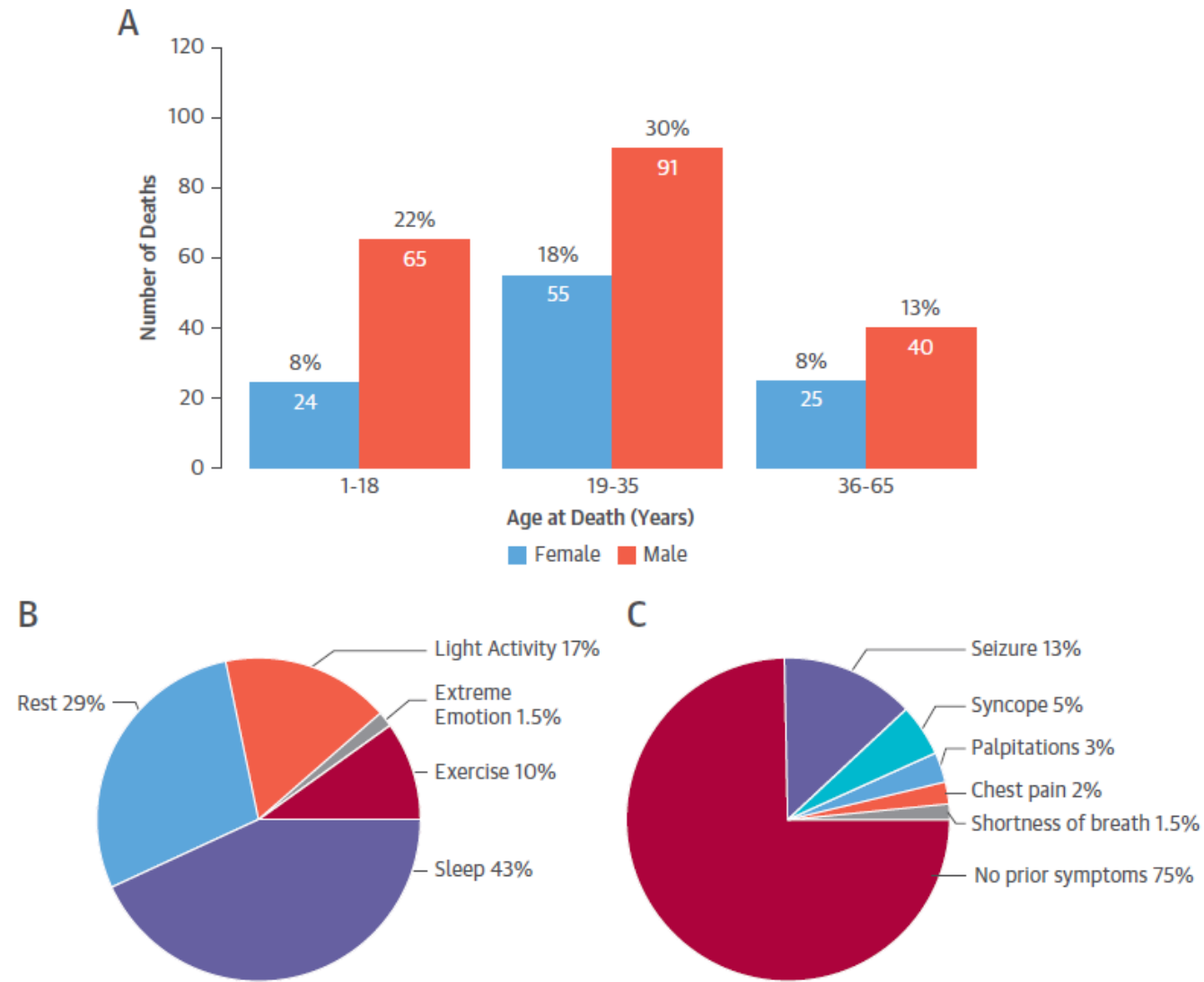
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Utility of Post-Mortem Genetic Testing in Cases of Sudden Arrhythmic Death Syndrome



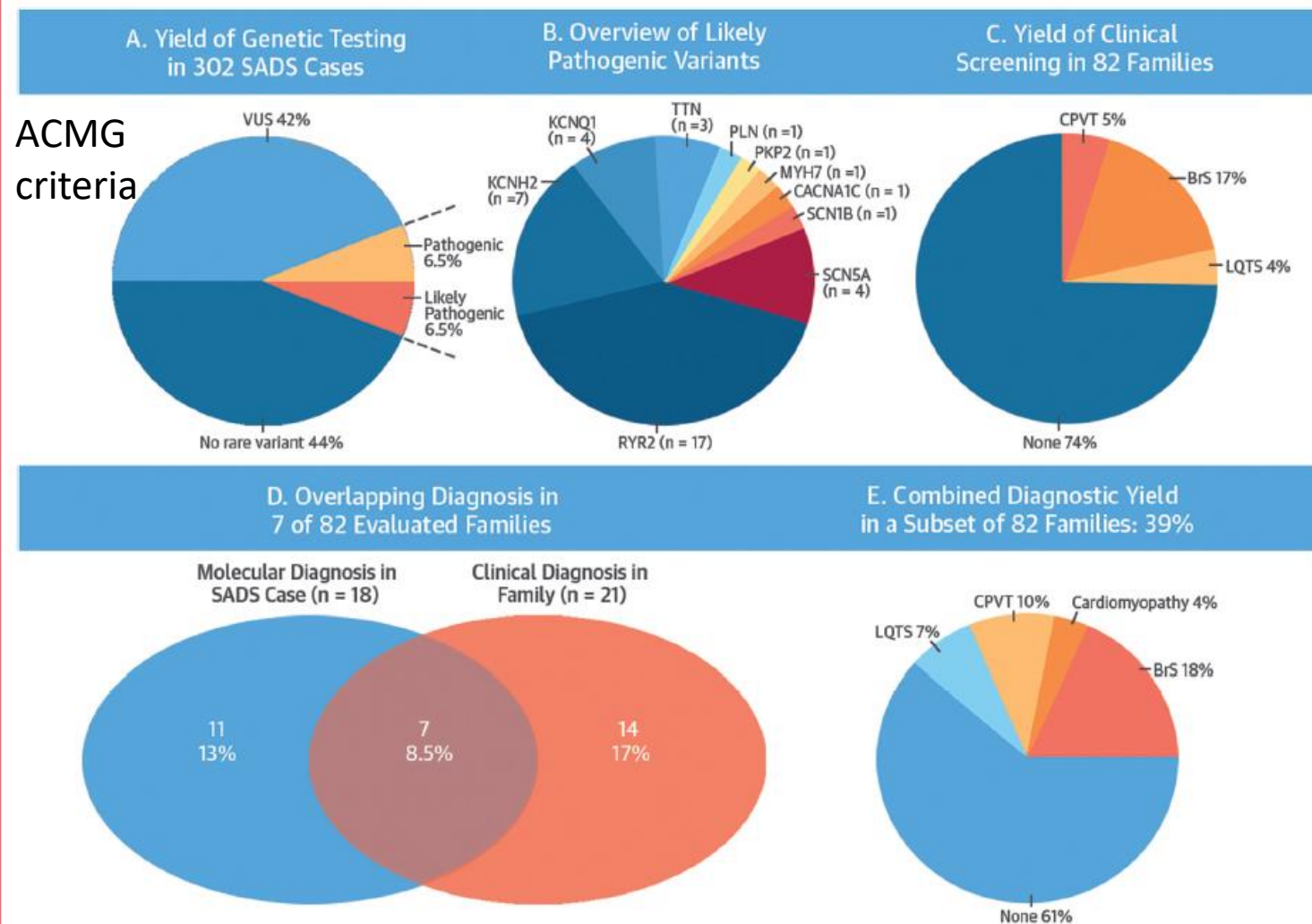
Najim Lahrouchi, MD,^a Hariharan Raju, MBChB, PhD,^{b,c} Elisabeth M. Lodder, PhD,^a Efstathios Papatheodorou, MD,^{b,c} James S. Ware, PhD,^{d,e} Michael Papadakis, MBBS, MD,^{b,c} Rafik Tadros, MD, PhD,^{a,f} Della Cole, BSc,^{b,c} Jonathan R. Skinner, MBChB, MD,^g Jackie Crawford,^g Donald R. Love, PhD,^g Chee J. Pua, PhD,^h Bee Y. Soh, PhD,^h Jaydutt D. Bhalshankar, PhD,^h Risha Govind, MSc,^{d,e} Jacob Tfelt-Hansen, MD, DMSc,ⁱ Bo G. Winkel, MD, PhD,ⁱ Christian van der Werf, MD, PhD,^a Yanushi D. Wijeyeratne, BMBS,^{b,c} Greg Mellor, MBChB, MD,^{b,c} Jan Till, MD,^{c,d,e} Marta C. Cohen, MD, DMJ (PATHOL),^j Maria Tome-Esteban, MD, PhD,^{b,c} Sanjay Sharma, MBChB, MD,^{b,c} Arthur A.M. Wilde, MD, PhD,^{a,k} Stuart A. Cook, MD, PhD,^{d,h,l} Connie R. Bezzina, PhD,^a Mary N. Sheppard, MB, BCh, BAO, MD,^{b,c} Elijah R. Behr, MBBS, MD^{b,c}

