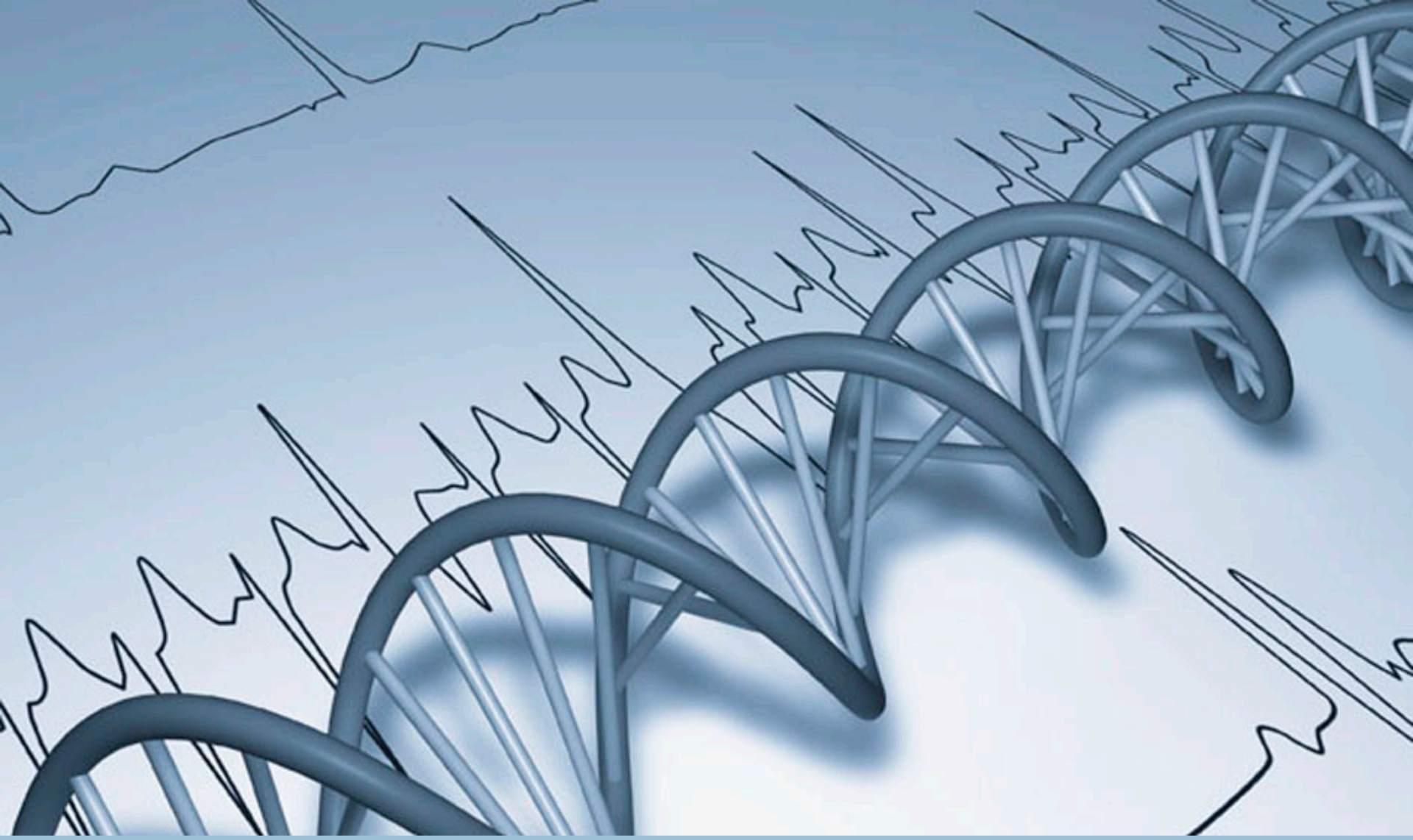
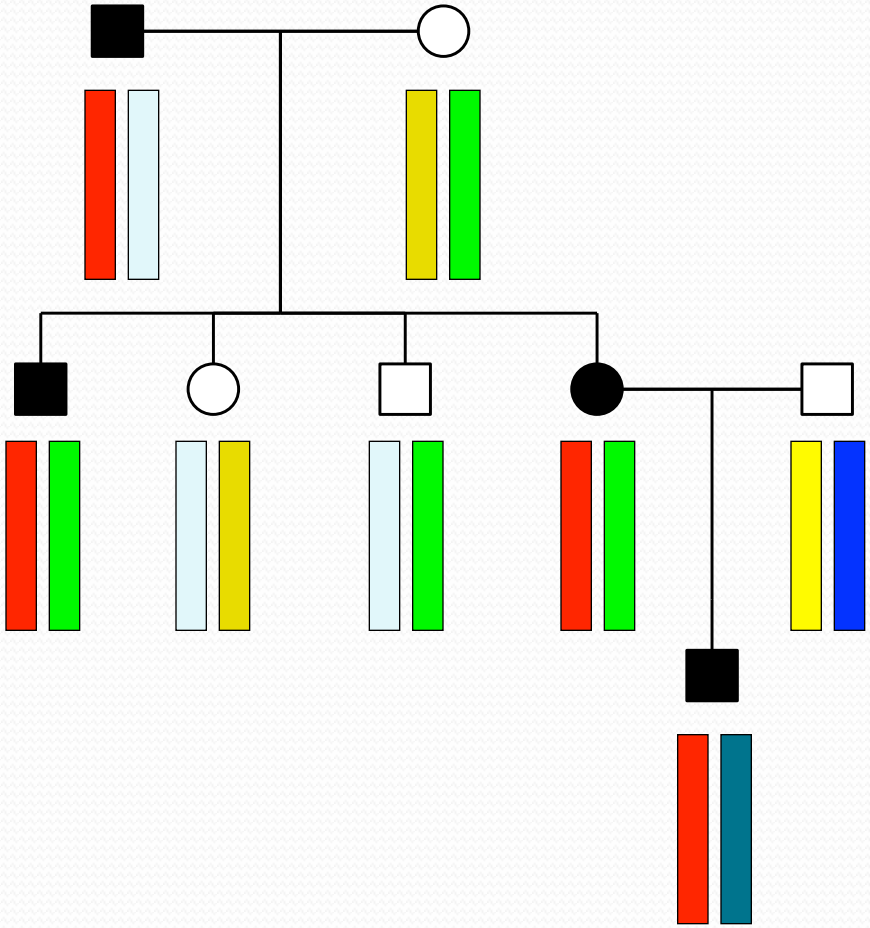


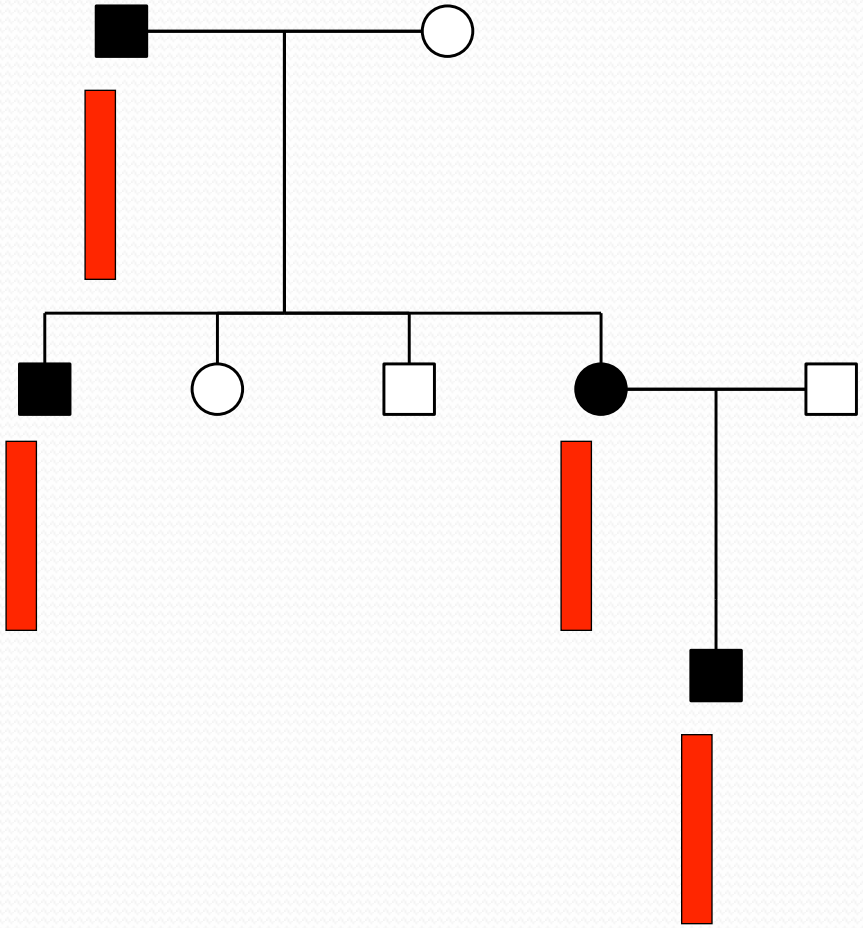
How to Interpret Genetic Data

Z. Bhuiyan, MBBS, Ph.D

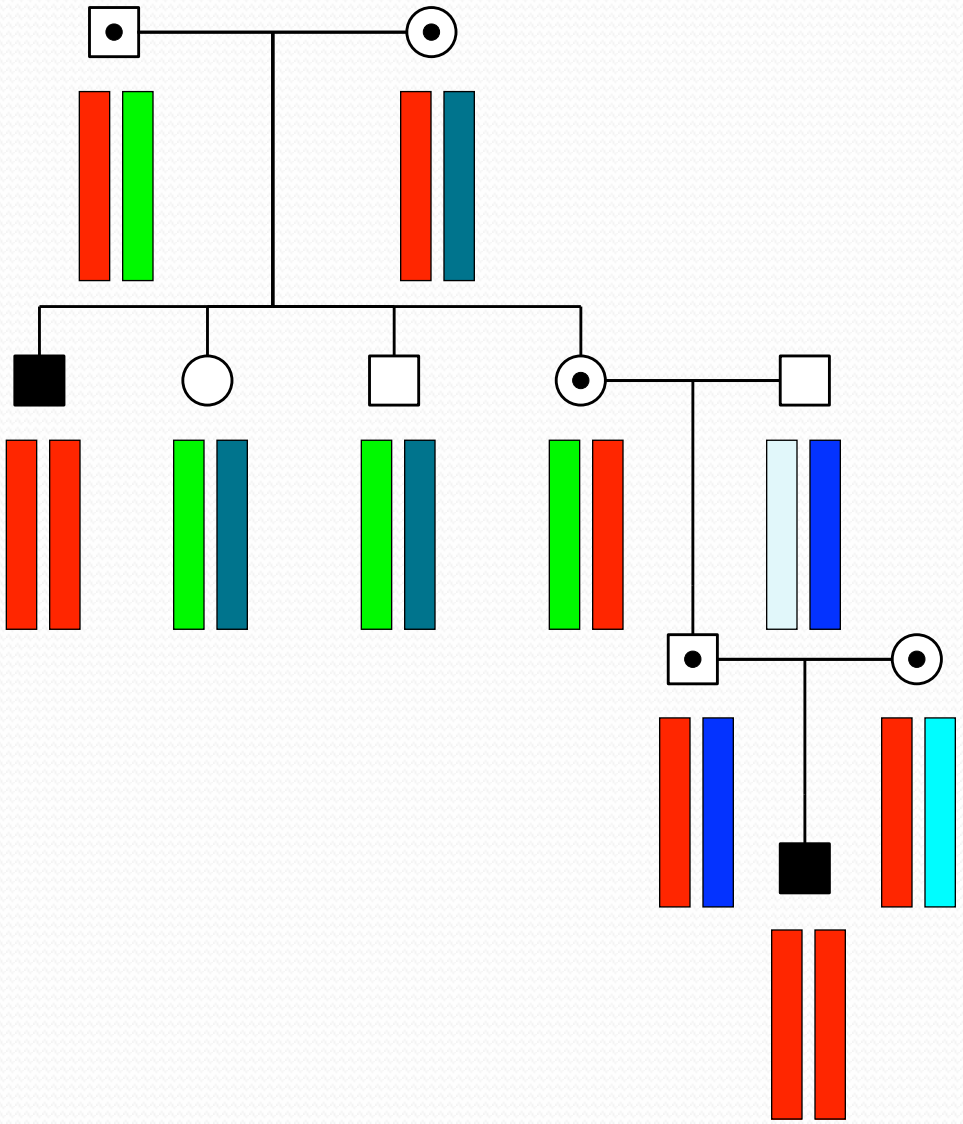
Disease Transmission from One Parent (Autosomal Dominant)



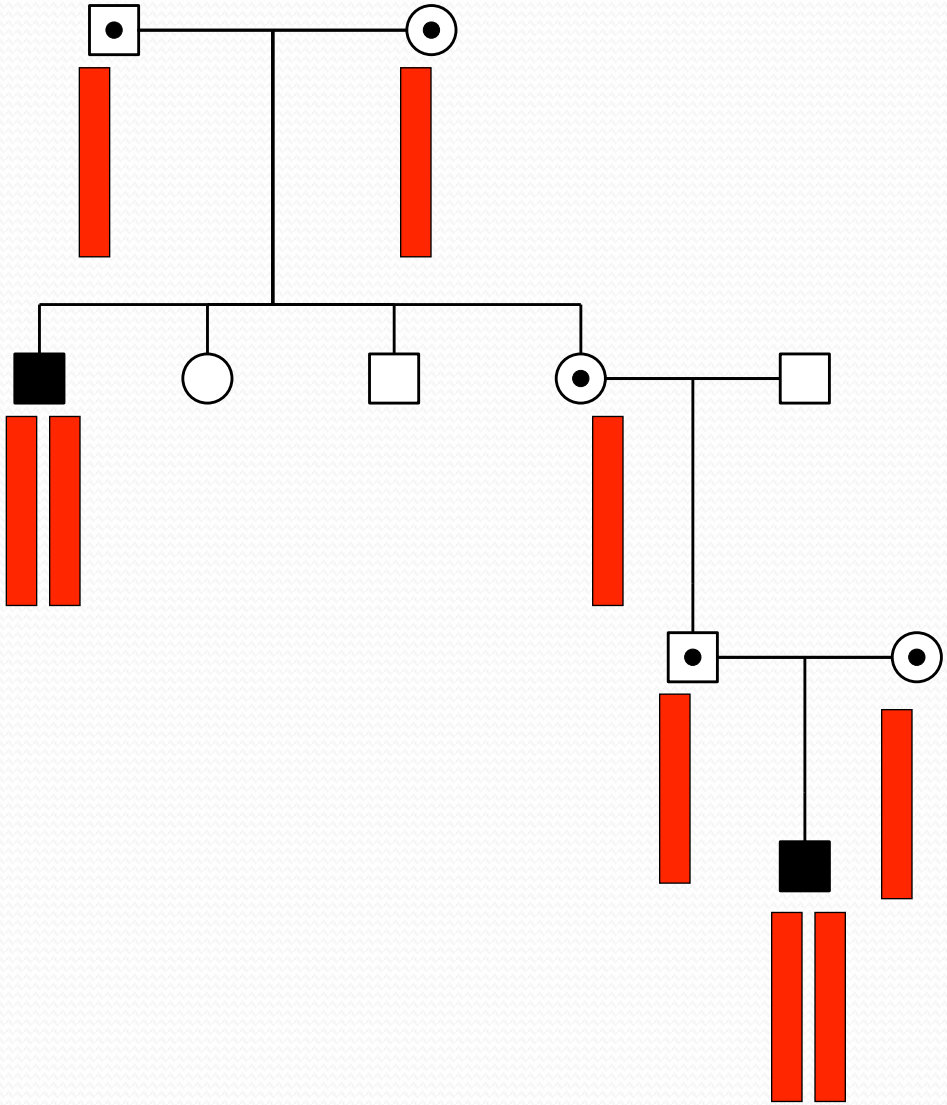
Disease Transmission from One Parent (Autosomal Dominant)



