Inherited arrhythmia syndromes I:

What you always wanted to know, but were afraid to ask:

The basics of inherited arrhythmia syndromes and genetics

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Clinical Geneticist

Amsterdam, the Netherlands

PEDIRHYTHM VII

Thessaloniki, Greece feb 4-7 201

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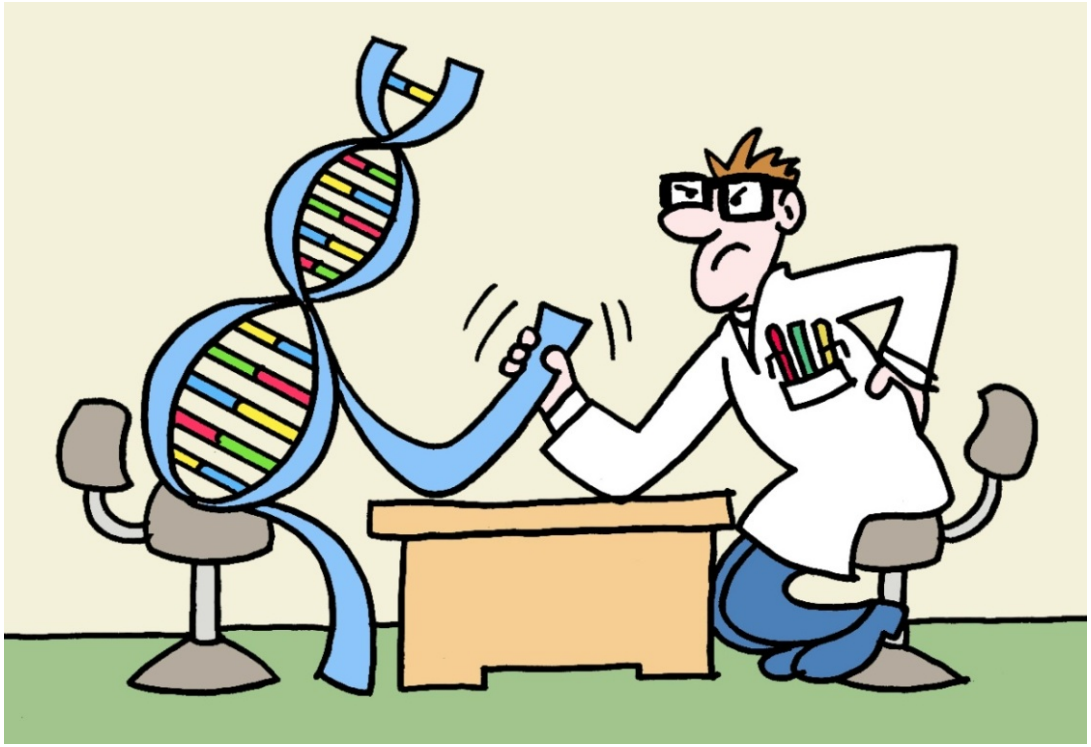

Genetics and mutations

Patterns of inheritance and pitfalls

DNA diagnostics

Limitations techniques

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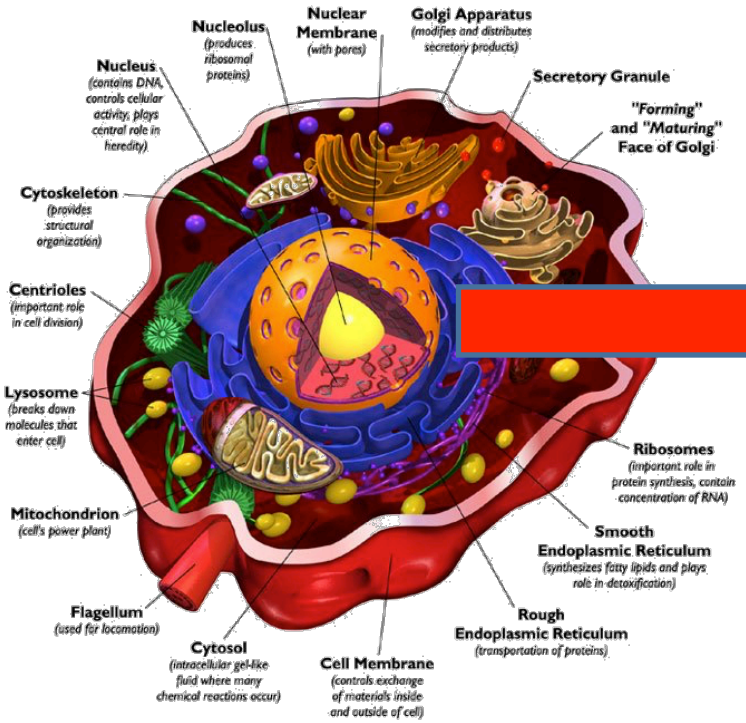
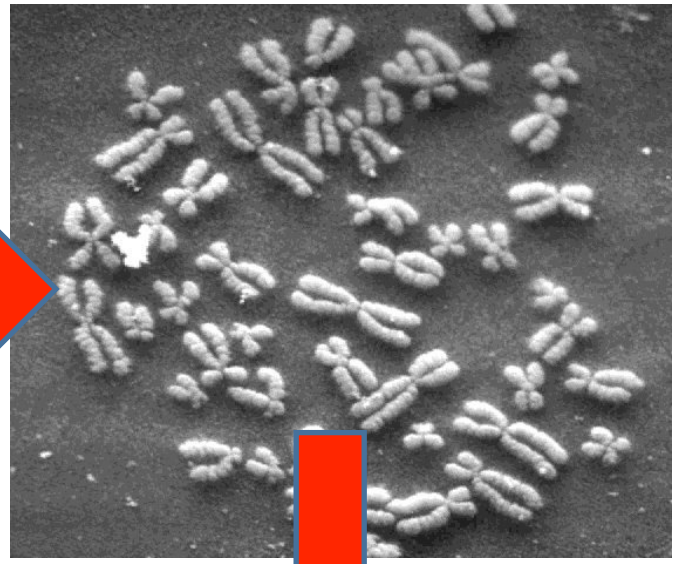
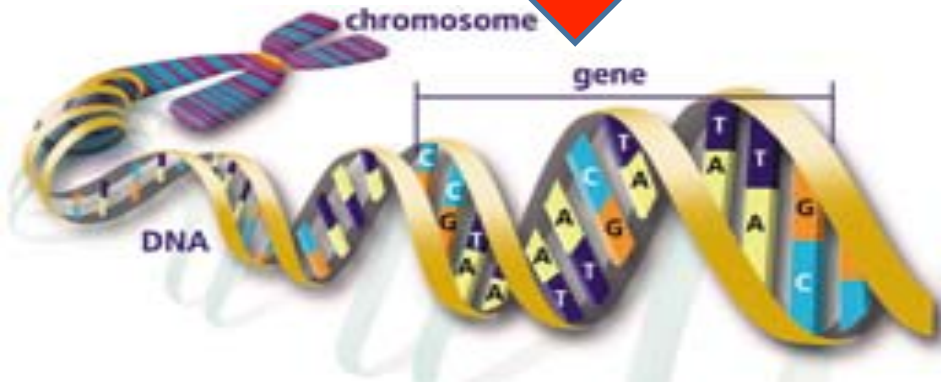