FIGURE 1 SADS: Demographic and Clinical Characterization



Sudden arrhythmic death syndrome (SADS) (A) more often affected males and individuals 35 years and younger; (B) 72% of SADS deaths occurred during sleep or rest. (C) Whereas three-quarters exhibited no symptoms before death, 13% had a history of seizures.

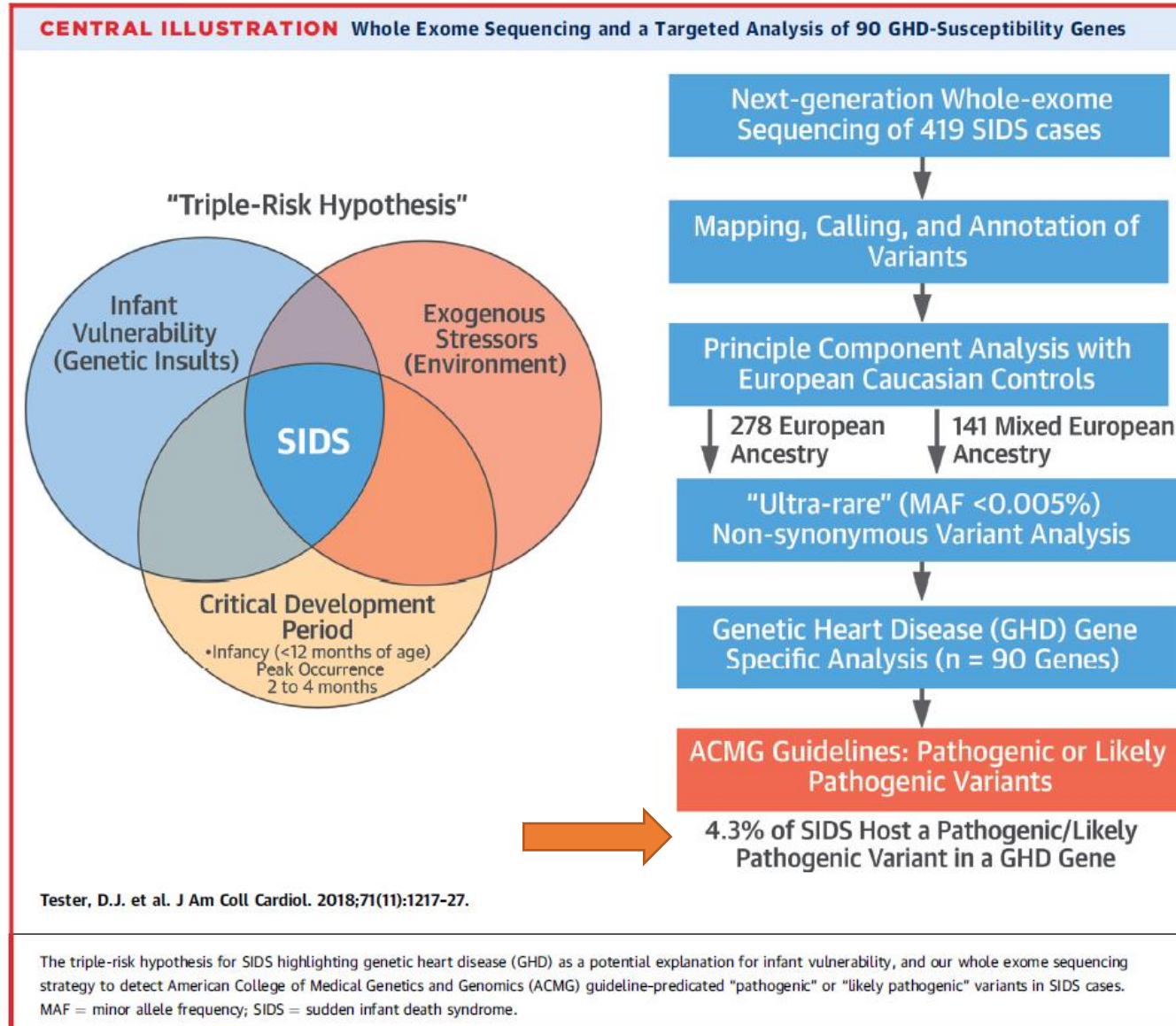
CENTRAL ILLUSTRATION Sudden Arrhythmic Death Syndrome: Genetic Testing and Clinical Screening



Lahrouchi, N. et al. J Am Coll Cardiol. 2017;69(17):2134-45.