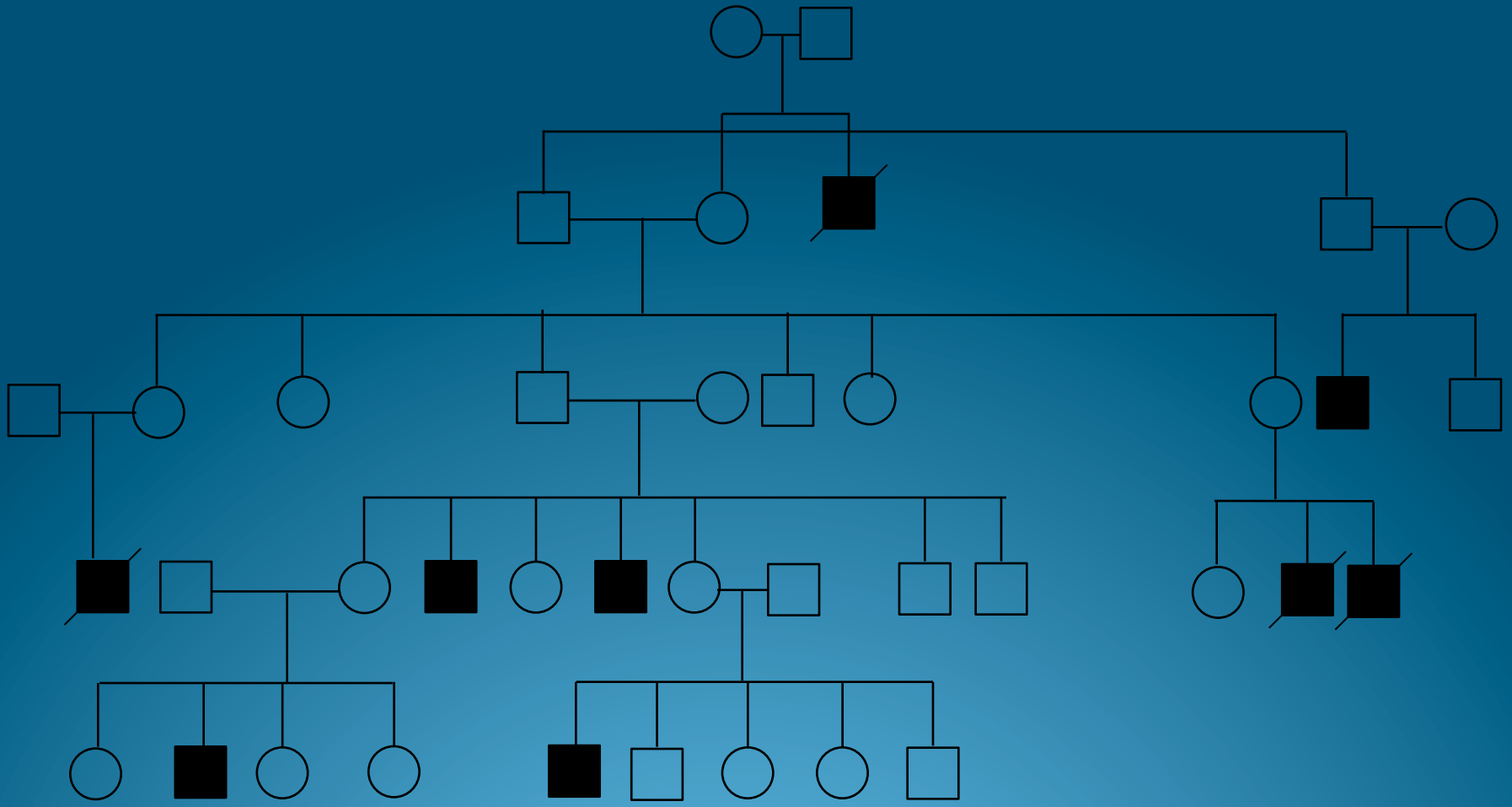
Disease Transmission from Both Parents (Autosomal Recessive)



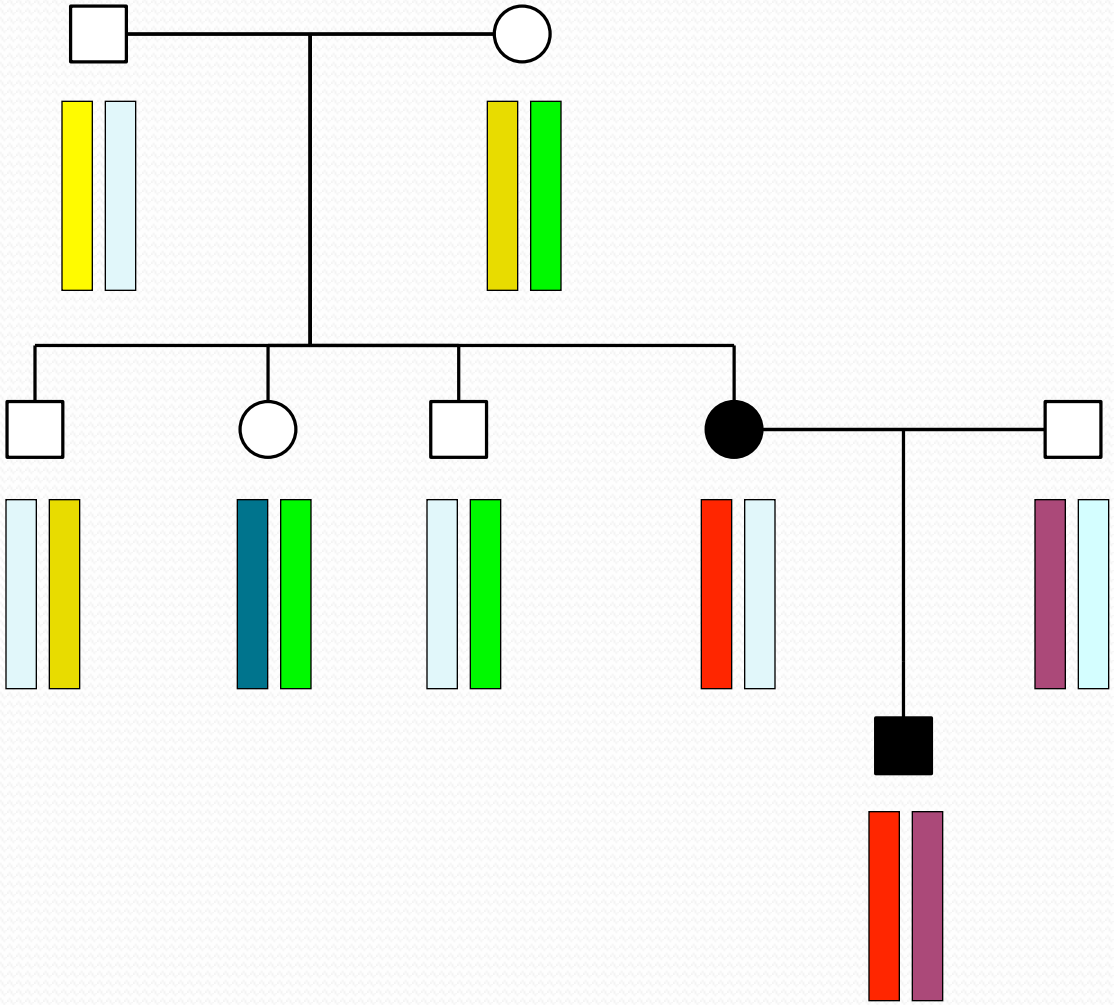
Disease Transmission from Both Parents (Autosomal Recessive)



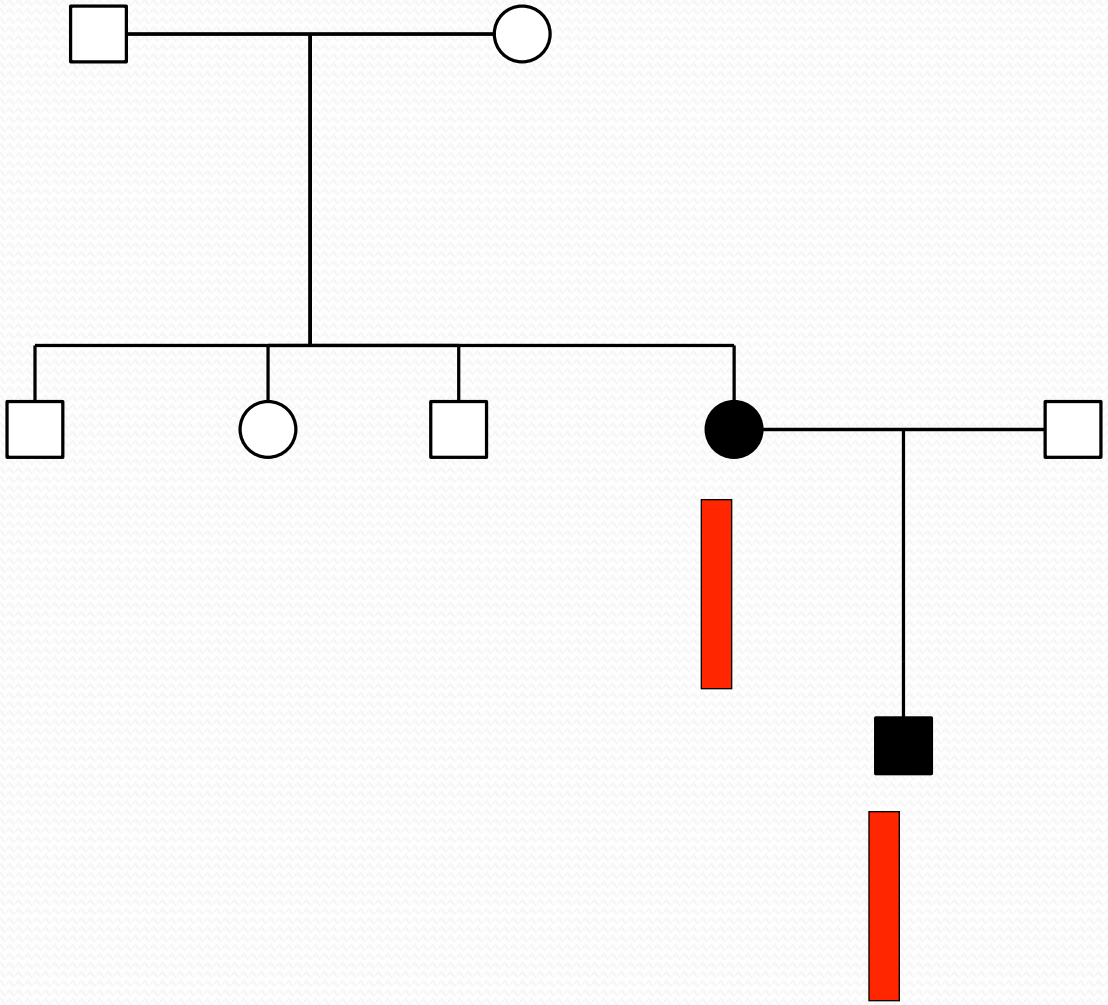
Disease Transmission from Carrier Mother (X linked)



Disease Transmission: *De Novo* Origin



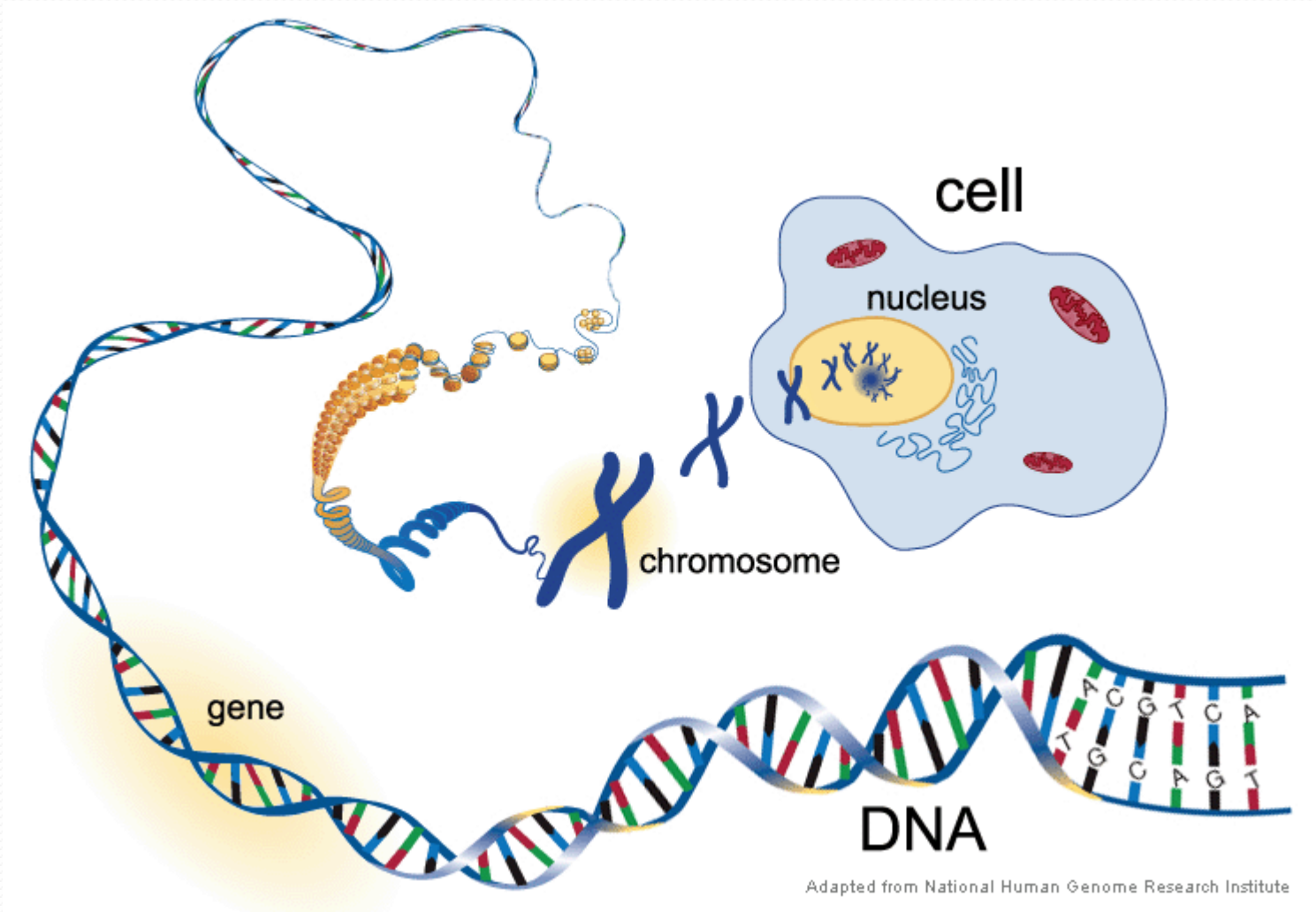
Disease Transmission: *De Novo* Origin



Gonadal Mosaicism

Courtesy: Nottingham University Hospitals NHS Trust

Cell, Chromosome and Gene



Different types of Mutations

Normal or Wild type

TCCTTCCGCAGGCGCACGGACAAGGACACG
S F R R R T D K D T

Missense

TCCTTCCGCAGGCGCACGGACACGGACACG
S F R R R T D T D T

Insertion of a nucleotide

TCCTTCCGCAGGCGCACGGACTAAGGACACG
S F R R R T D X

Non-sense

CAGAGGCAGATGACGCTG
Q R Q M T L

CAGAGGTAGATGACGCTG
Q R X M T L

Deletion of nucleotides

TCCAGTGGCCCCTCCAGCCCTGAGAGC
S S G P S S P E S

TCCAGTGGCCCCTCCAGCTGAGAGC
S S G P S S X

Factors to consider while analysing genomic data

- **Clinical phenotype**
- **Type of Mutation/Variation**
- **Frequency of the variation in Genome data base**
- **Involved Gene**
- **Mutation previously reported or not**
  Is the published data convincing ?
- **Is there any functional analysis data available**
- **Location of the Mutation in the Gene**
- **Conservation of the involved amino acid**
- **Family history for the disease**