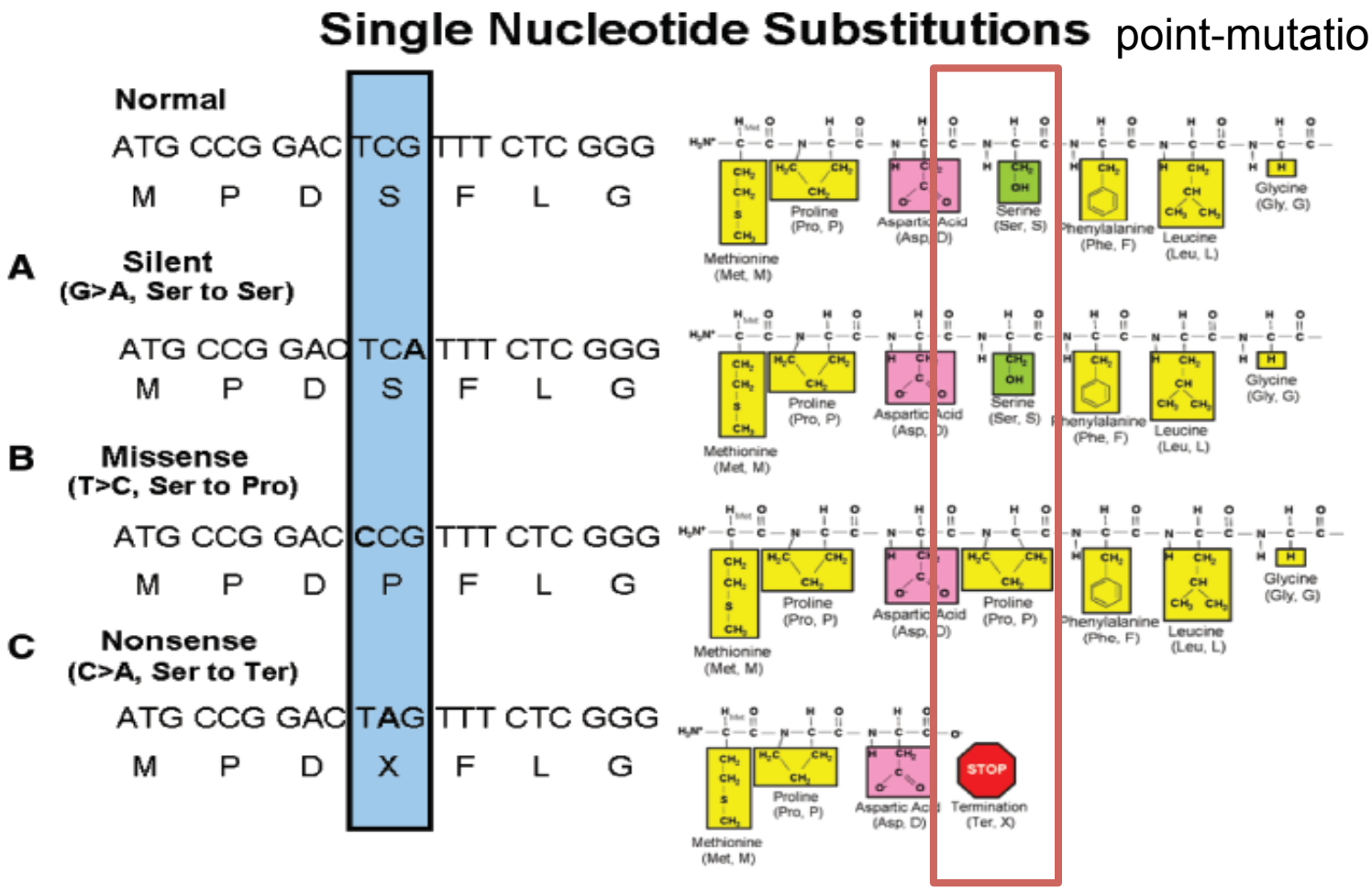
The basics

GENETICS AND MUTATIONS

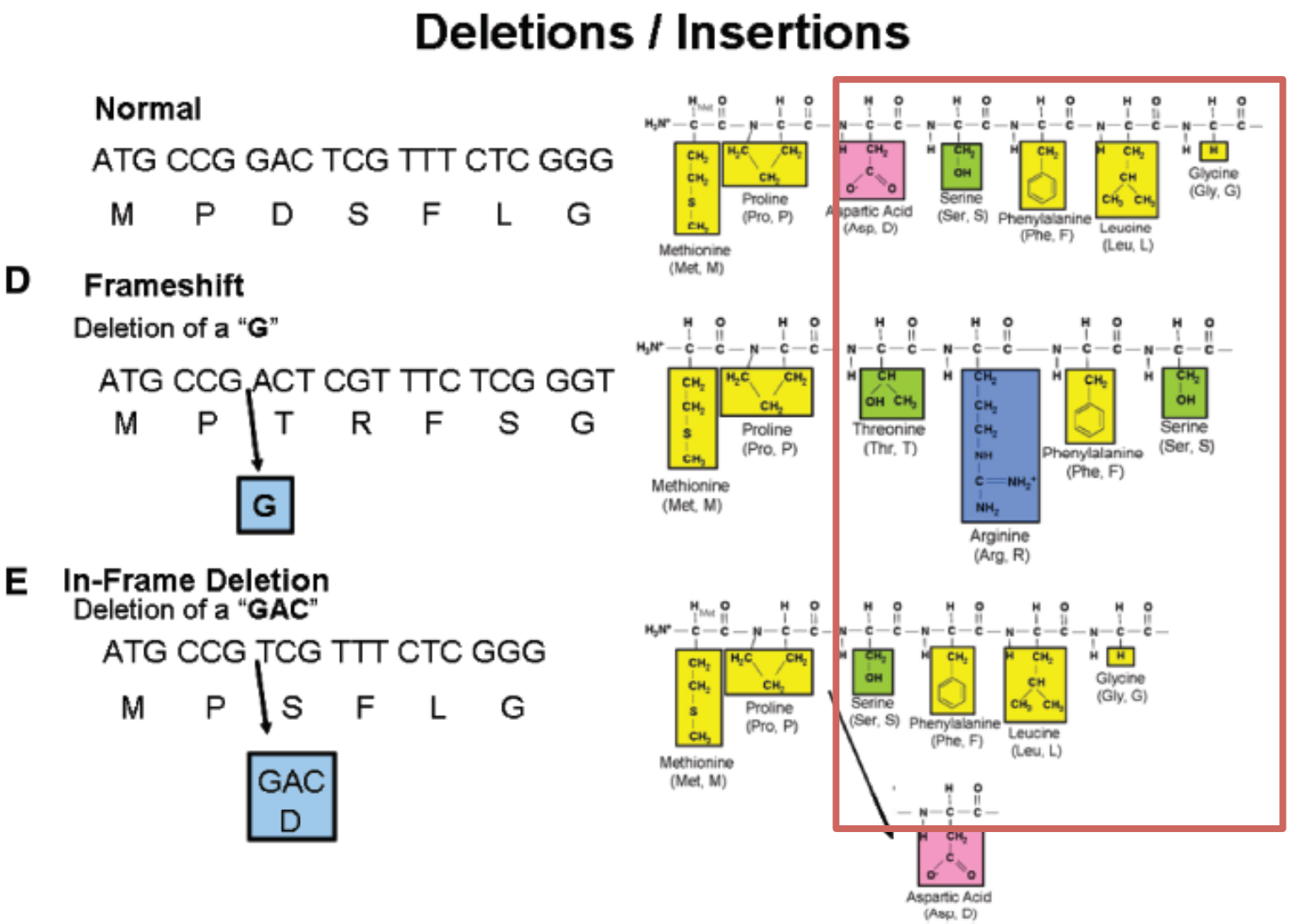


Variations in your DNA

- mutation= variation in DNA that affects function



Deletions-insertions



What determines pathogenicity?