(A to E) Genetic testing via next-generation sequencing in 302 sudden arrhythmic death syndrome (SADS) cases identified pathogenic and likely pathogenic variants in 13% of cases. Clinical screening of relatives in 82 families found 35 relatives from 21 families with a clinical diagnosis of a primary electrical disease: Brugada syndrome (BrS); catecholaminergic polymorphic ventricular tachycardia (CPVT); and long-QT syndrome (LQTS); there was overlap between molecular and clinical diagnosis in 7 families. Combining molecular autopsy with clinical evaluation increased diagnostic yield in surviving families to 39%. VUS = variant of unknown significance.

Molecular autopsy in SIDS



Case 1

- Male, °1990, Belgian nationality
- Sudden death during sleep 7/2014
- No history of Cardiac or Neurologic disease

Case 1

- Post mortem examination
 - Congestive organs: protracted death
 - Microscopy of heart and brain: normal
 - Toxicology

Case 1

- Post mortem examination

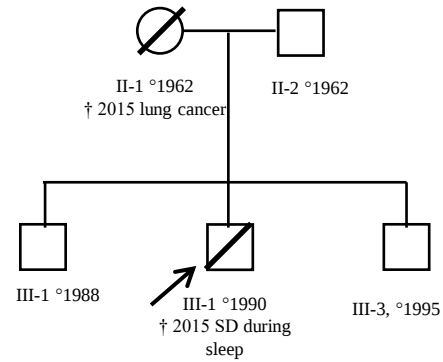
❖ Substantie	BC ^{II}	UC	Interpretatie op basis van BC
Alcohol	0.08 g/L	afwezig	Stadium 1 (4 % dronken ¹²) : geen significante invloed ¹³
Opiaten: morfine	Spoortje	11.4ng/mL	Narcotisch analgeticum Alkaloïde stof die voorkomt in opium en gekend metaboliet van heroïne (maar MAM negatief)
Opiaten: codeïne	Spoortje	8.9ng/mL	Narcotisch antitussivum Alkaloïde stof die in heroïne en morfinepreparaten voorkomen Morfine als metaboliet
Kinine (Quinidine)	/	Aantoonbaar	Anti-aritmicum, ikv hospitaalmilieu en bij REA/EHBO Behandeling van Malaria Soms als versnijmiddel van drugs, zoals opiaten
Laudanosine	/	Aantoonbaar	Metaboliet van atracurium, een spierverslappende stof (curariserend middel), ikv anesthesie Ook aanwezig in opium
Theophylline	/	Aantoonbaar	thee of luchtwegverwijdende medicatie
Nicotinamide	Aantoonbaar	Aantoonbaar	Vitamine B3

Case 1

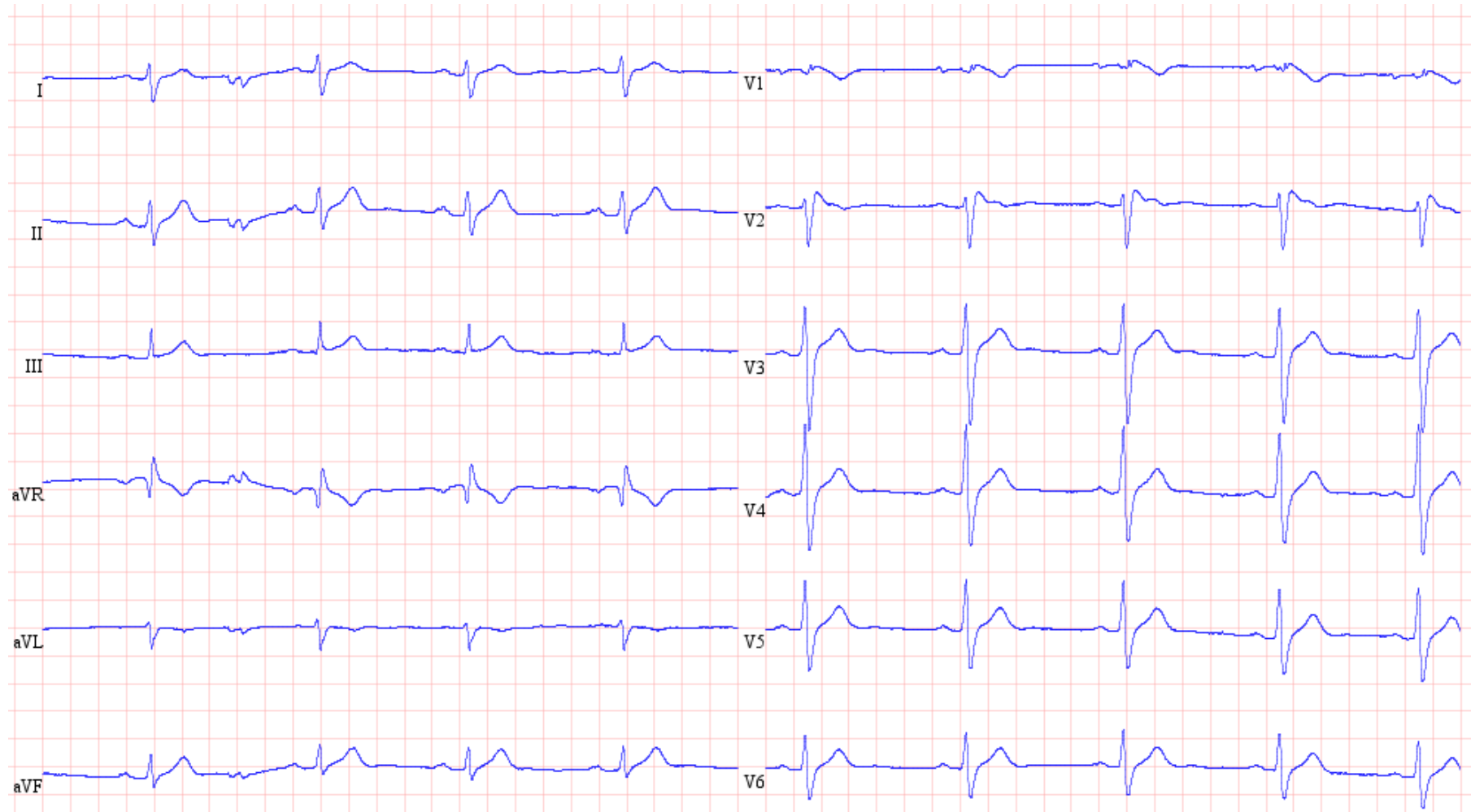
- Aspecific findings, cause of death:
 - DD toxic substance induced
 - DD SADS