Splice Site Mutation

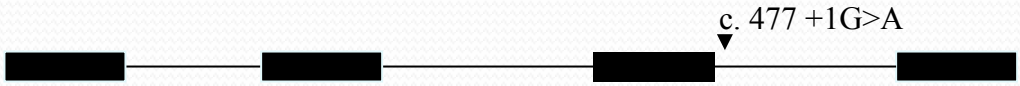
gttatgaaaacatgtcagatagcatcagttctgtagaactgatttgattaaacagttacagaagcaaaagtttgatgtattataatgatgta
cacatctctgtttccttatggtaacctcagTTTGAAGTAGATCTGTACCAAAATGAAACAGCCTGTCAGAGT
CCTTTGGATTATCAGTACCGTCAGGAGATCCTGAAGCTGGAGAATTCGGGTGGCAAGAAAA
ACCGACGAATCTTTACCTACACTGACTCTGATAGATACACCAATTTGGAGGAGgtattgttctttcta
gggtctcatagcttccatctttgcttcctctagctttttgcctcactgagttctgtgtgttatgtcagagcctagtaaggggagacaagtagcc
tgtctaggaa

AATTTGGAGGAG
N L E E

AATTTGGAGGAA
N L E E

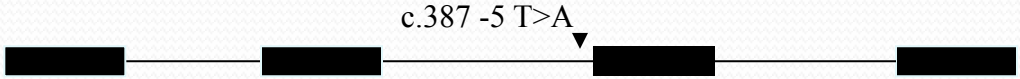
Splice Site Mutations their Consequence

Splicing Result



Complete skipping
of exon-2

Zehelein J et al. 2006. J Biol Chem.



90% skipping of exon-2
10% normal splicing

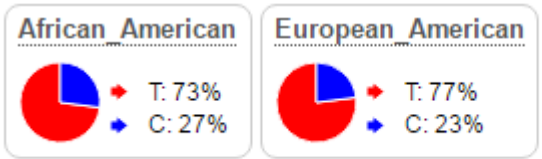
Bhuiyan et al. 2007

ExAC Browser (Beta) | Exome Aggregation Consortium

7:150645534 T / G (rs1805123)	7	150645534	p.Lys897Thr	PASS	missense	MNP	21515	114962	2260
7:150645534 T / TGTCCG (rs1805123)	7	150645534	p.Lys897ThrfsTer79	PASS	frameshift		2	114962	0
7:150645537 T / C	7	150645537	p.Asp896Gly	PASS	missense		1	116448	0

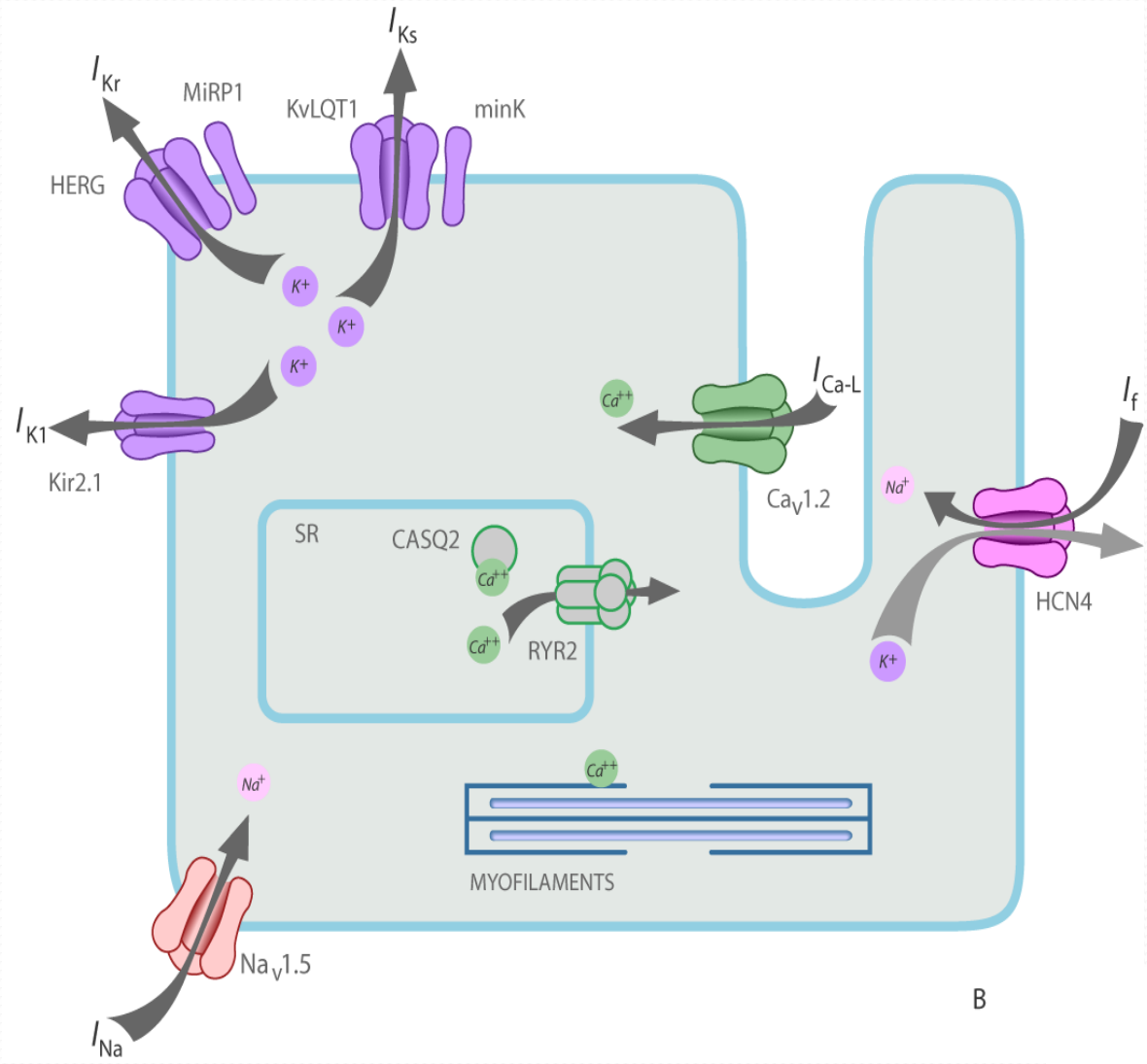
Population genetics

NHLBI Exome Sequencing Project allele frequencies

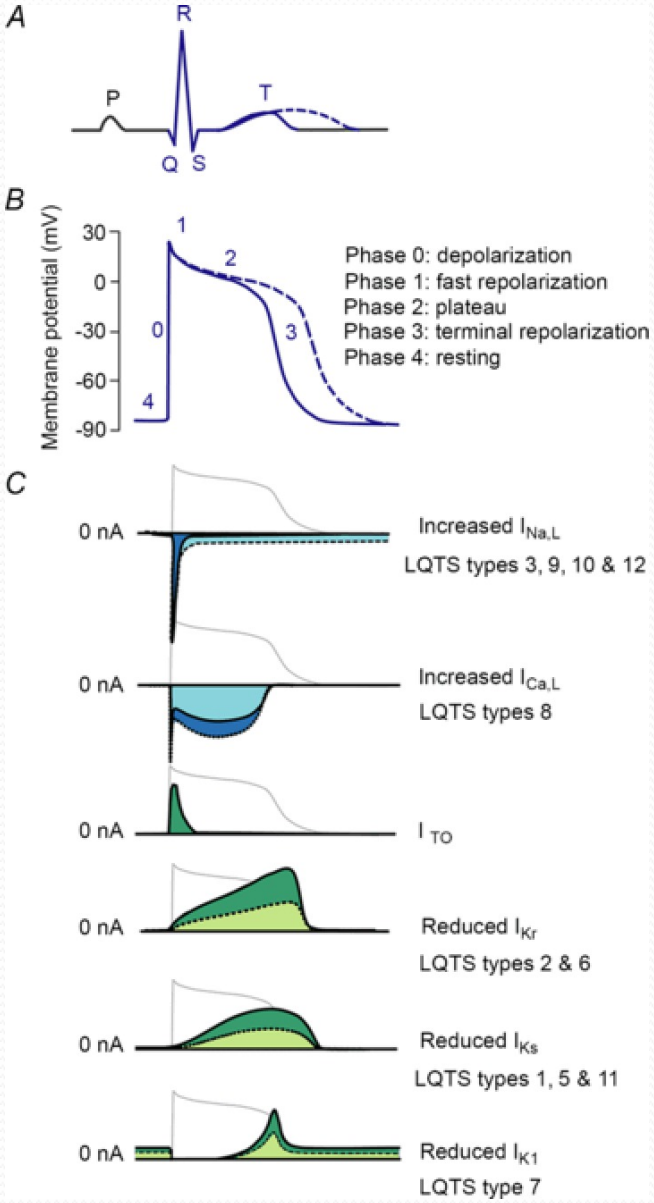


H558R:SCN5A

Ion Channels in the Heart



Cardiac Ion Channels and long QT syndromes



SCN5A, *CAV3*, *SCN4B*, *SNTA1*

CACNA1C

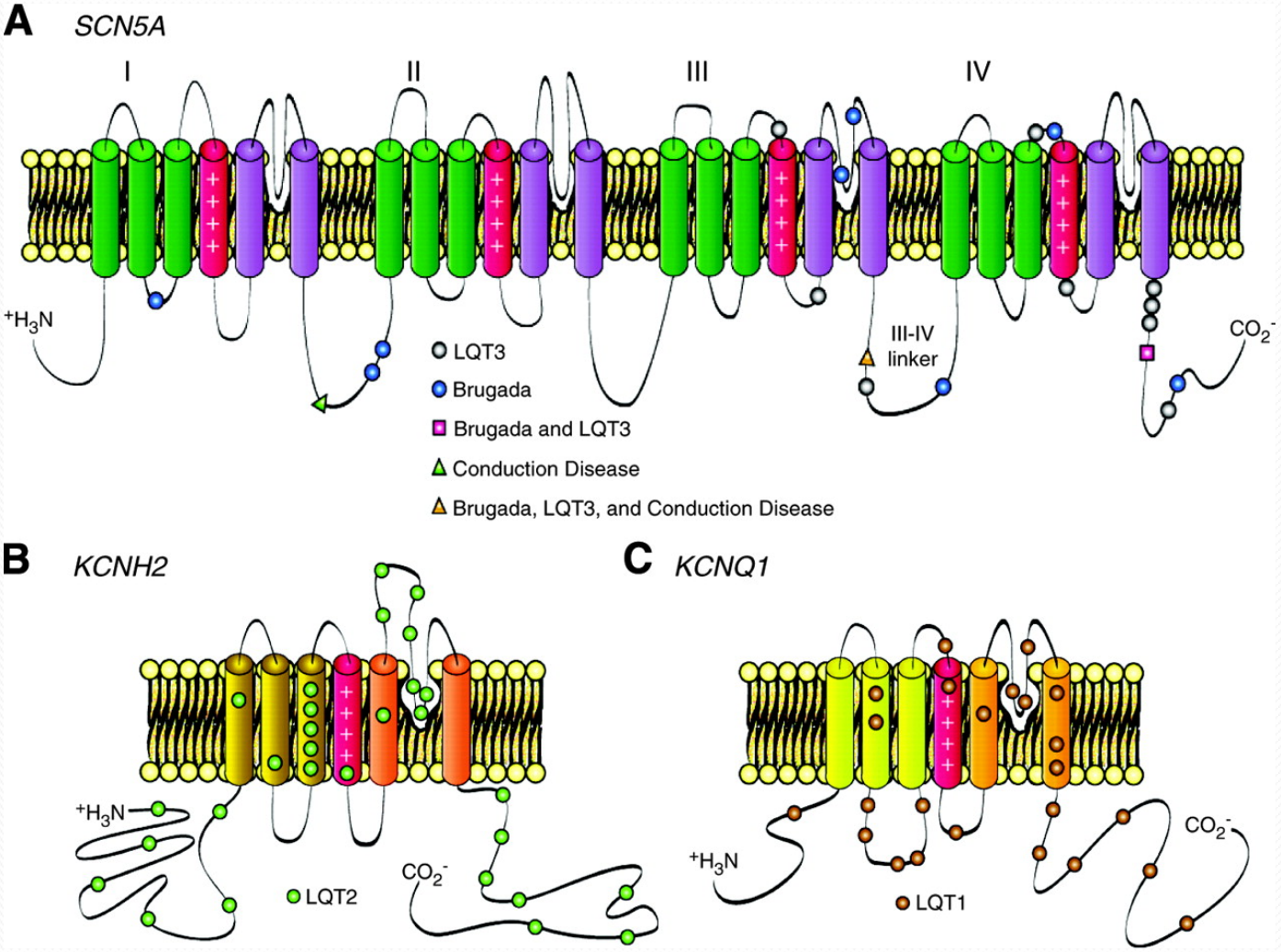
KCNH2, *KCNE2*

KCNQ1, *KCNE1*, *AKAP9*

KCNJ2

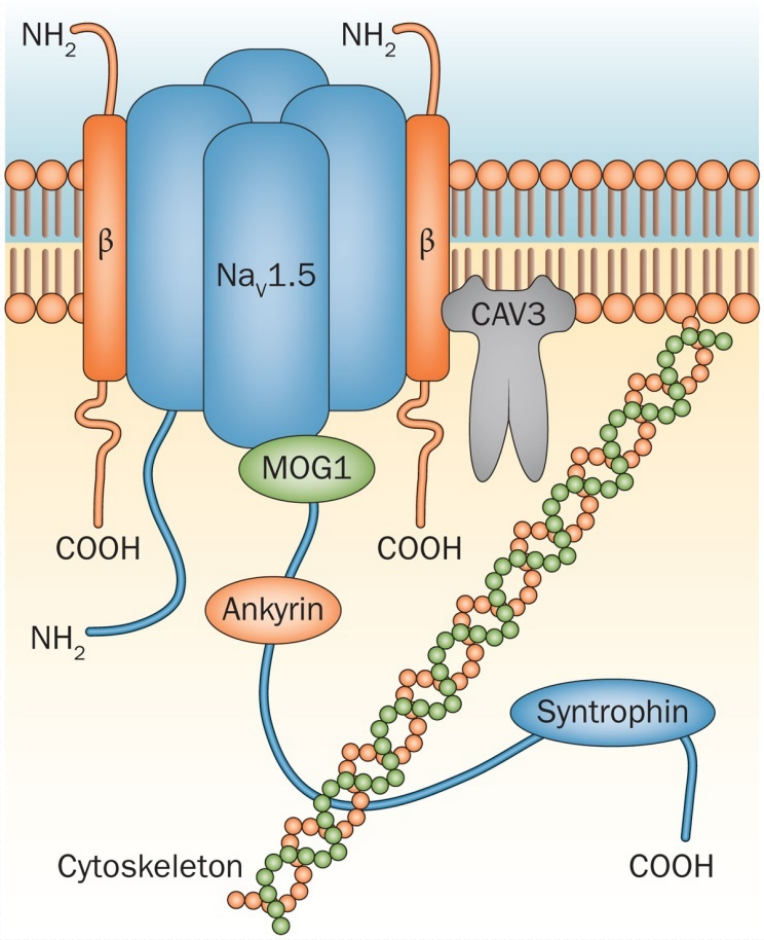
Amin et al. J Physiol. 2013; 591: 4125–4139.