-Nonsense/ affecting splicing
-In silico predictions
-Amino-acid properties
-Evolutionary conservation
-Functionally important domain
-Absence in control group
-Functional data
-Co-segregation
-Described before as.....
-De novo

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Classification variants

- class 5 pathogenic (>99%)
- class 4 likely pathogenic (95-99%)

- class 3 variant unknown significance (5-95%)

- class 2 unlikely to be pathogenic (1-5%)
- class 1 not pathogenic (<1%)

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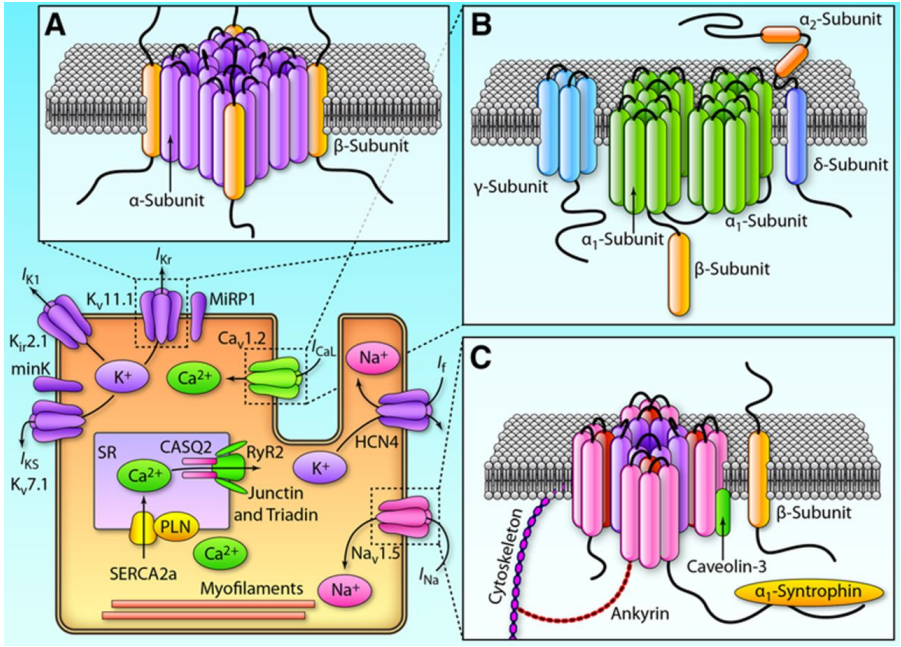
Arrhythmias: channelopathies



- Genes encoding different subunits
  sodium/potassium/calcium channels
- Associated proteins
- Trafficking proteins

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Arrhythmias: channelopathies

Disease	yield	major genes	Minor genes	elusive
LQTS	80%	**KCNQ1, KCNH2, SCN5A, (75%)**	KCNE1(<1%), KCNE2(<1), CAV3(<1), SCN4B(0.1), SNTA1(<0.1), AKAP9(<0.1), CACNA2D1(<1), KCNJ5 (<1) , ANK2(<1)	20%
SQTS			*KCNQ1*, *KCNH2*, *KCNJ2, CACNA1C*, *CACNB2*, *CACNA2D1*	
Brugada	25%	**SCN5A (20-25%)**	*CACNA1C /A2D1/B2B (10%)* SCN1B (1%), SCN3B (1%), GPD1L, (<1) MOG1(<1%), SLMAP, KCNE3 (<1%), KCND3 (<1%), KCNE5, KCNJ8 (<1%) *TRPM4 (5)*	60-65%
CPVT	50%	**RyR2 (50%)**	CASQ2, TRDN, CALM1/2, KCNJ2	50%
WPW			LAMP2, PRKAG2, mt	
AF/SSS			SCN5A, HCN4,	
conduction disease			SCN5A, TRPM4	

PATTERNS OF INHERITANCE AND PITFALLS

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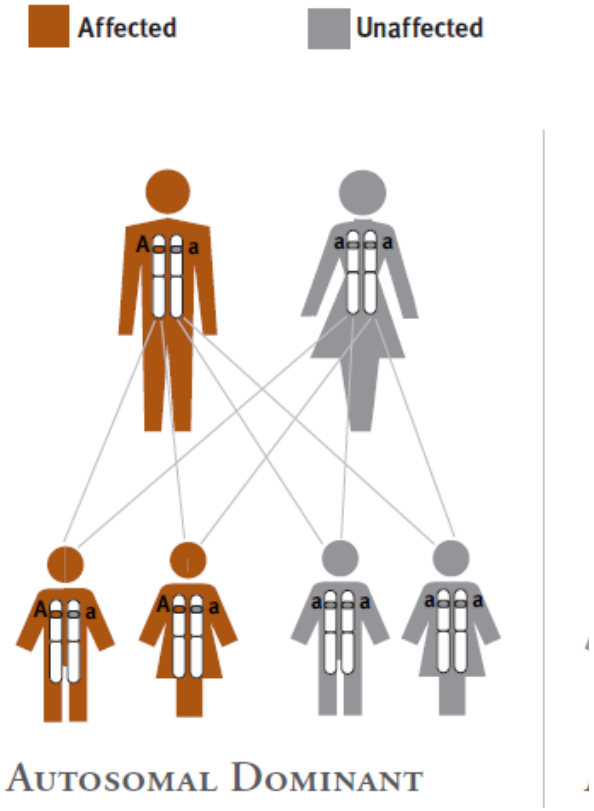
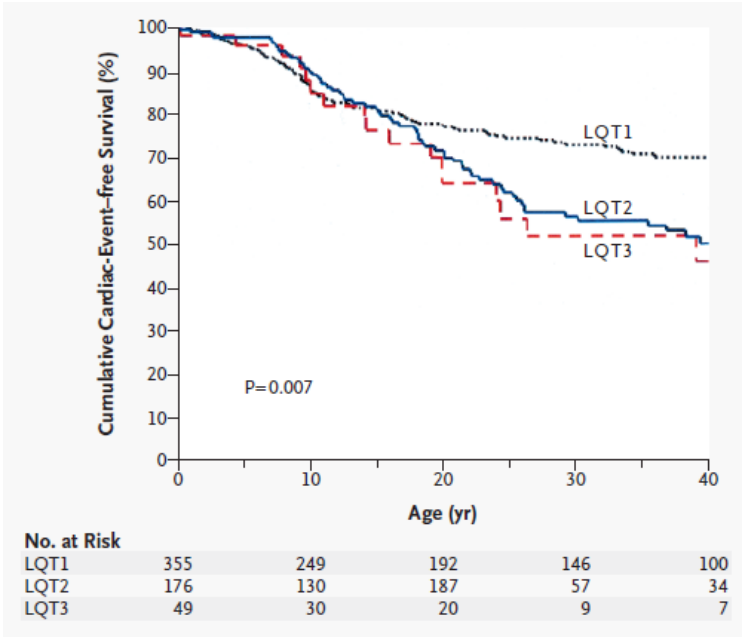