Case 1

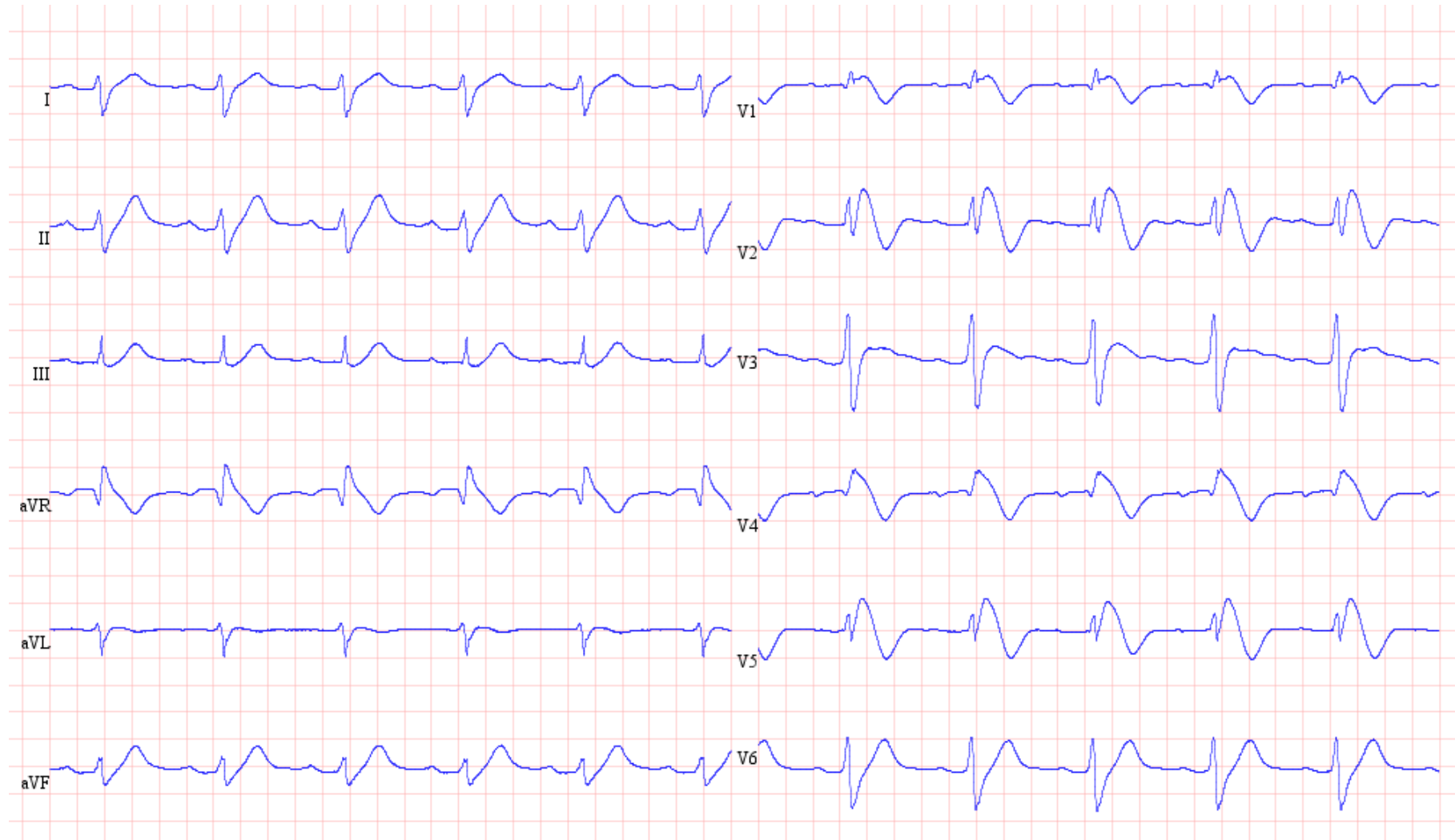
- Screening of first degree family members + molecular autopsy