Pore-forming (α) subunits of cardiac Nav (A) and Kv (B and C) channels linked to inherited arrhythmias.



Jeanne M. Nerbonne, and Robert S. Kass Physiol Rev 2005;85:1205-1253

The $Na_v1.5$ (SCN5A) channel is part of a macromolecular complex



Liu, M. *et al.* (2014). *Nat. Rev. Cardiol.* doi:10.1038/nrcardio.2014.85

Predominant Genes in Long QT Syndrome

• Type 1	*KCNQ1* (KvLQT1)	50%
• Type 2	*KCNH2* (HERG)	35%
• Type 3	*SCN5A*	8%

Genes in Long QT Syndrome

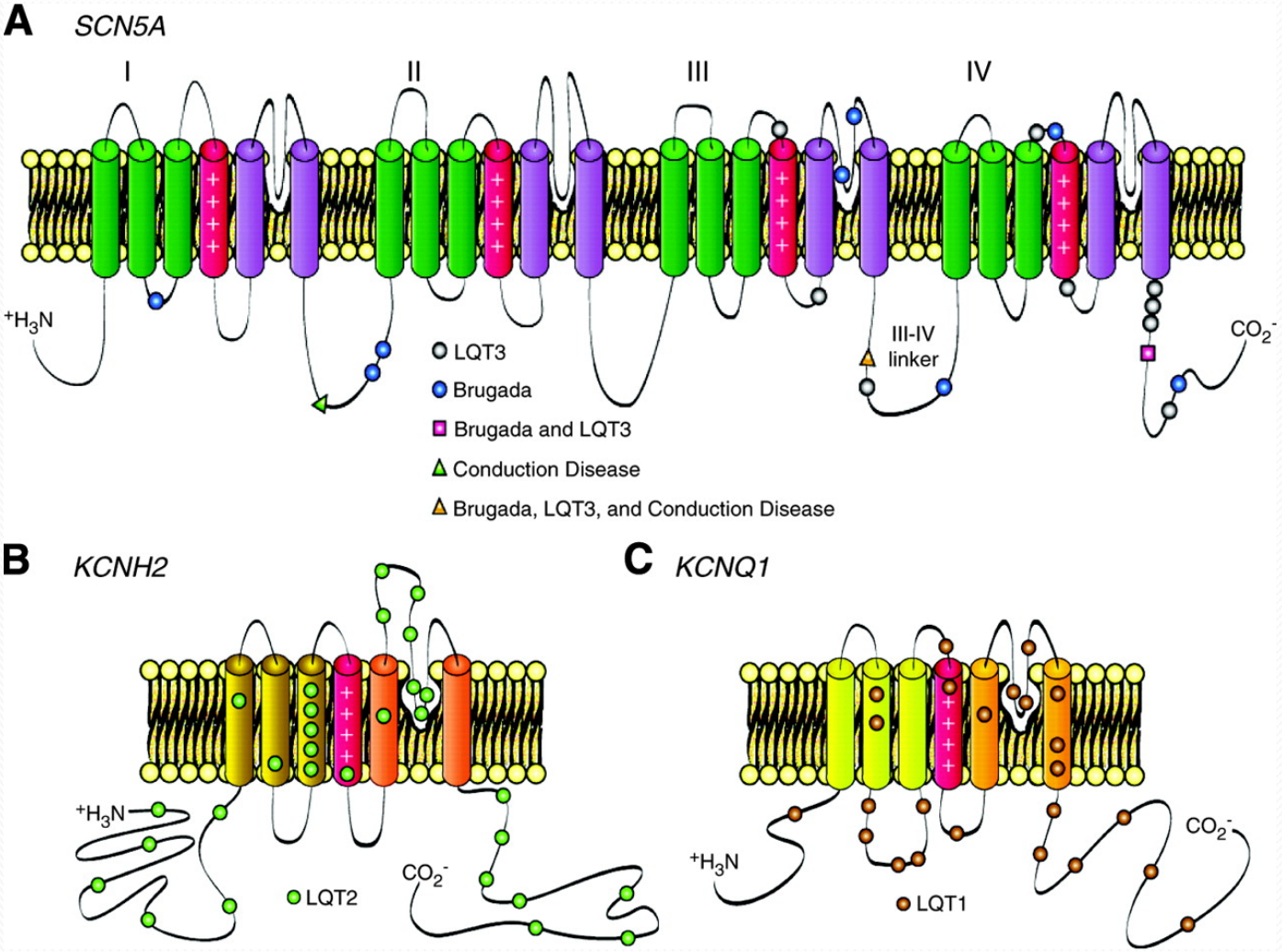
Remaining LQTS	Gene	Protein	Current
Type 4	Ankyrin B	Ankyrin	Na+/K+ ATPase and others
Type 5	KCNE1	MinK	Iks ↓
Type 6	KCNE2	MiRP1	Ikr ↓
Type 7	KCNJ2	Kir2.1	Ik1 ↓
Type 8	CACNA1C	CaV1.2	ICa-L ↑
Type 9	CAV3	Caveolin 3	INa ↑
Type 10	SCN4B	SCNβ4 subunit	INa ↑
Type 11	AKAP-9	Yotiao	Iks ↓
Type 12	SNTA-1	Syntrophin-α1	INa ↑
Type 13	KCNJ5	Kir3.4	IkAch ↓
Type 14	CALM1	Calmodulin 1	Defective Ca2+ signalling
Type 15	CALM2	Calmodulin 2	
Type 16	CALM3	Calmodulin 3	

Genes in Long QT Syndrome

Remaining LQTS Type 4	Gene Ankyrin B	Protein Ankyrin	Current Na+/K+ ATPase and others
Type 5	KCNE1	MinK	Iks ↓
Type 6	KCNE2	MiRP1	Ikr ↓
Type 7	KCNJ2	Kir2.1	Ik1 ↓
Type 8	CACNA1C	CaV1.2	ICa-L ↑
Type 9	CAV3	Caveolin 3	INa ↑
Type 10	SCN4B	SCNβ4 subunit	INa ↑
Type 11	AKAP-9	Yotiao	Iks ↓
Type 12	SNTA-1	Syntrophin-α1	INa ↑
Type 13	KCNJ5	Kir3.4	IkAch ↓
Type 14	CALM1	Calmodulin 1	Defective Ca2+ signalling
Type 15	CALM2	Calmodulin 2	
Type 16	CALM3	Calmodulin 3	

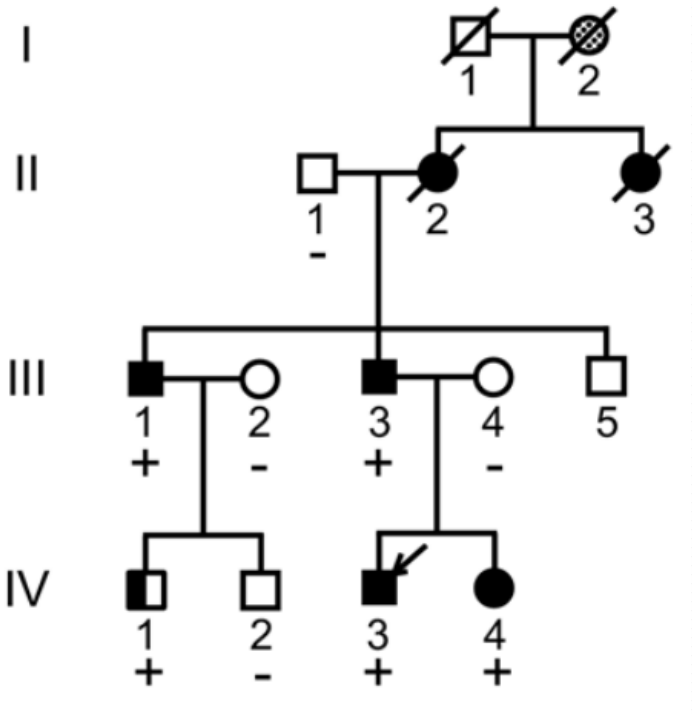
Type 17 or CPVT3: Bi-allelic mutation in *TECRL* gene (Trans-2,3-Enoyl-CoA Reductase-Like)

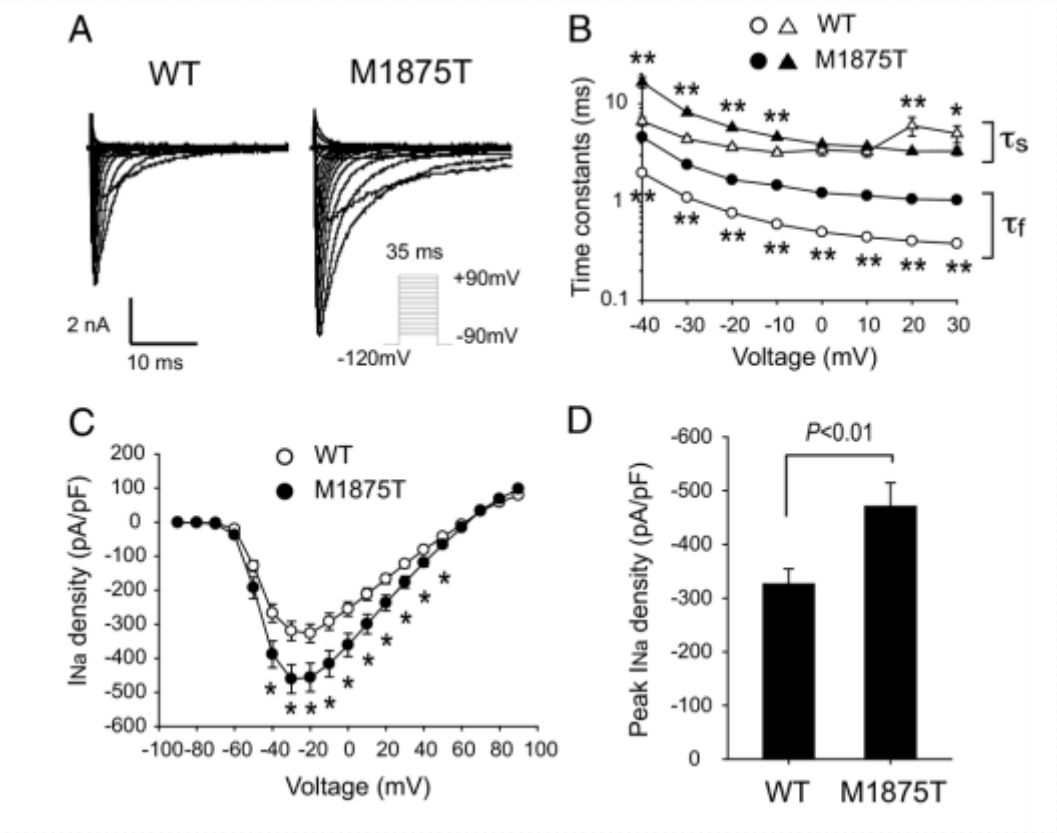
Pore-forming (α) subunits of cardiac Nav (A) and Kv (B and C) channels linked to inherited arrhythmias.



Jeanne M. Nerbonne, and Robert S. Kass. Physiol Rev 2005;85:1205-1253

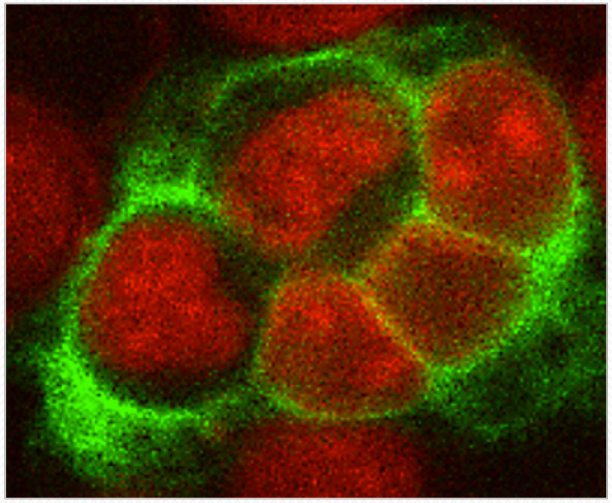
Gain of Function Mutation in SCN5A



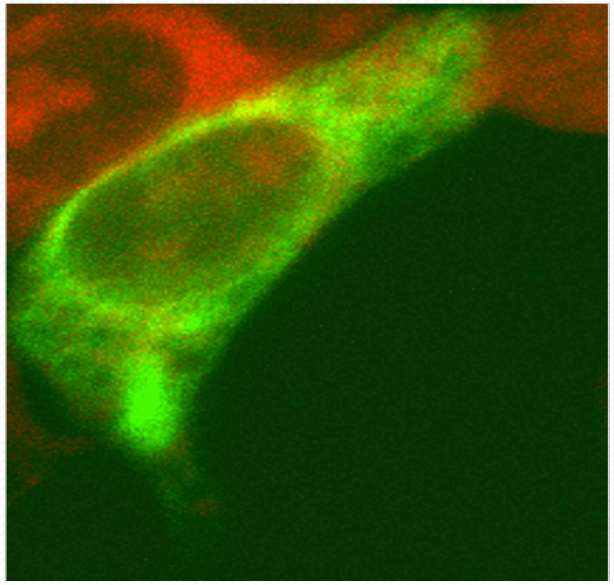


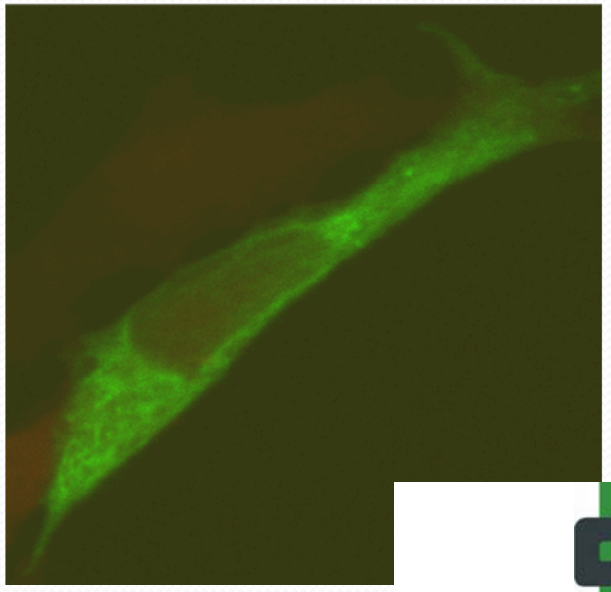
Makiyama et al. J Am Coll Cardiol. 2008;52:1326–34