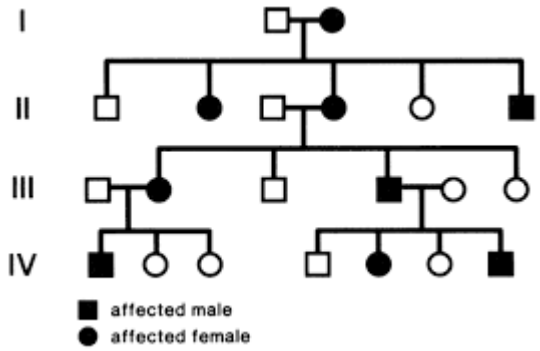
AUTOSOMAL DOMINANT

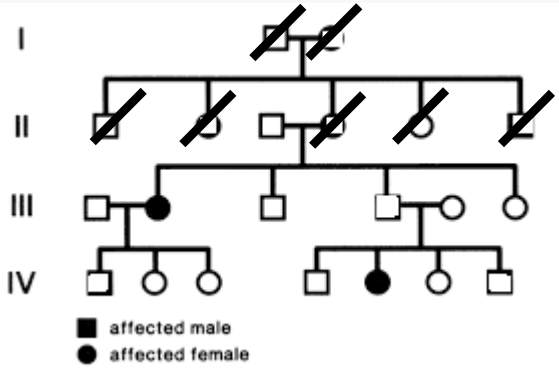
Variable expressivity

Reduced penetrance



No. at Risk					
LQT1	355	249	192	146	100
LQT2	176	130	187	57	34
LQT3	49	30	20	9	7





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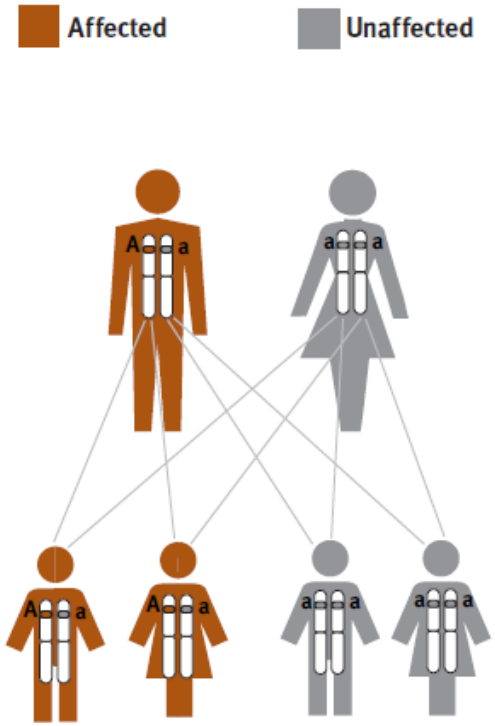
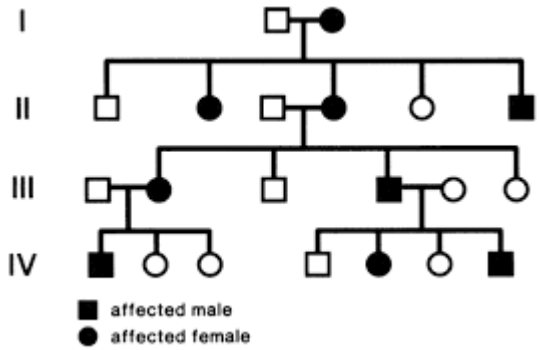


AUTOSOMAL DOMINANT



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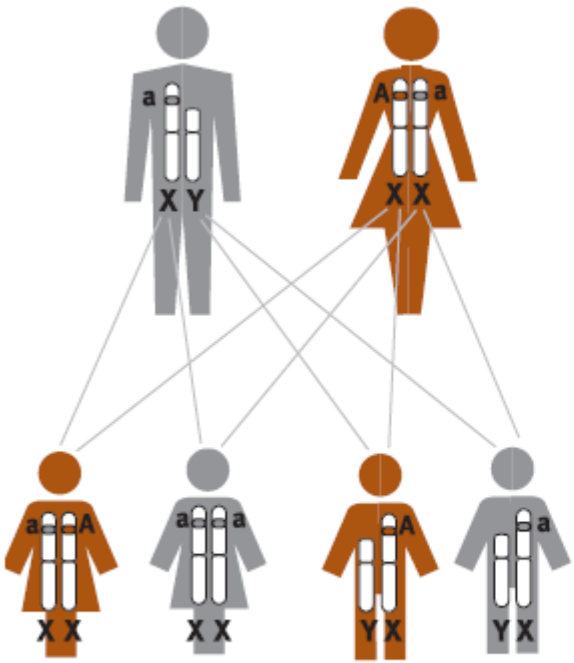
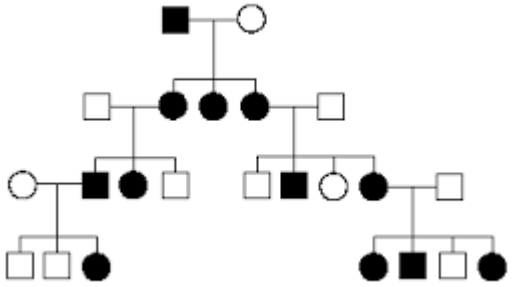
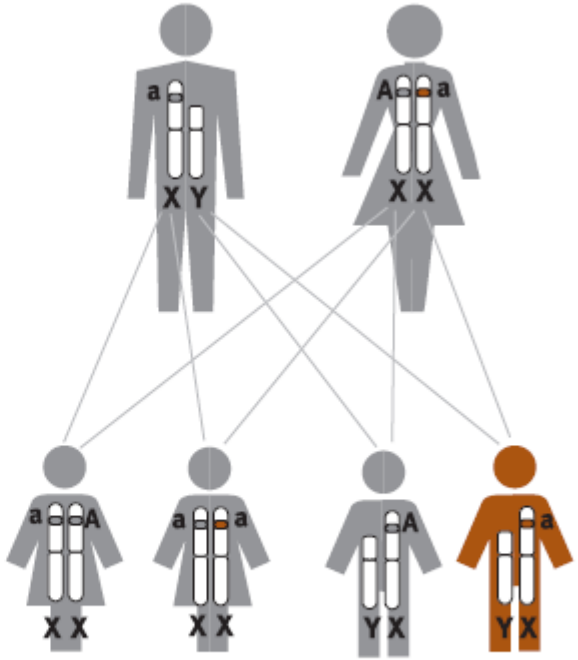
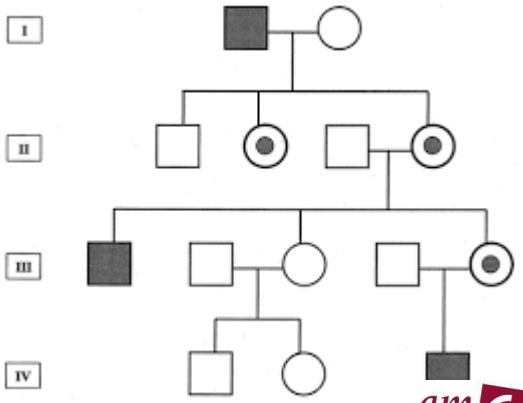


X-linked Dominant





X-linked Recessive



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Why and how?