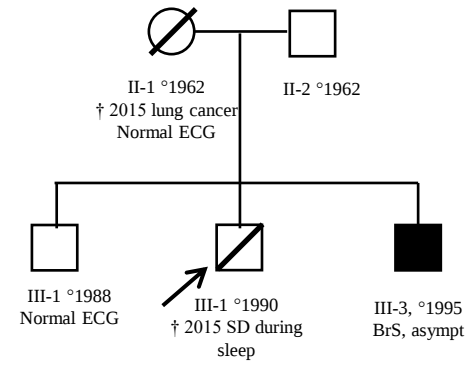
Case 1 – III-3



Case 1 – III-3

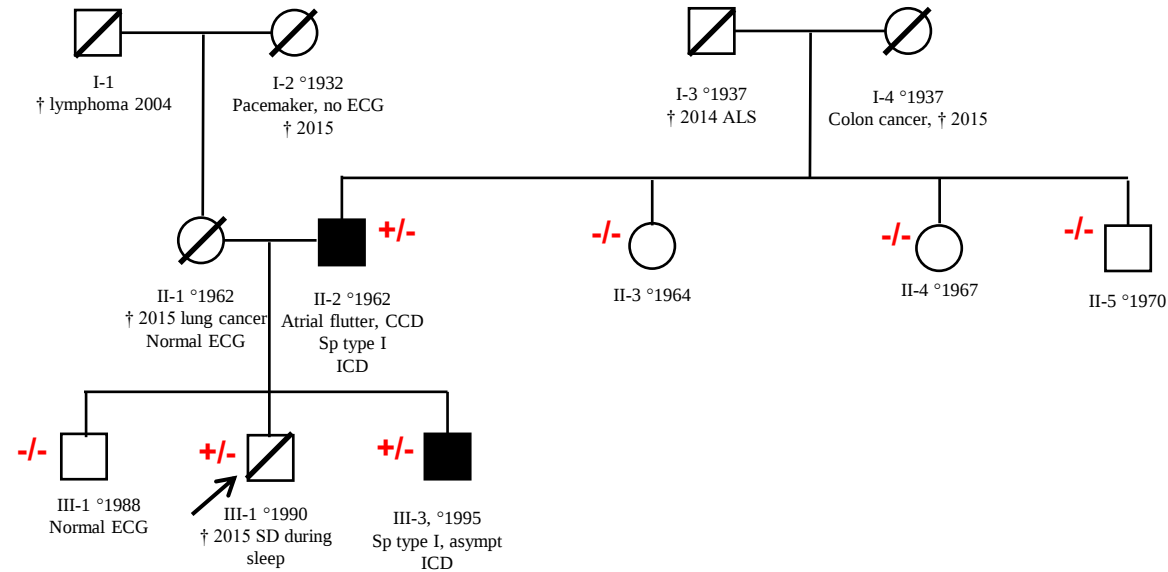


Case 1



Case 1 – Molecular Autopsy

SCN5A c.2658T>A or p.His886Gln

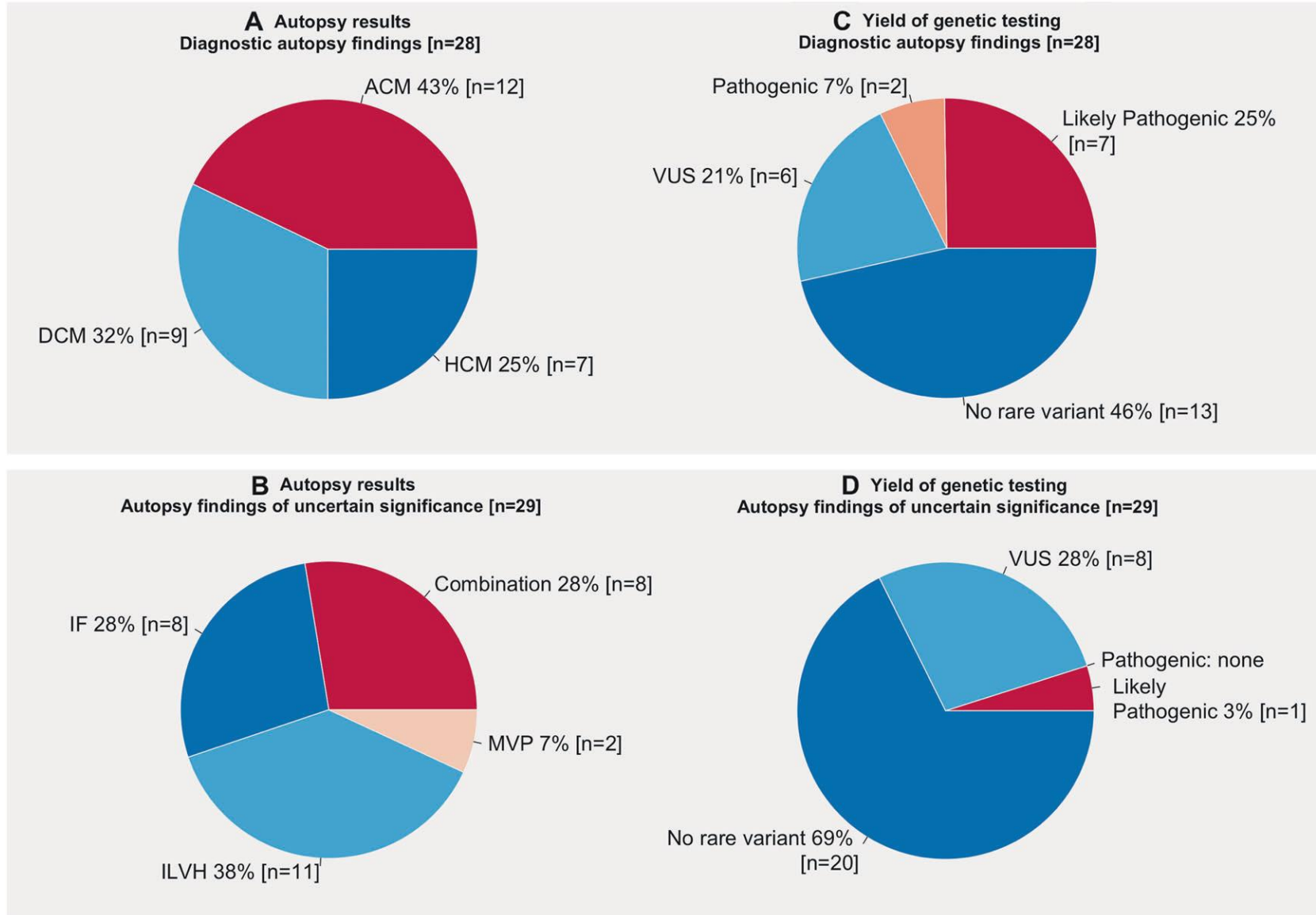


2th possibility: Autopsy result: structural abnormalities

- Cardiomyopathy or structural findings with uncertain significance
 - HCM versus idiopathic LVH
 - ARVC versus fatty infiltration of RV
 - ...



Molecular autopsy in individuals with structural abnormalities



Case 2

- Male, °1980, Italian origin
- Sudden death 1/2015 while taking a shower
- No history of cardiac or neurologic disease

Case 2

- Post mortem examination:
 - dilated right ventricle
 - Heart microscopy:
 - Right ventricle: fatty infiltration + fibrosis, no inflammation
 - Left ventricle: decreased number of myocytes + fibrosis, no fatty infiltration