What Happens Inside a Single Cell



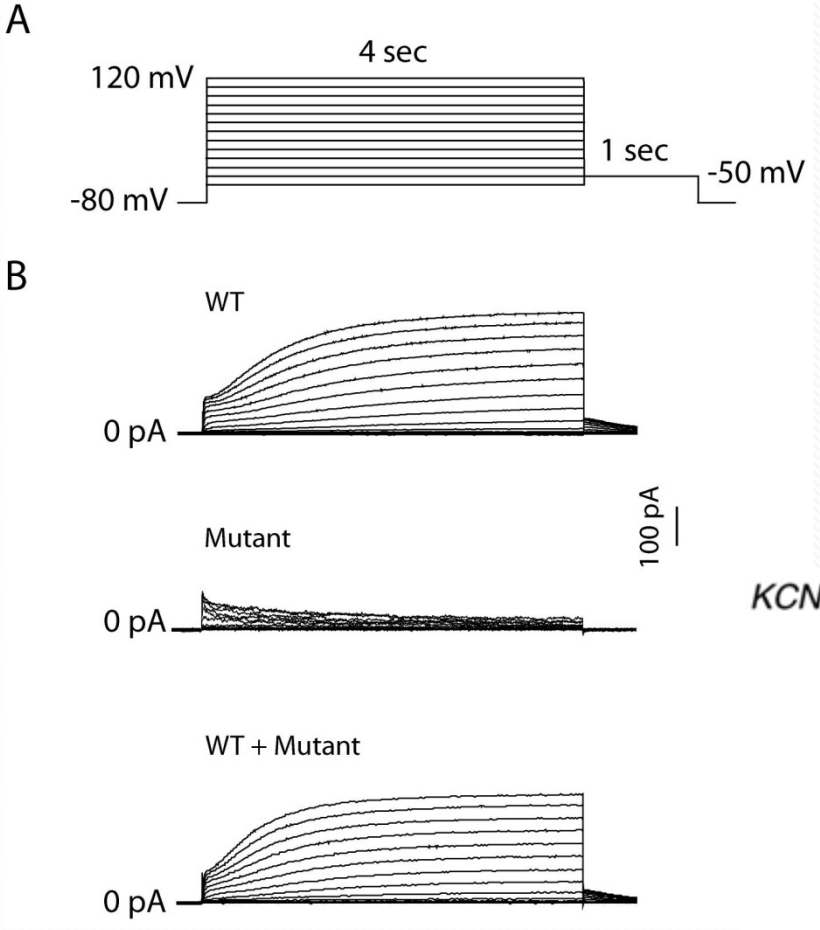
Wild type

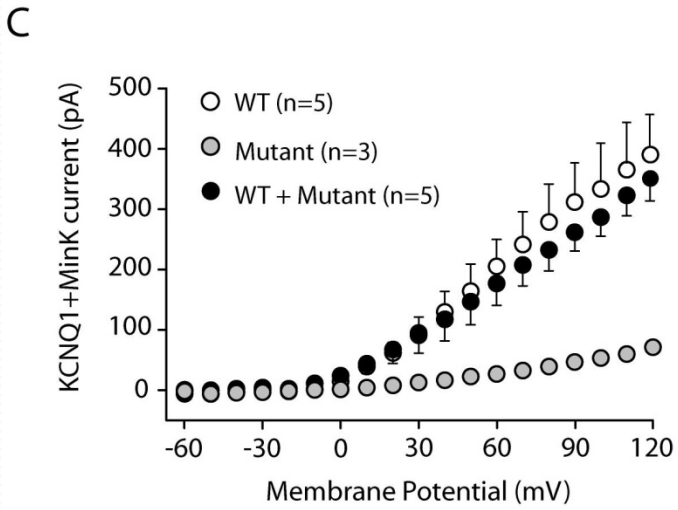


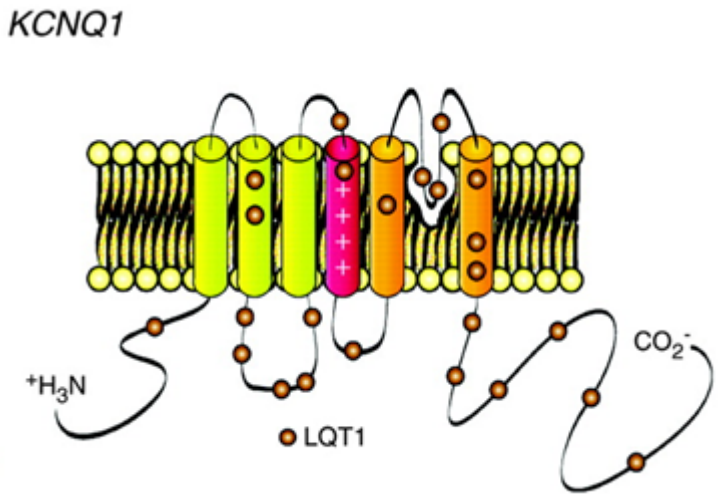


Mutant

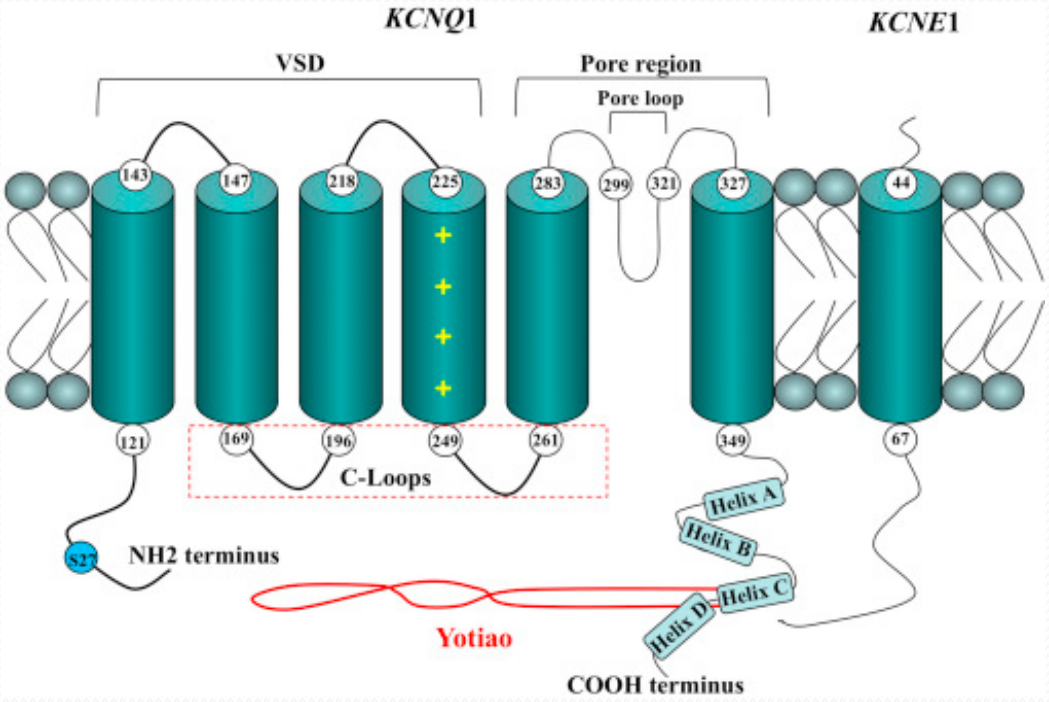
Diminution or Loss of IKs current in LQT1 due to KCNQ1 Mutations







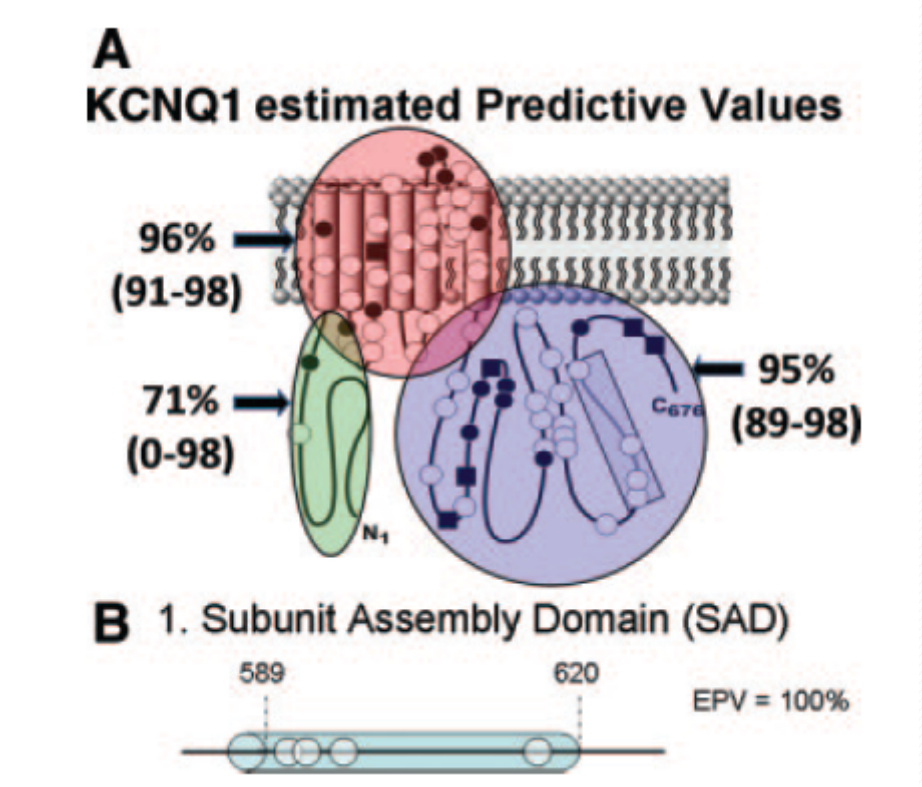
Predicted topology of the IKs channel



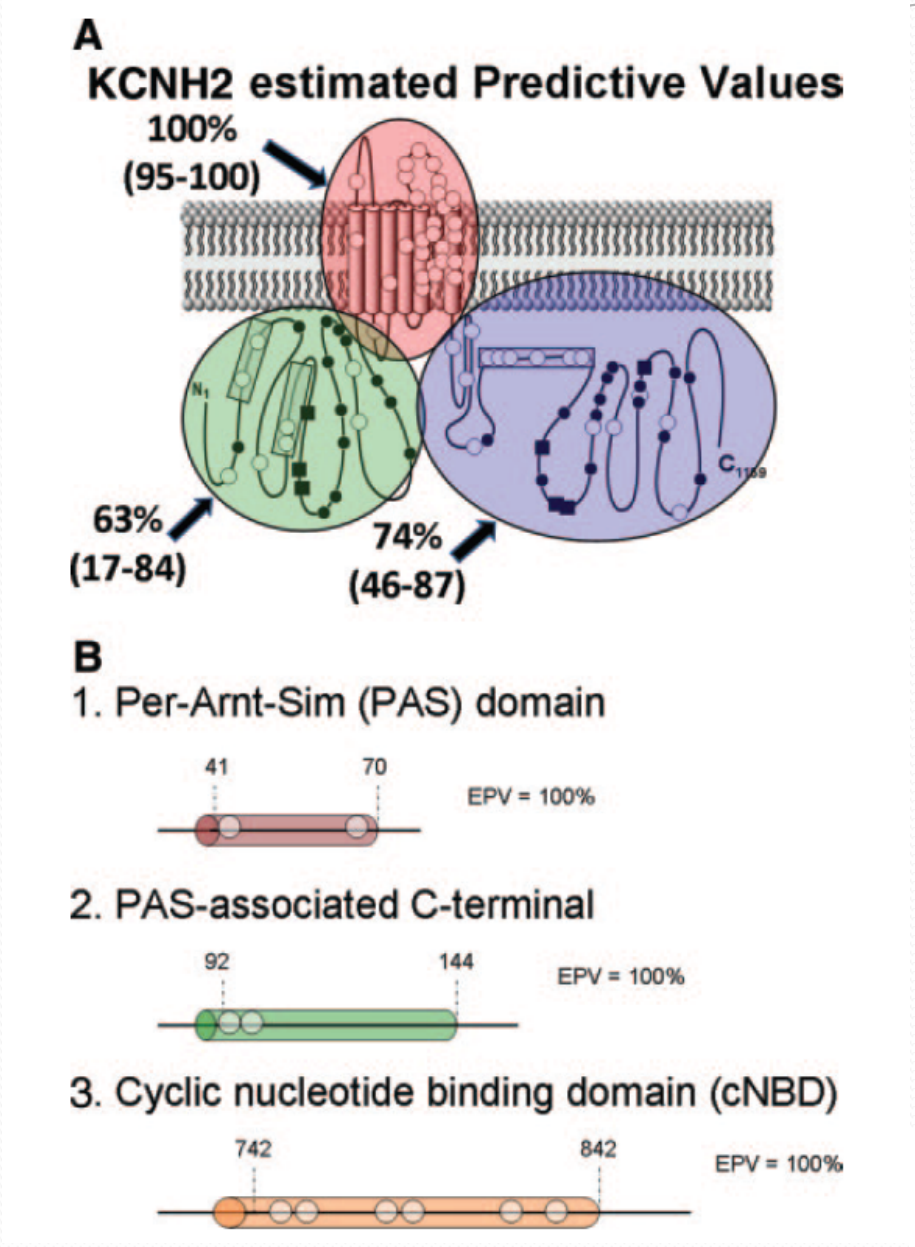
Amino terminus: 1-121
Transmembrane, Linker and Pore: 122-348
C-terminus: 349-676

Wu et al. Molecular pathogenesis of long QT syndrome type 1. Journal of Arrhythmia. 2016;32: 381–388

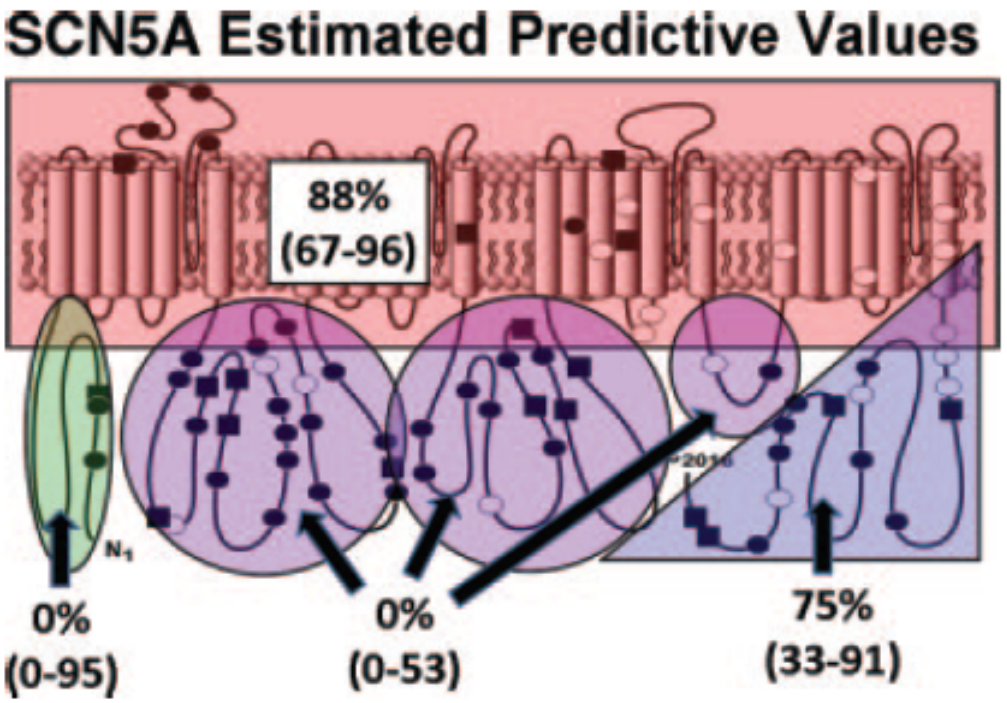
Pathogenicity prediction for Missense Mutations in KCNQ1