DNA DIAGNOSTICS

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Why DNA-analysis?

- Confirmation (Dx/pattern of inheritance/PNDx)
- Diagnostic (borderline cases; criteria)
- **Facilitates cascade genetic screening**
  - early detection
  - dismiss non-carriers from follow-up
- Study genotype-phenotype relations
  - risk stratification?
  - gene (mutation) dependent therapy?

HRS/EHRA Expert Consensus Statement on the State of Genetic Testing for the Channelopathies and Cardiomyopathies

This document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA)

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Section # – Disease	Diagnostic	Prognostic	Therapeutic
Section I – LQTS	+++	+++	++
Section II – CPVT	+++	+	-
Section III – BrS	+	+	-
Section IV – CCD	+	+	+
Section V – SQTS	+/-	-	-
Section VI – AF	-	-	-
Section VII – HCM	+++	++	+
Section VIII – ACM/ARVC	+	+/-	-
Section IX – DCM	+/-	-	-
Section IX – DCM + CCD	++	++	+
Section X – LVNC	+	-	-
Section XI – RCM	+	+	+

Figure 1 Impact of genetic testing for the index case. The relative strength (– = negligible to +++ = strong) regarding the contribution/impact of the genetic test result for the index case for each disease in each of the three categories (diagnostic, prognostic, and therapeutic) was voted upon by each writing member and >90% consensus was achieved for each "cell." The level of evidence for each cell's designation is the same as for the entire document, Level of Evidence C.

How? Genetic analysis in cardiac disease

- <1995: nothing
- 1995-2012: 1-2 *genes* at a time; sequentially. Max ca. 6 genes

- since 2012: *panels* 23-100+ genes

(cardiomyopathy/ arrhythmia/ aorta/ combinations)

- >2015 *Exome sequencing* ±20.000 genes/ 2%DNA
- >2017? Genome sequencing

Exome sequencing: all genes; 2% of DNA

Myocardial and Pericardial Diseases
ESC Working Group

The current role of Next generation DNA sequencing in routine care of patients with hereditary cardiovascular conditions

A viewpoint paper of the European Society of Cardiology working group on myocardial and pericardial diseases and members of the European Society of Human Genetics

- Whole exome/genome sequencing is considered to be a diagnostic method in development and should be used for genetic diagnosis only if filtered against recognised disease genes. The coverage should allow identification of all exomic variants in these genes

Unsolicited findings:

- Before genetic testing it is important to inform the patient about the challenges in interpretation of sequencing results of multiple genes and discuss the implications of unsolicited findings
- In a clinical diagnostic setting only recognised disease genes should be investigated in patients fulfilling diagnostic criteria of a specific cardiovascular condition

Eur H J 2015;36:1367-70

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NOT EVERY GENE IS RELEVANT (YET)
NOT EVERY PUBLISHED VARIANT IS DISEASE CAUSING

Genetics and inherited arrhythmias

ANY CAVEATS?

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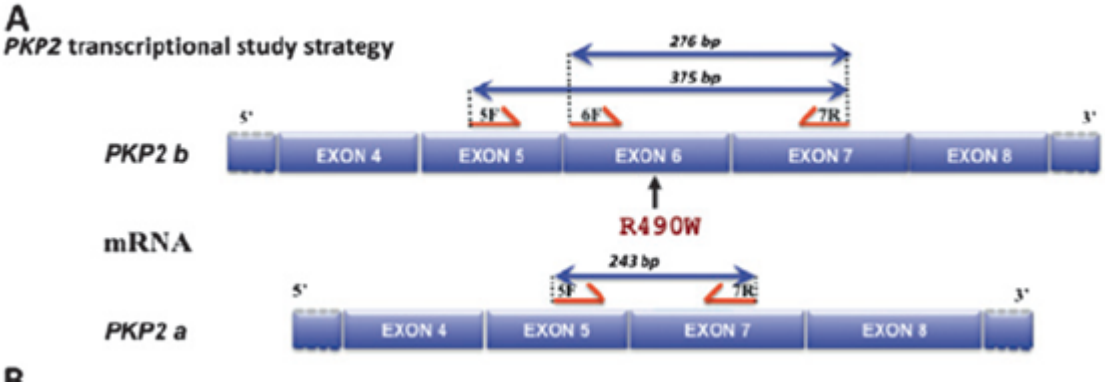
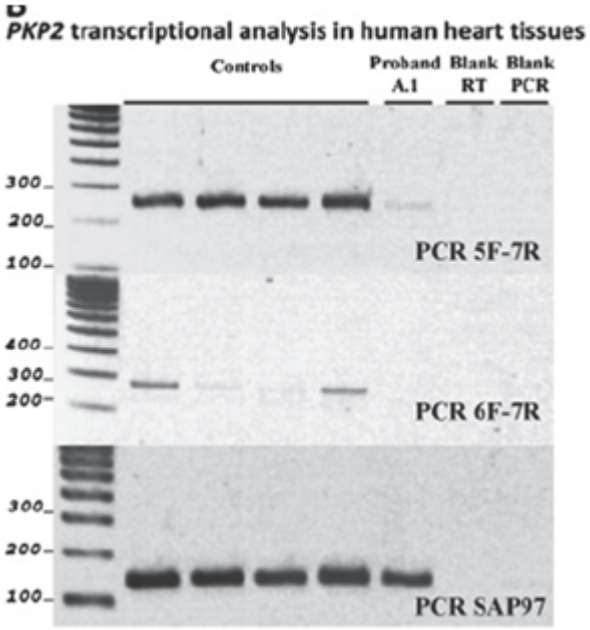
Interpretation Genetic Data

Plakophilin 2A is the dominant isoform in human heart tissue: consequences for the genetic screening of arrhythmogenic right ventricular cardiomyopathy

E Gandjbakhch,[1,2,3] P Charron,[1,2,3,4] V Fressart,[5] G Lorin de la Grandmaison,[6] F Simon,[5] F Gary,[1,2] A Vite,[1,2] B Hainque,[5] F Hidden-Lucet,[3] M Komaida,[1,2,3] E Villard[1,2]





Heart 2011; 97:844-9

Gene	Locus	Exon	Mutation	DNA Change	Protein Change	Type	Reported Classification	No of clinical reports	Details
PKP2	12p11	6	Intronic	c.1379-22G>A	-	Intronic	No known pathogenicity	1	Show Detai
PKP2	12p11	6	Splice	c.1379-1G>A	r.spl?	Splice site	Pathogenic	1	Show Detai
PKP2	12p11	6	G489R	c.1465G>A	p.Gly489Arg	Missense	Pathogenic	2	Show Detai
PKP2	12p11	6	R490W	c.1468C>T	p.Arg490Trp	Missense	Unknown/UV	3	Show Detai
PKP2	12p11	6	Splice	c.1510+5G>A	r.spl?	Splice site	Pathogenic	0	Show Detai
PKP2	12p11	6	Intronic	c.1510+78G>A	-	Intronic	No known pathogenicity	1	Show Detai

PITTFALLS DIFFERENT TECHNIQUES

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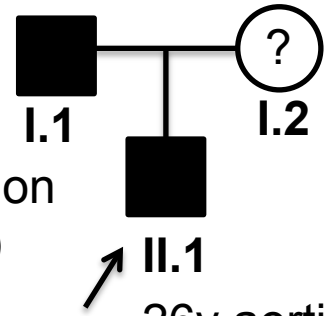
Older techniques (false negatives)