Case 2

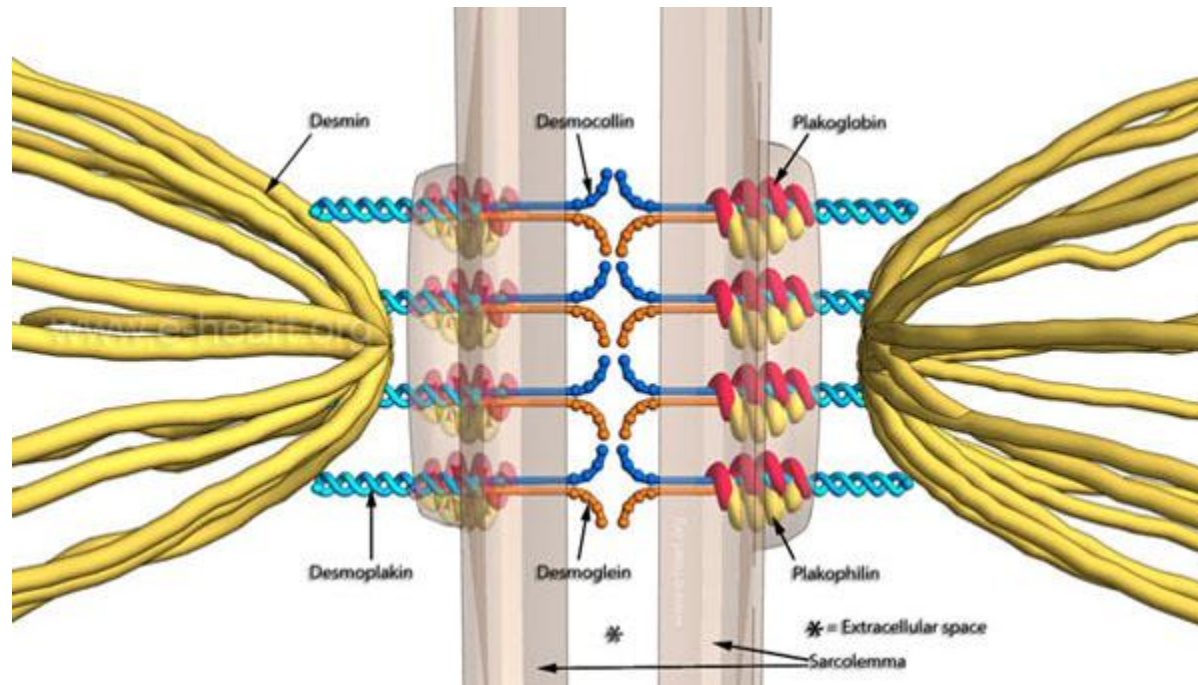
Diagnosis of ARVC

→ Cardiologic evaluation of first degree + symptomatic relatives

→ Molecular Autopsy

Case 2

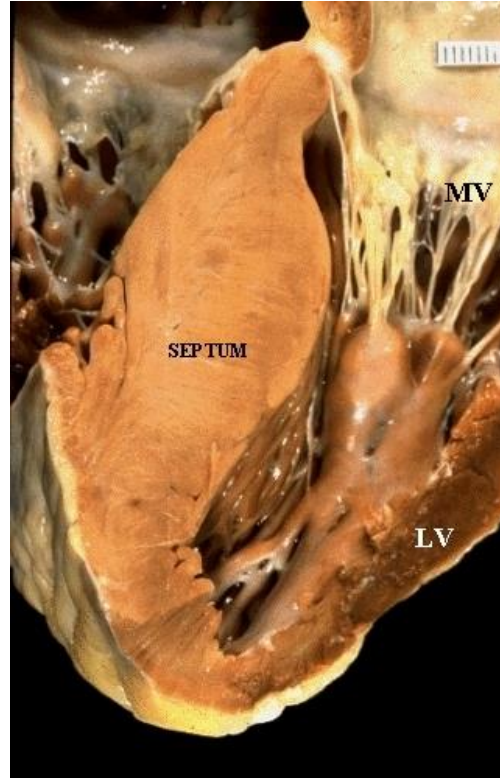
- Results molecular autopsy
 - No (likely) pathogenic variants
 - 2 variants of unknown significance
 - DSG2 c.1557C>A or p.Asp519Glu
 - RYR2 c.1310G>A or p.Ser437Asn



- **Co-Segregation analysis!**

Type of genetic test in molecular autopsy?

- Diagnostic autopsy: perform targeted genetic test



Hereditary cardiomyopathy:

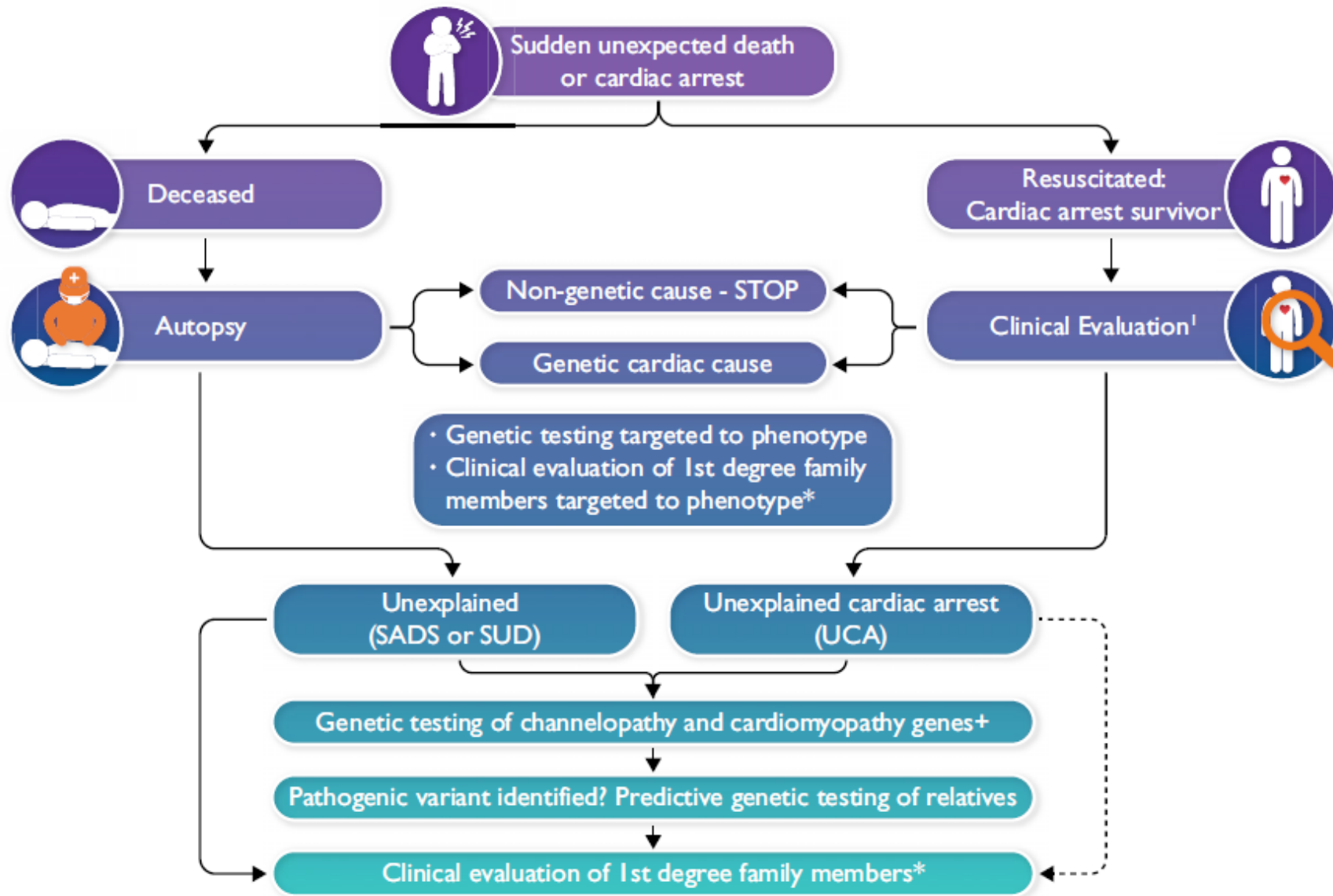
HCM

A(RV)C

LVNC

DCM

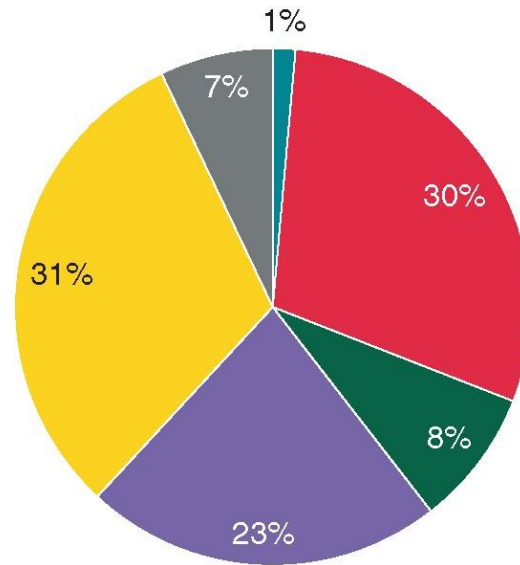
- Negative autopsy (SADS): perform broader genetic arrhythmia (+/- CMP) panel