Pathogenicity prediction for Missense Mutations in KCNH2

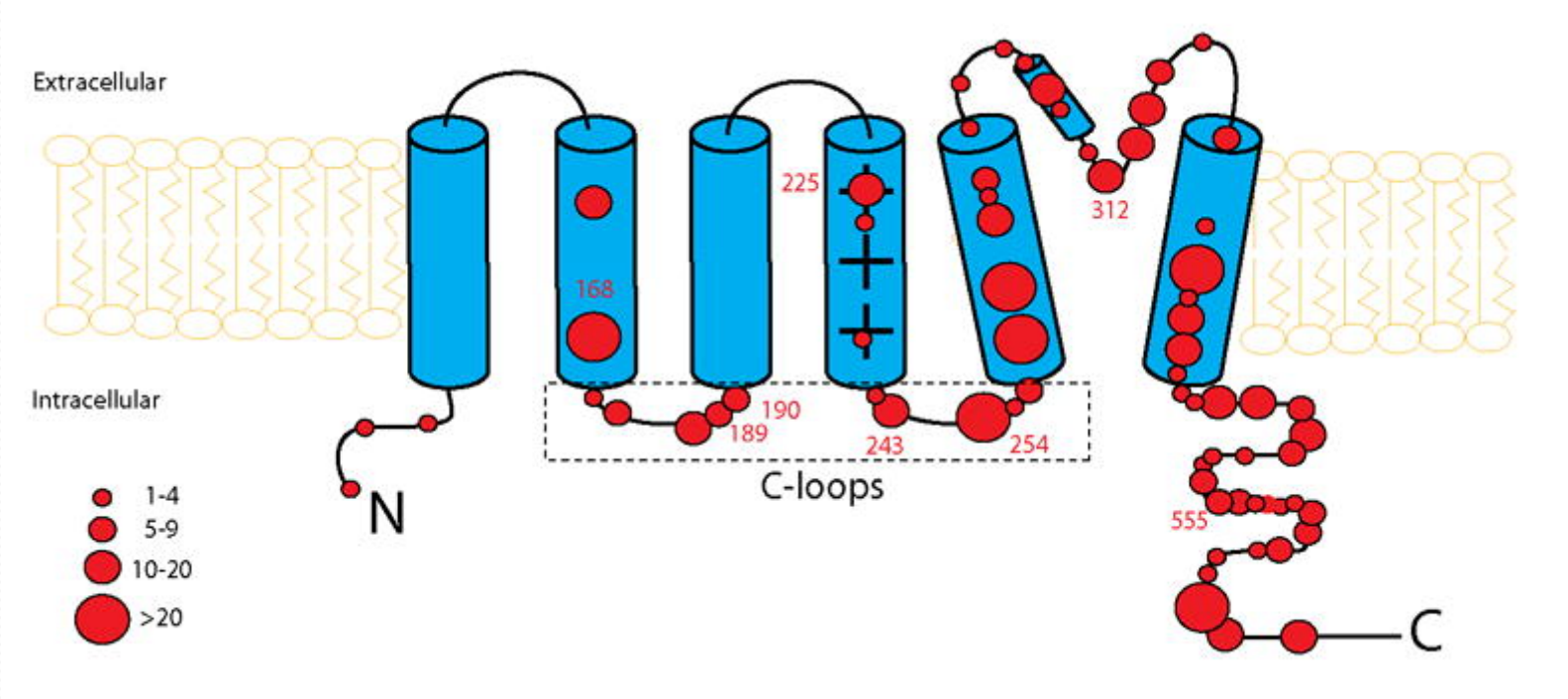


Pathogenicity prediction for Missense Mutations in SCN5A

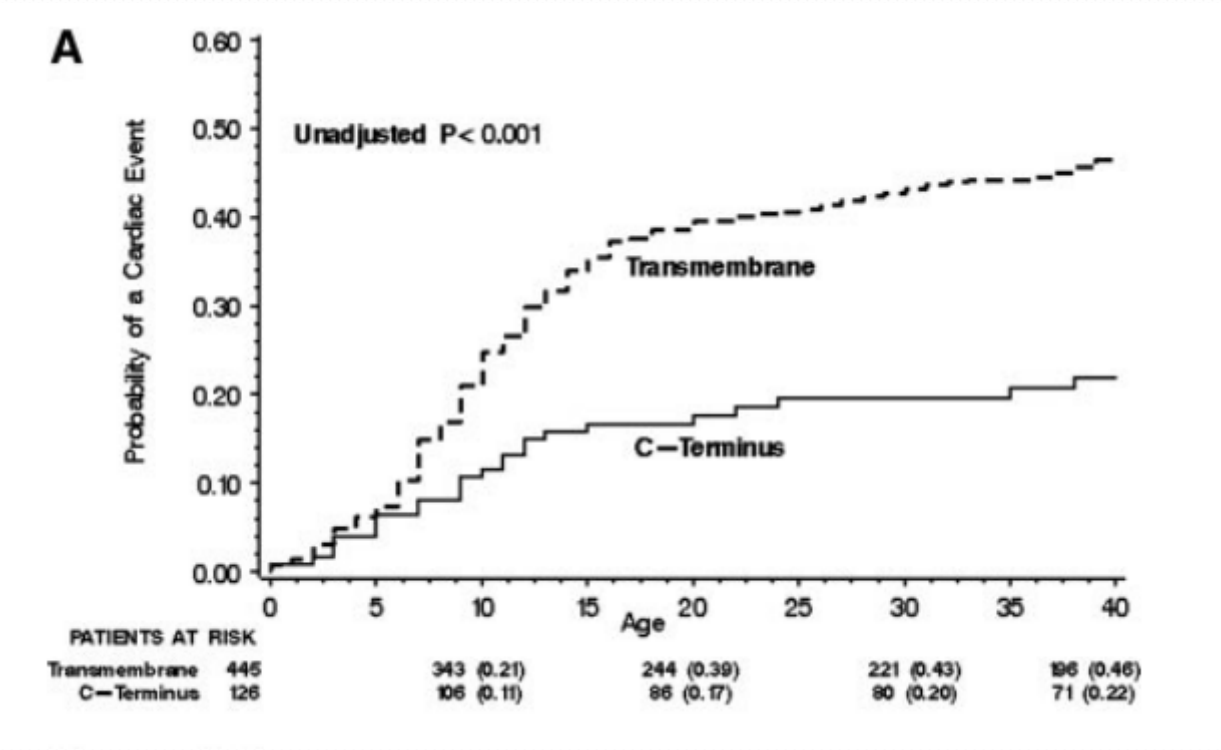


Transmembrane and Linker Region: EPV 88%

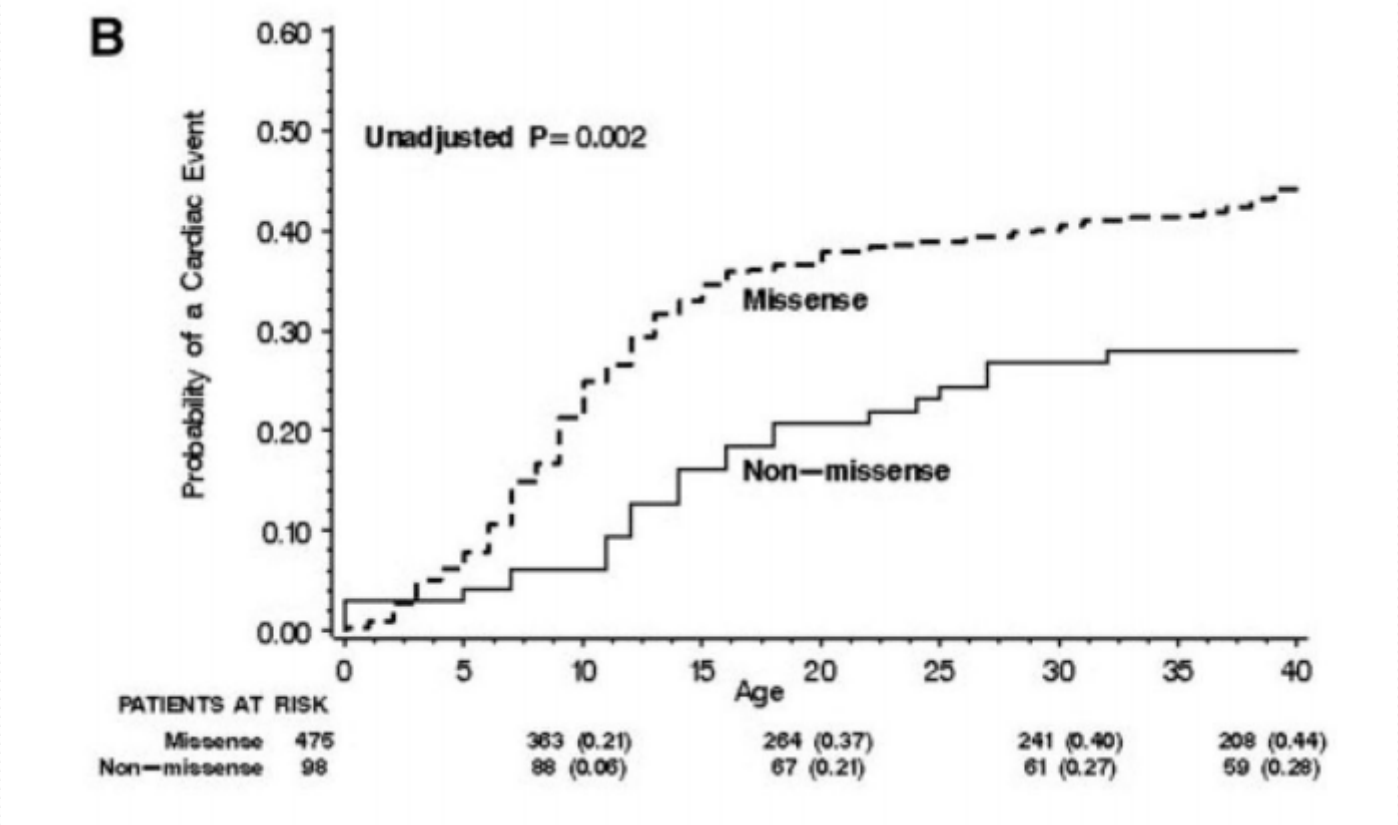
C-loop Mutations in KCNQ1 gene are more pathogenic