- Older techniques: (dHPLC), misses mutations
- Sanger sequencing:
  - misses large deletions/duplications (add MLPA)
  - Mosaicism missed
- Panels based upon WES: coverage

ACTA2:c.[116G>A];[=] p.(Arg39His)

✓ Sanger: not detected



60y aortic dilatation
(root, ascending)

26y aortic dissection

ACTA2:c.[116G>A];

p.(Arg39His)

✓ NGS

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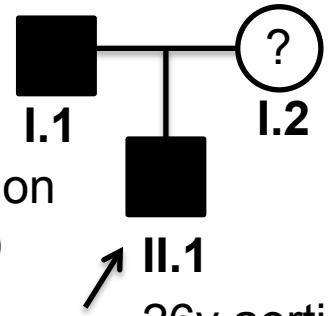
ACTA2:c.[116G>A];[=] p.(Arg39His)

- ✓ Sanger: not detected
- ✓ NGS: 4% (12/286 reads).



60y aortic dilatation
(root, ascending)

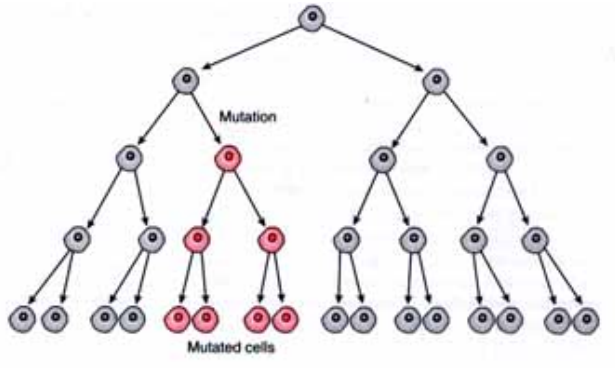
26y aortic dissection

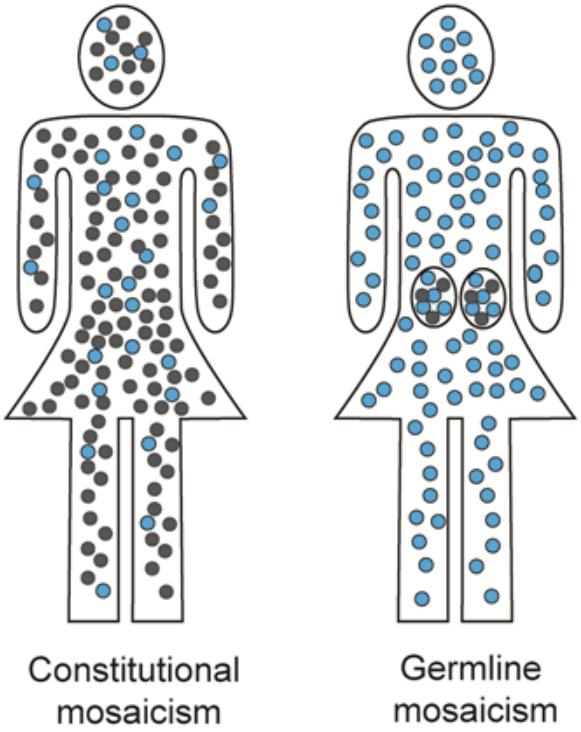
ACTA2:c.[116G>A];

p.(Arg39His)

- ✓ NGS

mosaicism





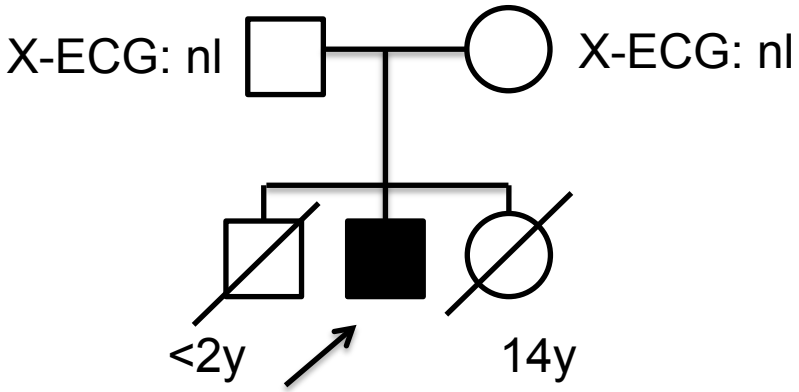
J Am Coll Cardiol. 2009 November 24; 54(22): 2065–2074. doi:10.1016/j.jacc.2009.08.022.

Comprehensive Open Reading Frame Mutational Analysis of the *RYR2*-Encoded Ryanodine Receptor/Calcium Channel in Patients Diagnosed Previously with Either Catecholaminergic Polymorphic Ventricular Tachycardia or Genotype Negative, Exercise-Induced Long QT Syndrome

Argelia Medeiros-Domingo, MD, PhD[*,1], Zahurul A. Bhuiyan, MD, PhD[*,2], David J. Tester, BS[1], Nynke Hofman, MSc[2], Hennie Bikker, PhD[2], J Peter van Tintelen, MD, PhD[3], Marcel M.A.M Mannens, PhD[2], Arthur A.M. Wilde, MD, PhD[2,4], and Michael J. Ackerman, MD, PhD[1,5,6]

RyR2: Y4149S

25% hair roots
20% leucocytes
15% buccal cells & skin



Recurrence risk in de novo mutations: 1-2% !