Survey on autopsy practices in Europe

A

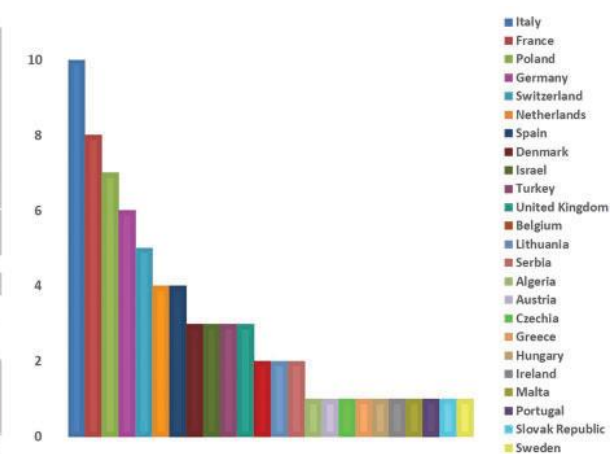
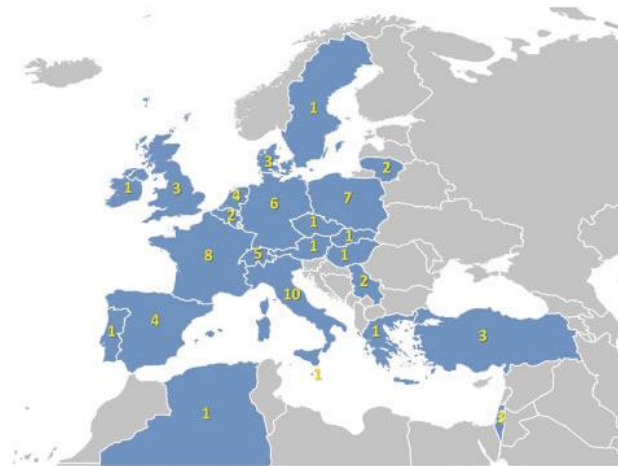
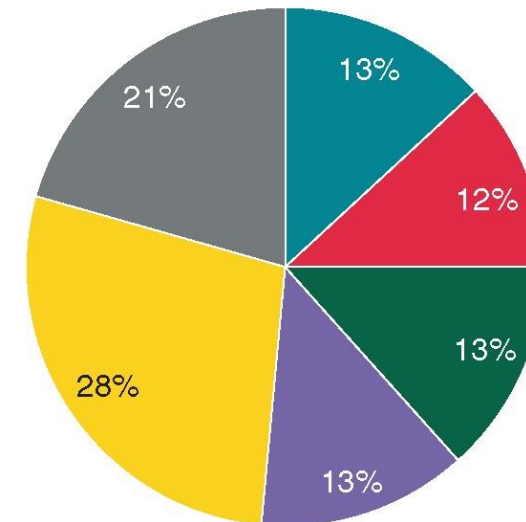
How frequently has an autopsy been undertaken in SUDY cases



- 100% of the cases
- 75-99% of the cases
- 50-74% of the cases
- 25-49% of the cases
- 25-49% of the cases
- never

B

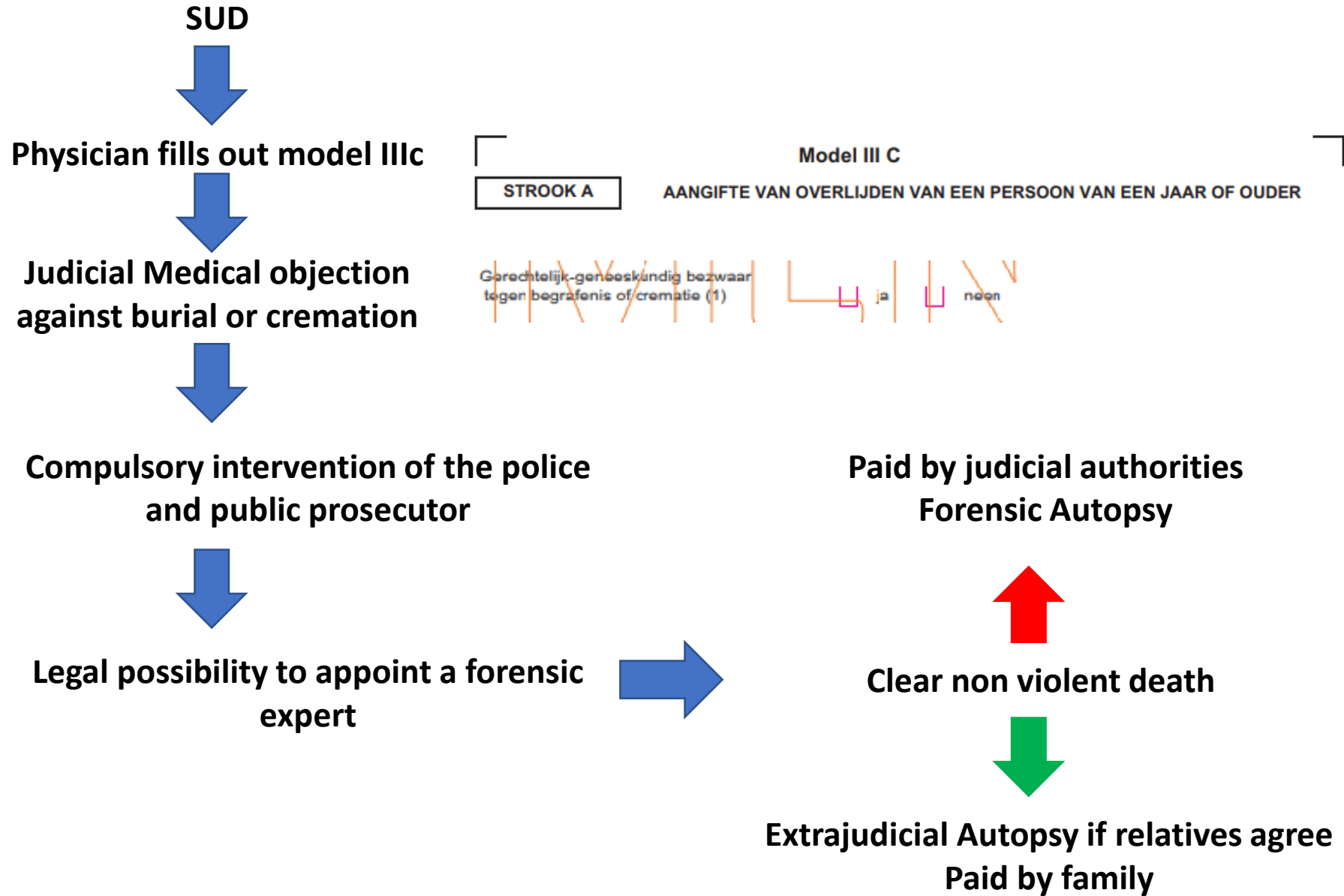
How often is post-mortem genetic testing requested in SADS cases