Transmembrane vs C-Terminus Mutations in KCNQ1

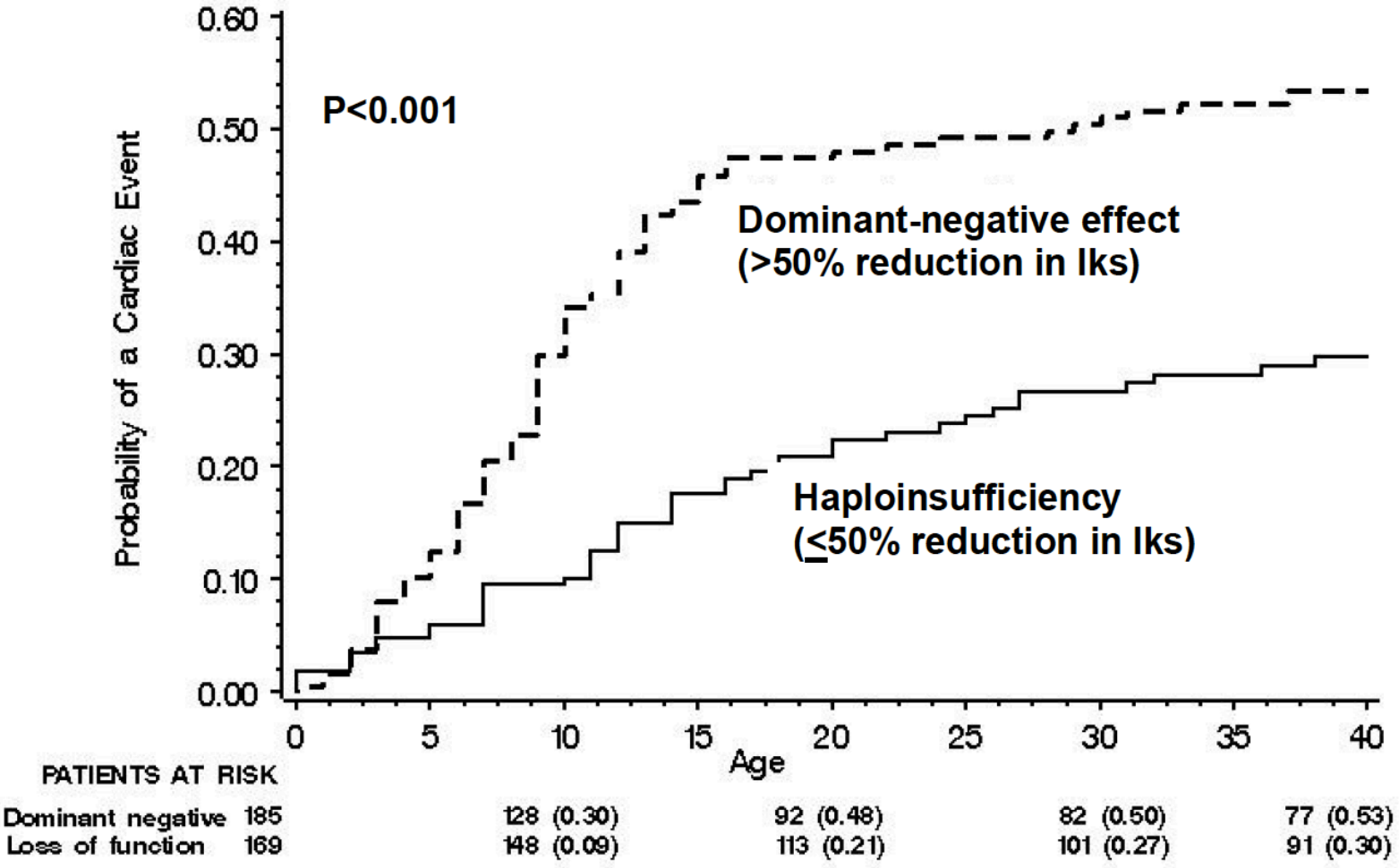


Missense vs Non-Missense Mutations in KCNQ1



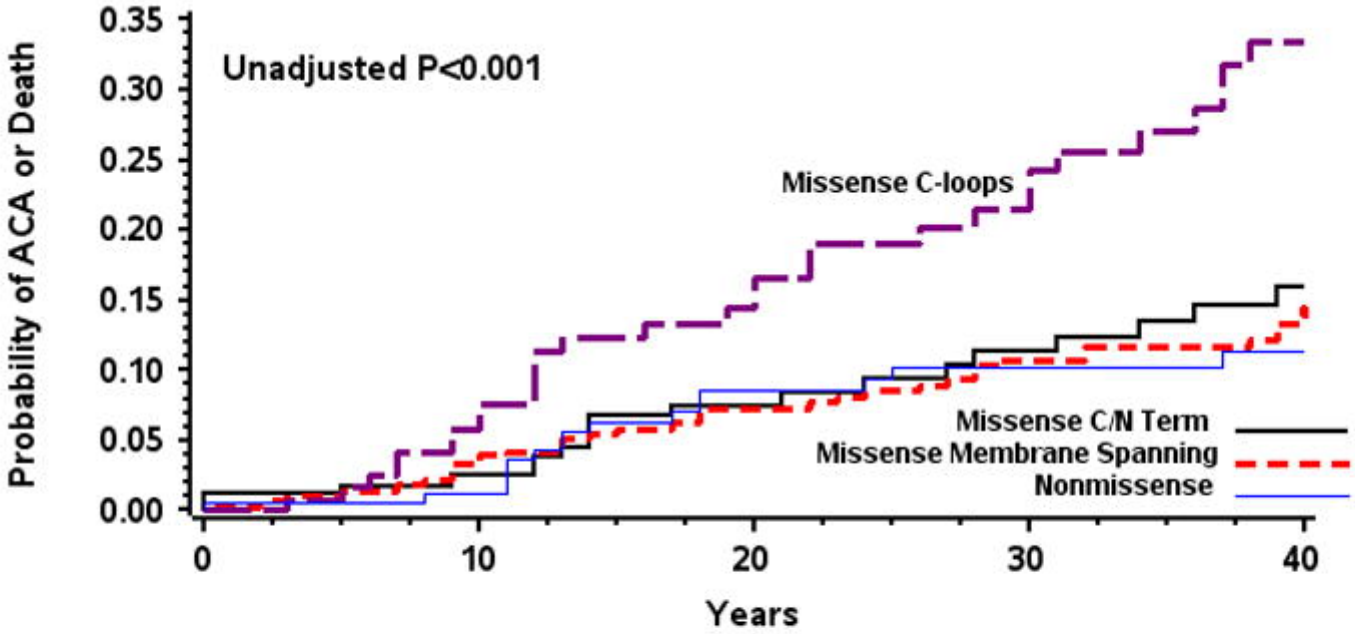
Moss et al. Circulation. 2007;115:2481-2489

Dominant negative vs Haploinsufficient Mutations in KCNQ1



Moss et al. Circulation. 2007;115:2481-2489

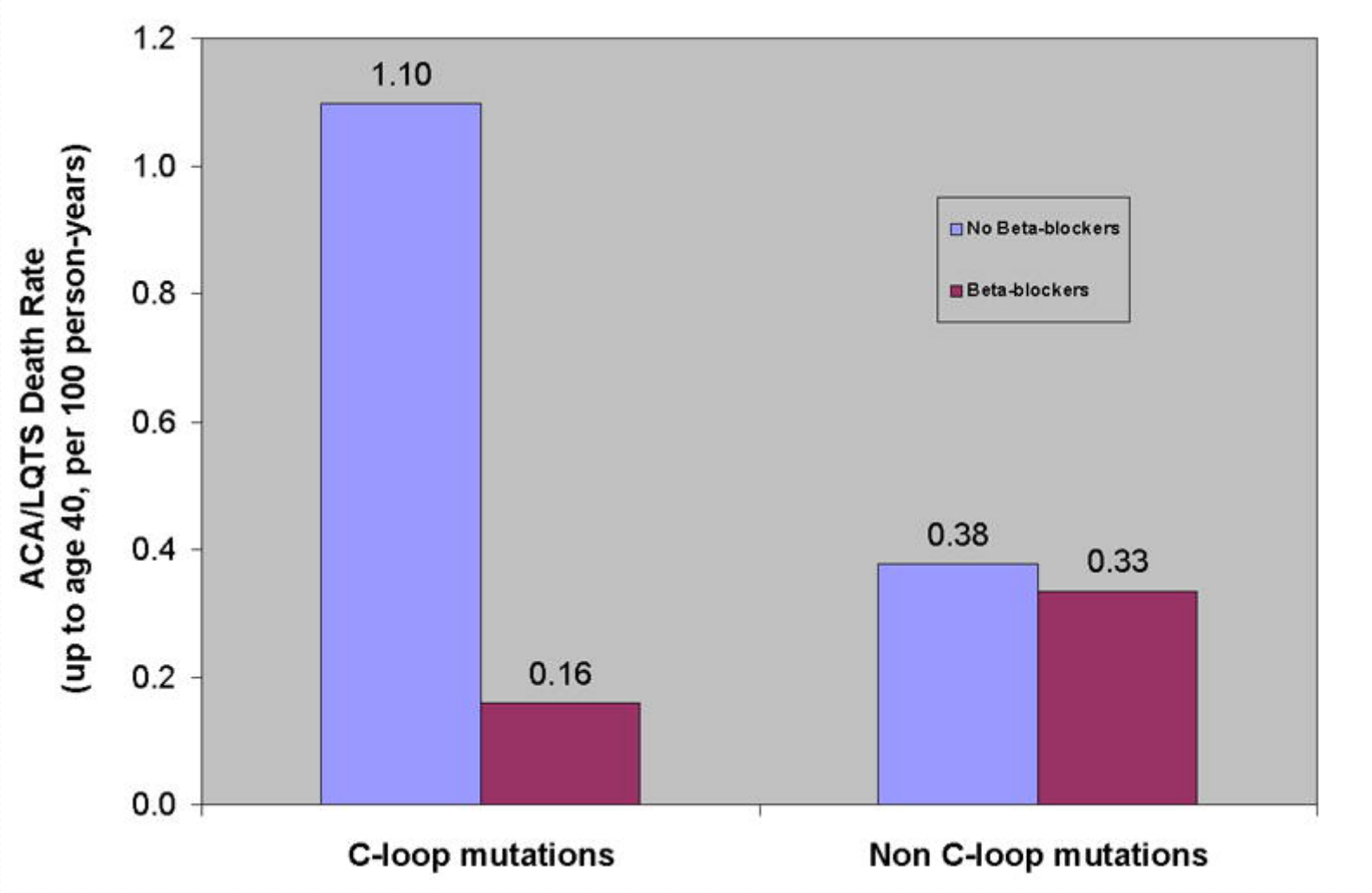
C-loop Mutations in KCNQ1 gene are more pathogenic



Patients at Risk					
C/N Term	172	145 (0.02)	108 (0.07)	88 (0.11)	62 (0.16)
Membr Spanning	376	328 (0.03)	242 (0.07)	192 (0.11)	154 (0.13)
C-loops	125	109 (0.06)	78 (0.14)	59 (0.22)	39 (0.33)
Nonmissense	186	157 (0.01)	119 (0.09)	95 (0.10)	69 (0.11)

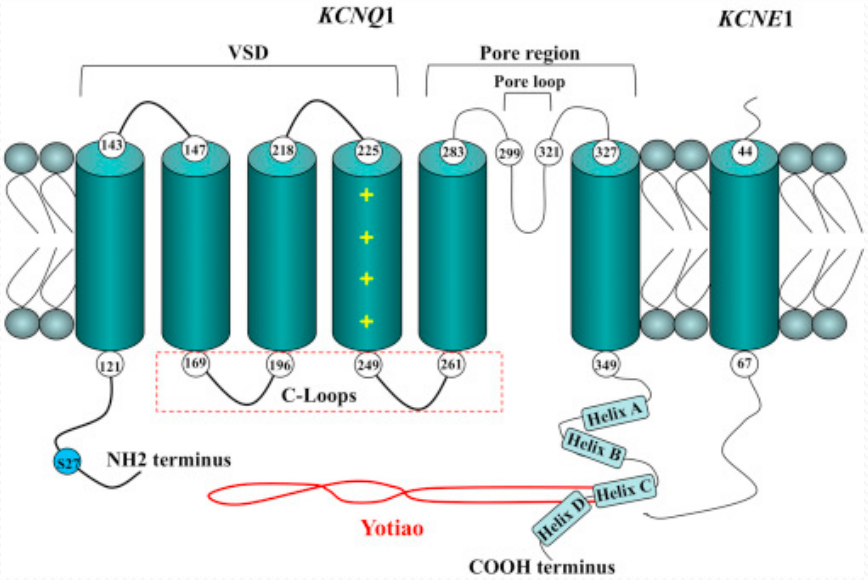
Barsheshet et al. Circulation. 2012; 125: 1988-1996

Risk for life-threatening cardiac events by mutation location and β-blocker treatment



Barsheshet et al. Circulation. 2012; 125: 1988-1996

Beta adrenergic stimulation and Protein kinase A mediated phosphorylation



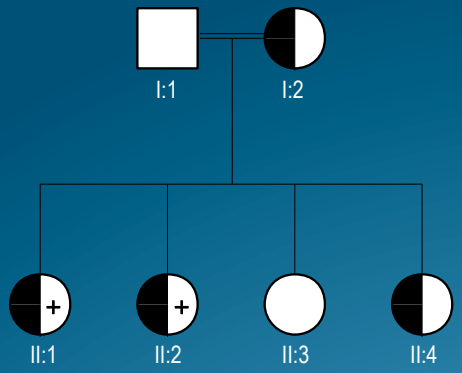
Beta-1 adrenergic receptor activation leads to activation of Protein Kinase A (PKA)

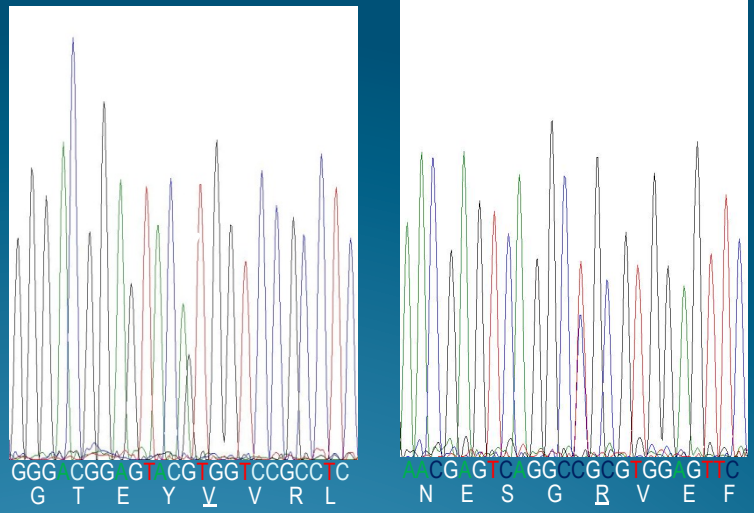
↓

PKA phosphorylates the KCNQ1 subunit and increases the IKs function

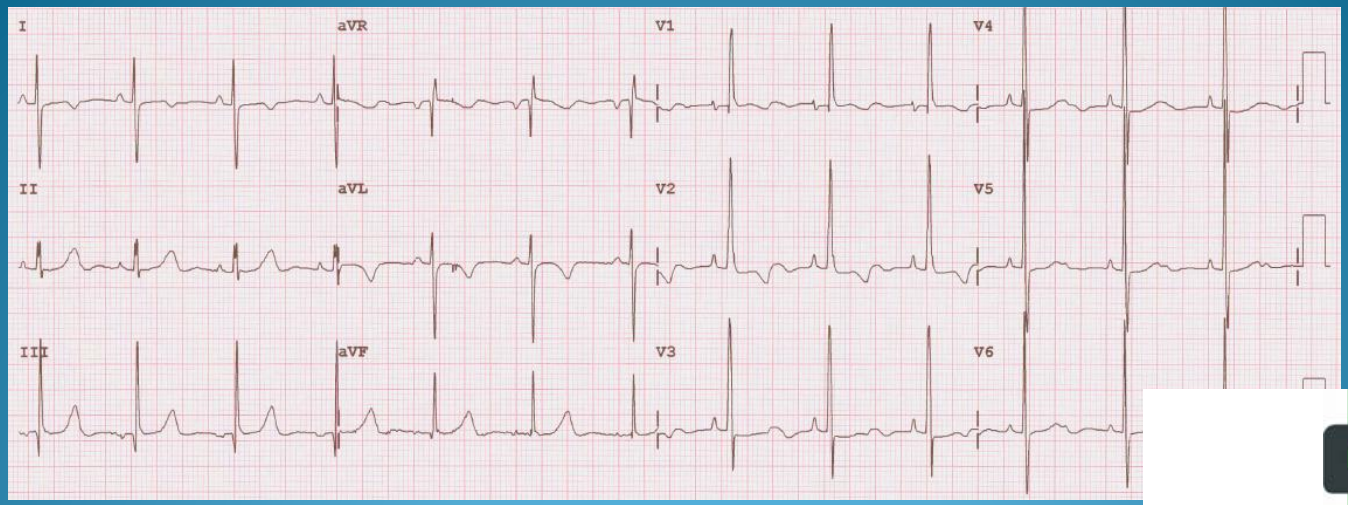
Wu et al. Molecular pathogenesis of long QT syndrome type 1. Journal of Arrhythmia. 2016;32: 381–388