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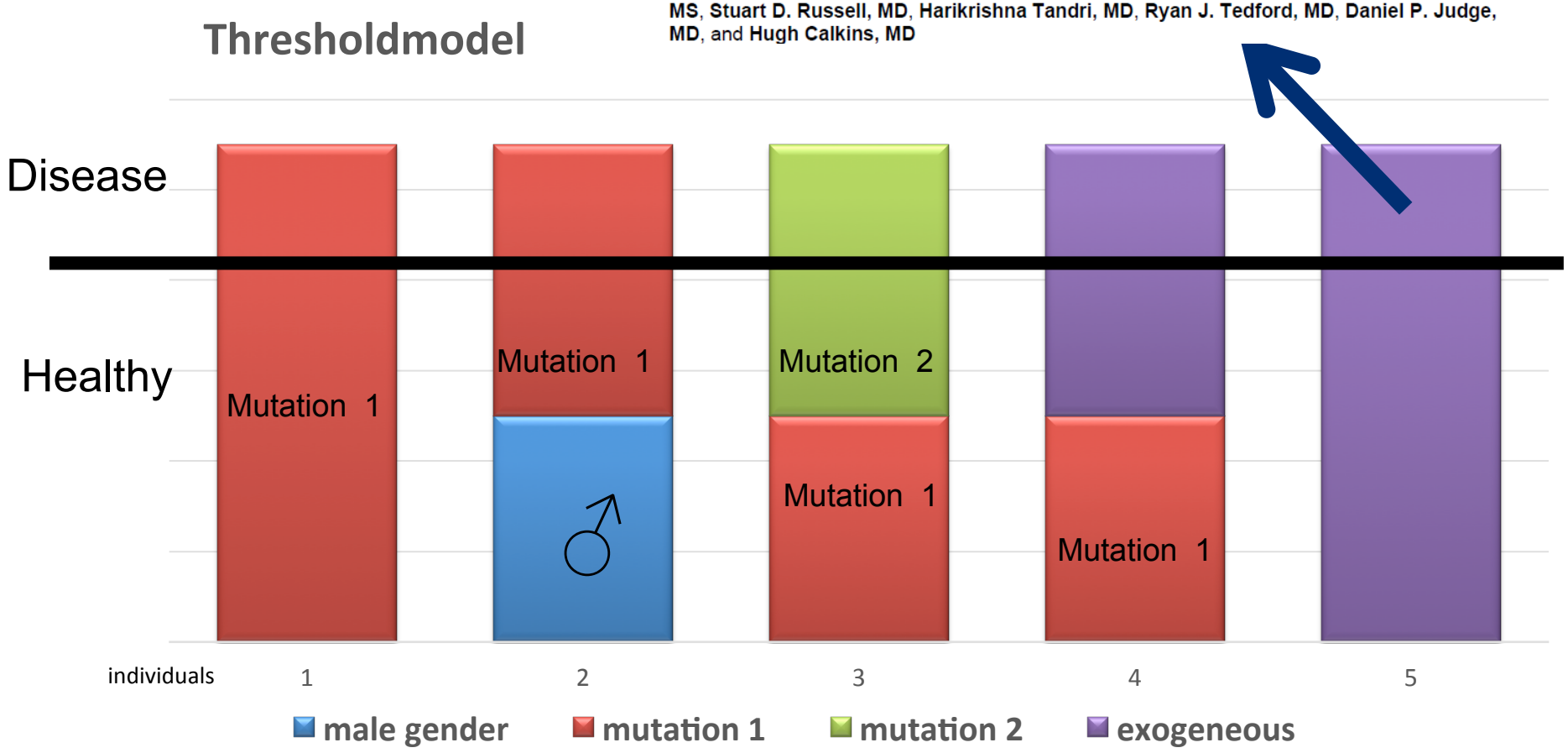
cardiogenetics: threshold-model:

gender +/- multiple genes +/- environmental influences

Exercise Increases Age-Related Penetrance and Arrhythmic Risk in Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy Associated Desmosomal Mutation Carriers

Cynthia A. James, ScM, PhD, Aditya Bhonsale, MD, Crystal Tichnell, MGC, Brittney Murray, MS, Stuart D. Russell, MD, Harikrishna Tandri, MD, Ryan J. Tedford, MD, Daniel P. Judge, MD, and Hugh Calkins, MD



SUMMARY

- Dominant: reduced penetrance/variability
- Genetics Dx mainly for family cascade-screening (counseling)
- Careful in interpreting genetic test results: dynamic new genes; published "mutations" *(false +)*
- Causal mutations can be missed (technique, mosaicism, deletions) *(false -)*
- Genetics points the gun, additional factors pull the trigger (threshold-model)

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Thank you

VUmc amc Durrer center for cardiogenetic research CVON Cardio Vasculair Onderzoek Nederland

Experimental Evidence Scoring

Evidence Category	Evidence Type	Score Range	Recommended points/ evidence	Points Given	Max Score
Function	Biochemical Function	½ - 2	½ point for each piece of evidence in any category	1.5	2
	Protein Interaction	½ - 2			
	Expression	½ - 2			
Functional Alteration	Patient cells	1 - 2	1 point	1	2
	Non-patient cells	½ - 1	½ point	NA	
Models & Rescue	Animal model	1 - 4	2 points	NA	4
	Cell culture model system	½ - 2	1 point	NA	
	Rescue in animal model	1 - 4	2 points	NA	
	Rescue in engineered equivalent	½ - 2	1 point	NA	
			Total Final Score	2.5	**0 - 8**

Assertion criteria	Genetic Evidence (0-12 points)	Experimental Evidence (0-6 points)	Total Points (0-18)	Replication Over Time (Y/N)
Description	Case-level, family segregation, or case-control data that support the gene-disease association	Gene-level experimental evidence that support the gene-disease association	Sum of Genetic & Experimental Evidence	> 2 pubs w/ convincing evidence over time (>3 yrs)
Assigned Points	**9.75**	**2.5**	**12.25**	**Y**

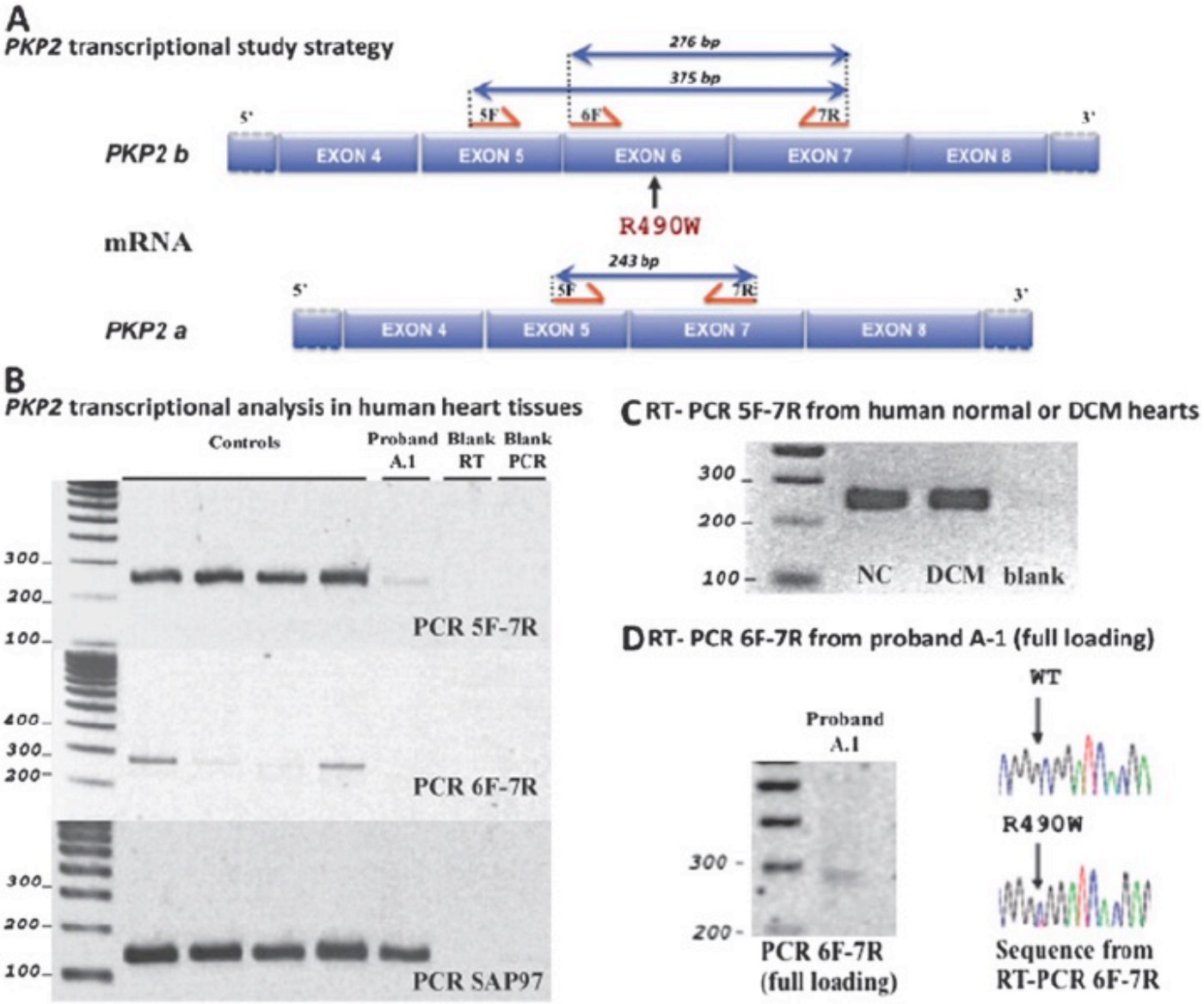
CALCULATED CLASSIFICATION	**LIMITED**	**1-6**
	MODERATE	**7-11**
	STRONG	**12-18**
	DEFINITIVE	**12-18 AND replication over time**