SCD in Belgium

- Incidence SCD 1-35 YO: 1-3/100000 individuals per year¹
- According to Statbel on 1/1/2021: 4,753,818 inhabitants aged 1-35
- → 50-150 SCD every year

Regulation in Belgium



Reimbursement in Belgium

- **Detailed autopsy according the guidelines¹**
 - Full autopsy
 - Macroscopy
 - Histology
 - Radiology (RX/CT)
 - Biochemistry
 - Microbiology
 - Toxicology

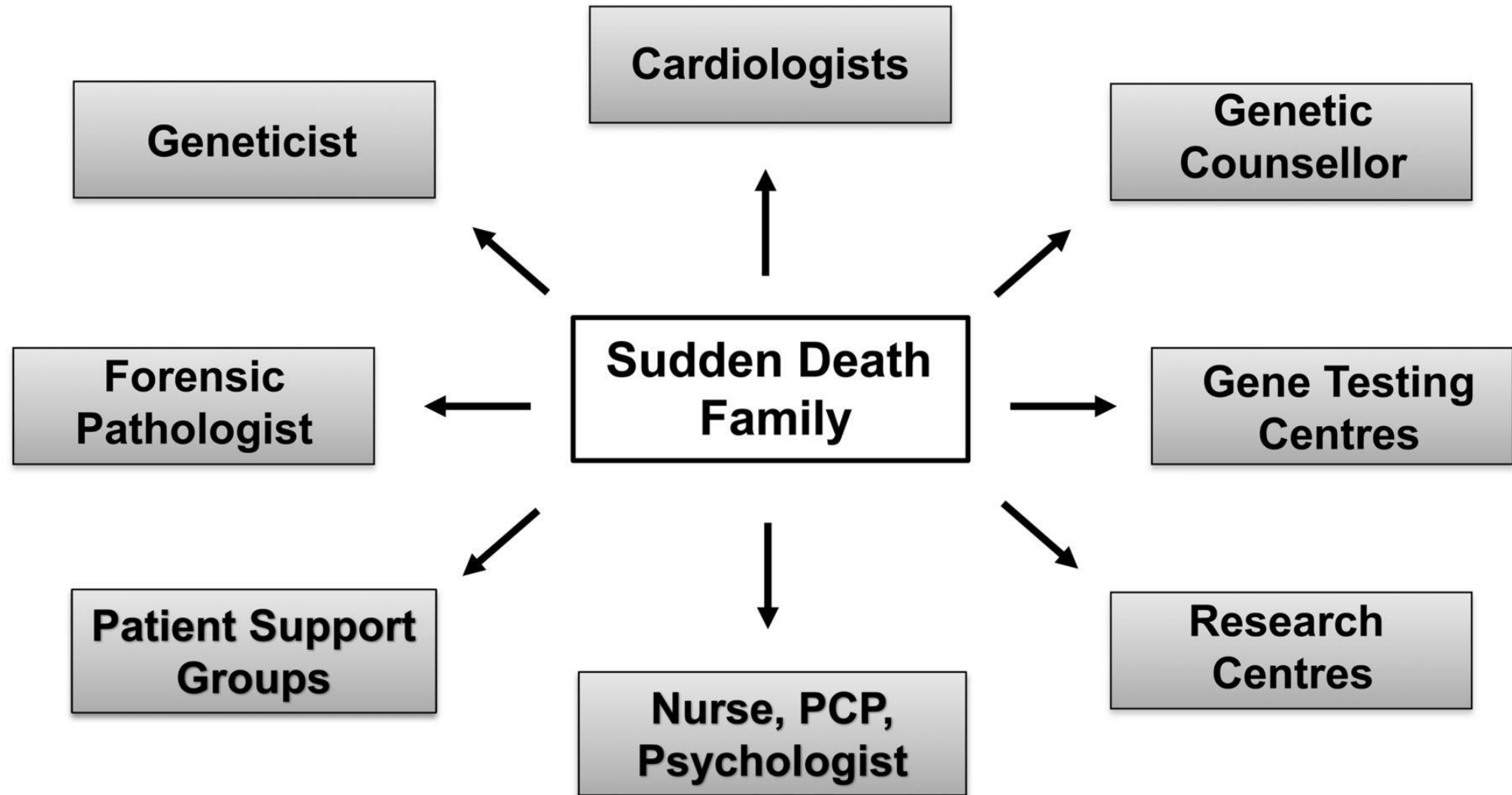
→ **Cost 2864 euro + VAT**
- Reimbursement clinical autopsy: **+/- 580 euro**
- Cost Molecular autopsy: +/- 1200 euro (almost fully reimbursed if charged <2Y after death)

Molecular autopsy Achilles heels

- Cost autopsy – Quality of autopsy
- Quality of the DNA sample
 - No Paraffin embedded material!¹
 - Good: EDTA tubes, fresh frozen tissue, bloodspot
- Interpretation of the molecular autopsy
 - “X” does not always mark the spot! (Mike Ackerman – genetic purgatory)
 - Signal to noise ratio (SADS is not a good phenotype! Yield +/- 15%)
 - VUS

¹Carturan E et al. Am J Clin Pathol 2008: 391-7

Team Effort!



Conclusions molecular autopsy

- Indication 1: SADS – genetic yield +/- 15%
- Indication 2: identified CMP – genetic yield = yield of specific phenotype
- Management of SUDY families includes both molecular autopsy and clinical testing
- Team effort!

Cardiogenetics Team Leuven

Center for human Genetics



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Corveleyn



Cuno Kuiperi



Jeroen
Breckpot

Forensic medicine



Wouter Van
Den Bogaert

Cardiovascular diseases



Tomas Robyns



Rik Willems



Lucas Van Aelst



Johan Van
Cleemput

Radiology



Jan Bogaert

Pediatric Cardiology



Bjorn Cools



Ruth Heying



Benedicte
Eyskens



Thomas Salaets

Questions?