Phenotype is Extremely Important: Not all pathogenic mutations are pathogenic

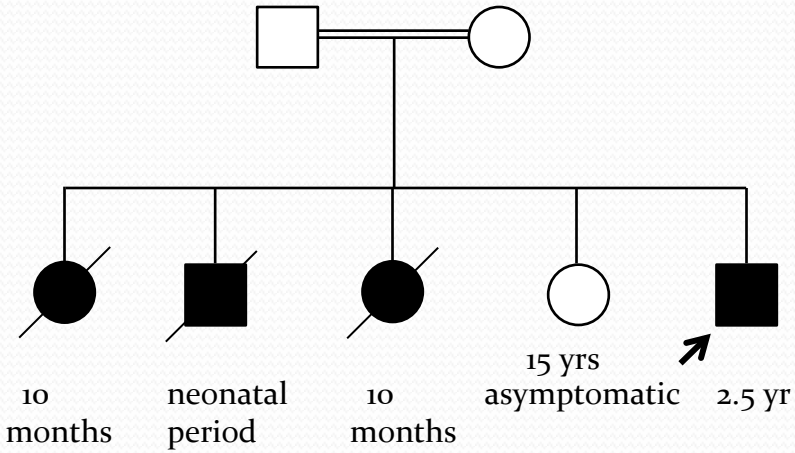




KCNQ1 gene



Family Pedigree



ECG of the Proband

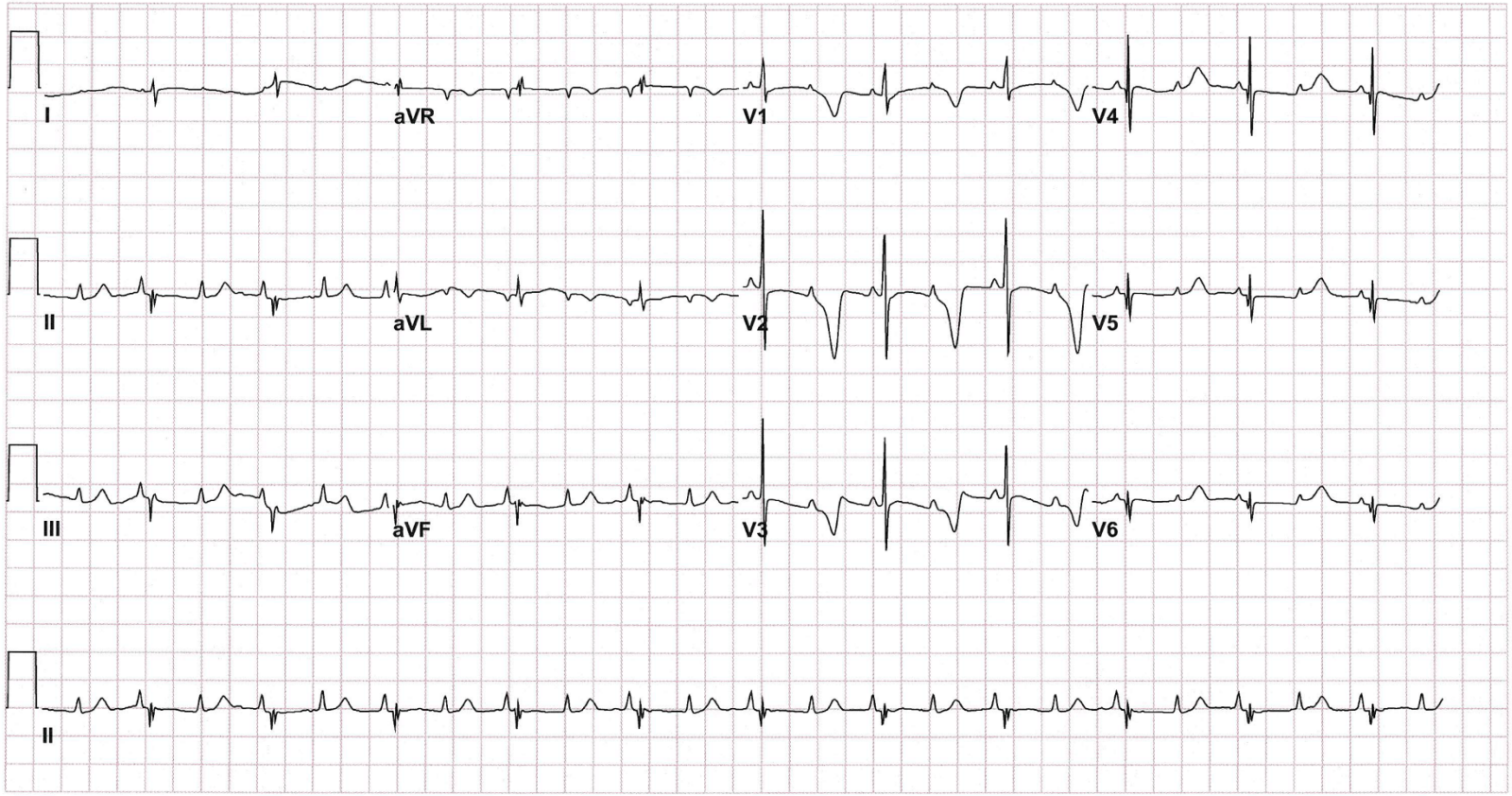
ID:2556797 23-MAY-2014 13:15:42 KING ABDULAZIZ CARDIAC CENTER

19-MAY-2014 (4 days)	Vent. rate	139	BPM	** ** ** ** * Pediatric ECG Analysis * ** ** ** **
Male Unknown	PR interval	116	ms	Sinus rhythm with Fusion complexes
	QRS duration	142	ms	Right atrial enlargement
Room:PCICU10	QT/QTc	332/505	ms	Left bundle branch block
Loc:29	P-R-T axes	83 89	27	No previous ECGs available

Technician:MARTHA BACOLOD
Test ind:PRE OP

Referred by: PEDS Confirmed By: : :

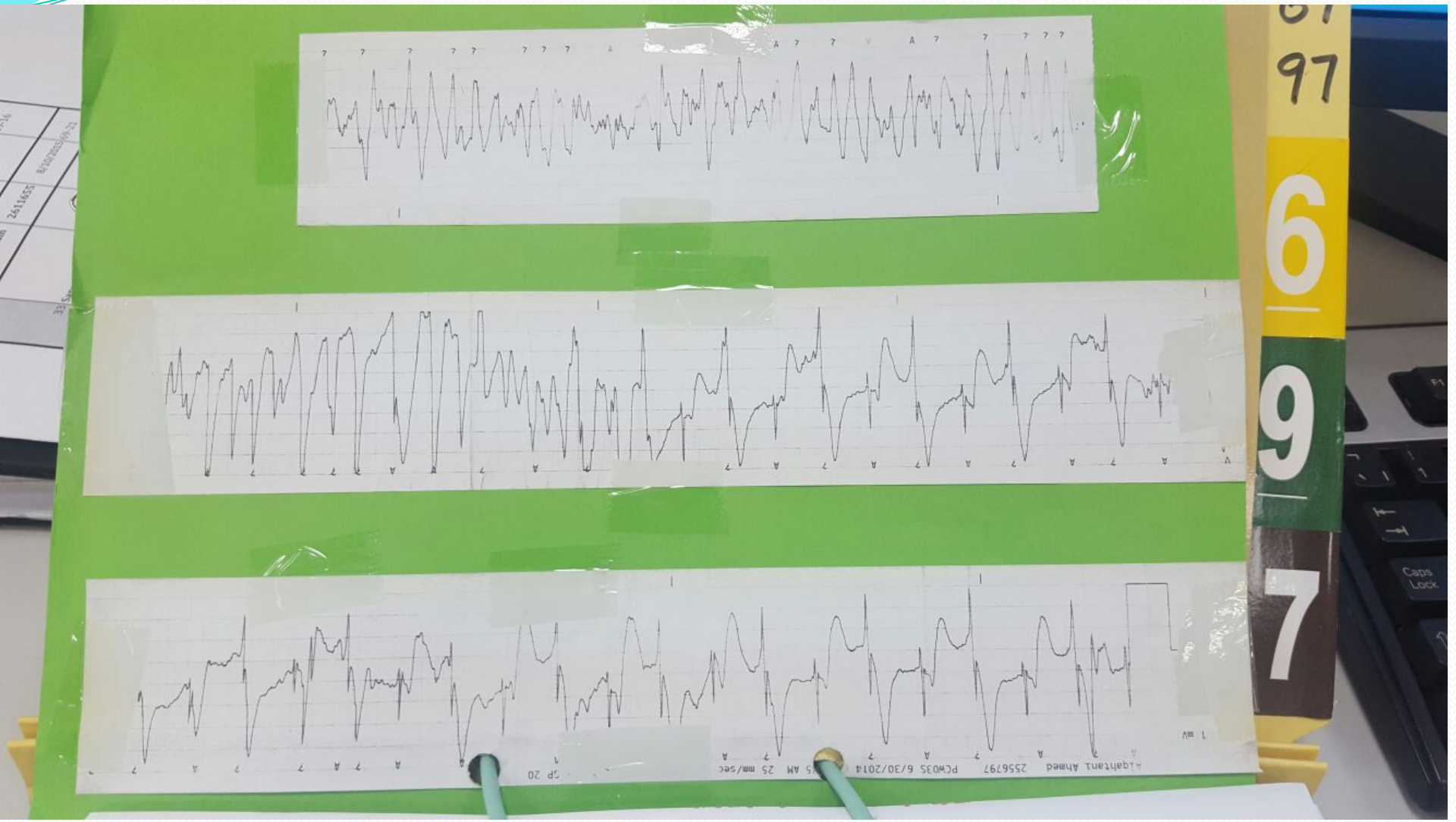
COMMENT: ECG 55-17:Yes



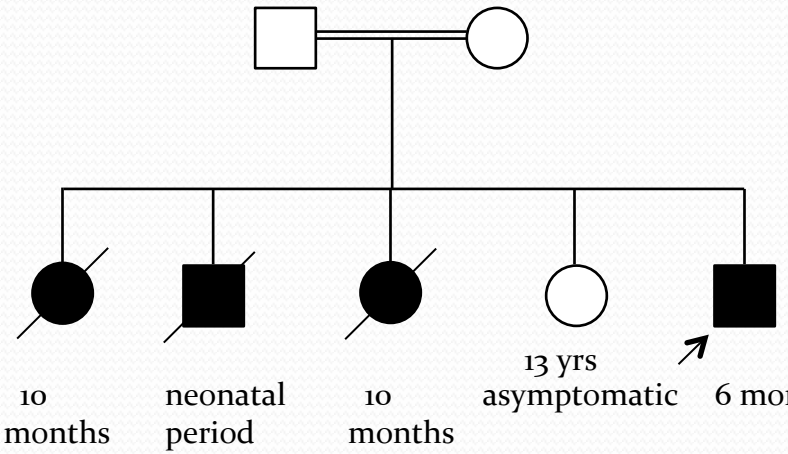
25mm/s 10mm/mV 40Hz 7.1.1 12SL 239 CID: 167

EID:222 EDT: 16:14 23-MAY-2014 ORDER: ACCOUNT: 2556797

ECG of the Proband



Dark Side of the Moon

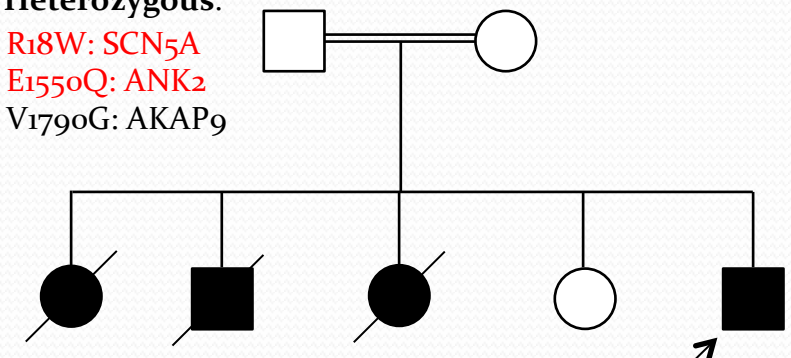


Heterozygous:

R18W: SCN5A
E1550Q: ANK2
V1790G: AKAP9

Dark Side of the Moon

Heterozygous:
R18W: SCN5A
E1550Q: ANK2
V1790G: AKAP9

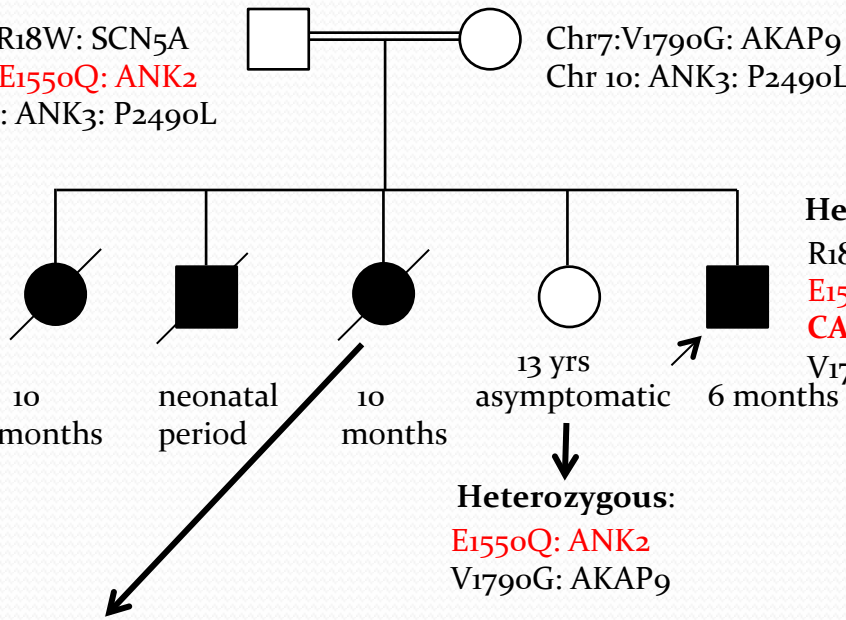


Heterozygous:
R18W: SCN5A
E1550Q: ANK2
V1790G: AKAP9

Moon Explored

Heterozygous:
Chr3: R18W: SCN5A
Chr4: E1550Q: ANK2
Chr 10: ANK3: P2490L

Heterozygous:
Chr7:V1790G: AKAP9
Chr 10: ANK3: P2490L



Heterozygous:
R18W: SCN5A
E1550Q: ANK2
CALM3: D130G
V1790G: AKAP9

Homozygous:
ANK3: P2490L
CCDC154: R386X

Heterozygous:
E1550Q: ANK2
V1790G: AKAP9

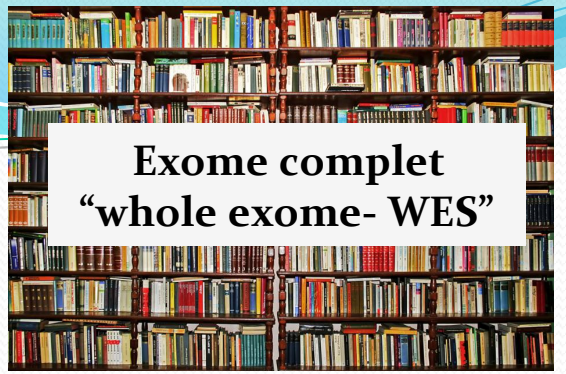
Heterozygous:
ANK2: E1550Q
CALM3: D130G
ANK3: P2490L

Homozygous:
Chr 10: ANK3: P2490L
Chr 16: CCDC154: R386X

Calm3 is Germline mosaic in sperm or oocytes

Exome clinique panels commerciaux

Analyse *in silico*
Panel de gènes selon la clinique



Exome complet “whole exome- WES”

- **Programmes commerciaux**
- **Pipeline bioinformatique “maison”** (basé sur des algorithmes “open source”)
- **Externaliser l’analyse (?)**

Données brutes FASTQ

…
ACGTTGC
A… ACGTTGC
A… ACGTTGC
A… ACGTTGC
A… ACGTTGC
A… ACGTTGC
A… ACGTTGC
A…

1. <u>Alignement</u> des séquences (BAM)
2. <u>Identification</u> des variants dans le panel de gènes

AGGTCGTTACGTACGCTAC
GACCTACATCAGTACATAG
GCATGACAAAGCTAGGTGT

Annotation et filtration

dbSNP, HGMD®, In house db, ClinVar, 1000 Genomes A Deep Catalog of Human Genetic Variation, SIFT, PolyPhen-2, ExAC

et plus….

- **qualité**
- **fréquence**
- **type de variant**
- **……**

Nr de variants

Variant(s) pathogène(s) ?

Classification, lien clinique

Rapport FAMH
DIE MEDIZINISCHEN LABORATORIEN DER SCHWEIZ
LES LABORATOIRES MÉDICAUX DE SUISSE
I LABORATORI MEDICI DELLA SVIZZERA

guidelines

généticien

Clinicien spécialisé

FAMH Expert d’laboratoire

Bioinformaticien

Conclusion

- Clinical Phenotype, Family History for the Disease, ECG phenotype, QTc interval
- Main Genes: KCNQ1, KCNH2, SCN5A
- Multiple Genes might be involved, which has to be considered
- Mutation severity should be evaluated
- Location of the Mutation is Important
- Electrophysiology data, In vitro functional data, Animal data is important.
- Overinterpretation could be dangerous


Thank you