Valid contradictory evidence? (Y/N)	List PMIDs and describe evidence:

CURATOR CLASSIFICATION	**STRONG???**
FINAL CLASSIFICATION	

Plakophilin 2A is the dominant isoform in human heart tissue: consequences for the genetic screening of arrhythmogenic rig

E Gandjbakhch,[1,2,3] P Charron, F Simon,[5] F Gary,[1,2] A Vite,[1,2]



Plakophilin-2 c.419C>T and risk of heart failure and arrhythmias in the general population

Alex Hørby Christensen[1], Pia Rørbæk Kamstrup[2], Estelle Gandjbakhch[3], Marianne Benn[4], Jan Skov Jensen[5], Henning Bundgaard[6], Eric Villard[3] and Anne Tybjærg-Hansen*[,7,8]

European Journal of Human Genetics (2016) 24, 732–738

- Multiple lines of evidence:
  - >10000 Controls 0.94%
  - No association with electro/echographic parameters
  - In vitro studies

ARTICLE RESPONSE

Nature Clinical Practice Cardiovascular Medicine (2008) **5**, E1
doi:10.1038/ncpcardio1434

Variations in *DSG2*: V56M, V158G and V920G are not pathogenic for arrhythmogenic right ventricular dysplasia/cardiomyopathy

Maximilian G Posch*, Matthias J Posch, Cemil Özcelik

ARTICLE

The p.A897KfsX4 frameshift variation in desmocollin-2 is not a causative mutation in arrhythmogenic right ventricular cardiomyopathy

Marzia De Bortoli[1], Giorgia Beffagna[1], Barbara Bauce[2], Alessandra Lorenzon[1], Gessica Smaniotto[1], Ilaria Rigato[2], Martina Calore[1], Ilena EA Li Mura[1], Cristina Basso[3], Gaetano Thiene[3], Gerolamo Lanfranchi[1,4], Gian Antonio Danieli[1], Andrea Nava[2] and Alessandra Rampazzo*[,1]

Conclusion: genetics ACM

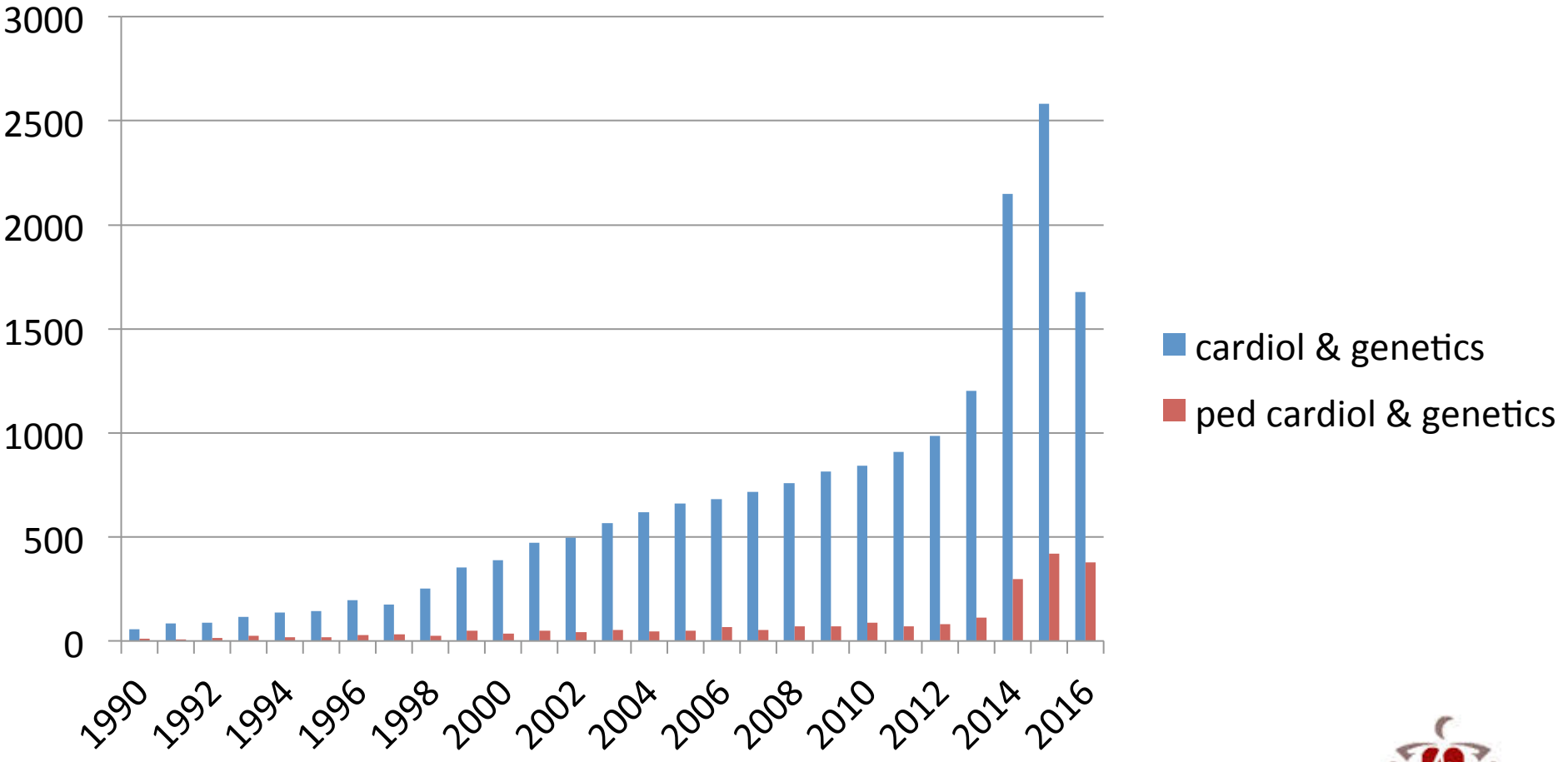
- Left-right-biventricular
- mainly desmosome related: mutations: ~50% (90% fam)
- Genotype-phenotype relationships:

  "double mutations" (screen all genes);

  DSP, PLN, TMEM43: LV involvement
- Careful in interpreting genetic test results: dynamic
- New monogenic-genes claims: be aware
- Highly penetrant dominant mutations rare; majority of mutations are RISK factors (threshold model)
- Counsel genotype, treat phenotype (limited role for genotyping in prediction of outcome; genotyping for cascade screening)

VUmc amc

Pubmed: nº publications -pediatric- cardiology & genetics



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