GENETIC AND ACQUIRED CAUSES OF SUDDEN DEATH

Jeffrey A. Towbin, M.D.

Professor, Pediatric Cardiology and Molecular & Human Genetics

Baylor College of Medicine

Texas Children's Hospital

Houston, Texas USA

BCM Baylor College of Medicine

Phoebe Willingham Muzzy Pediatric Molecular Cardiology Laboratory

John Welsh Cardiovascular Diagnostic Laboratory

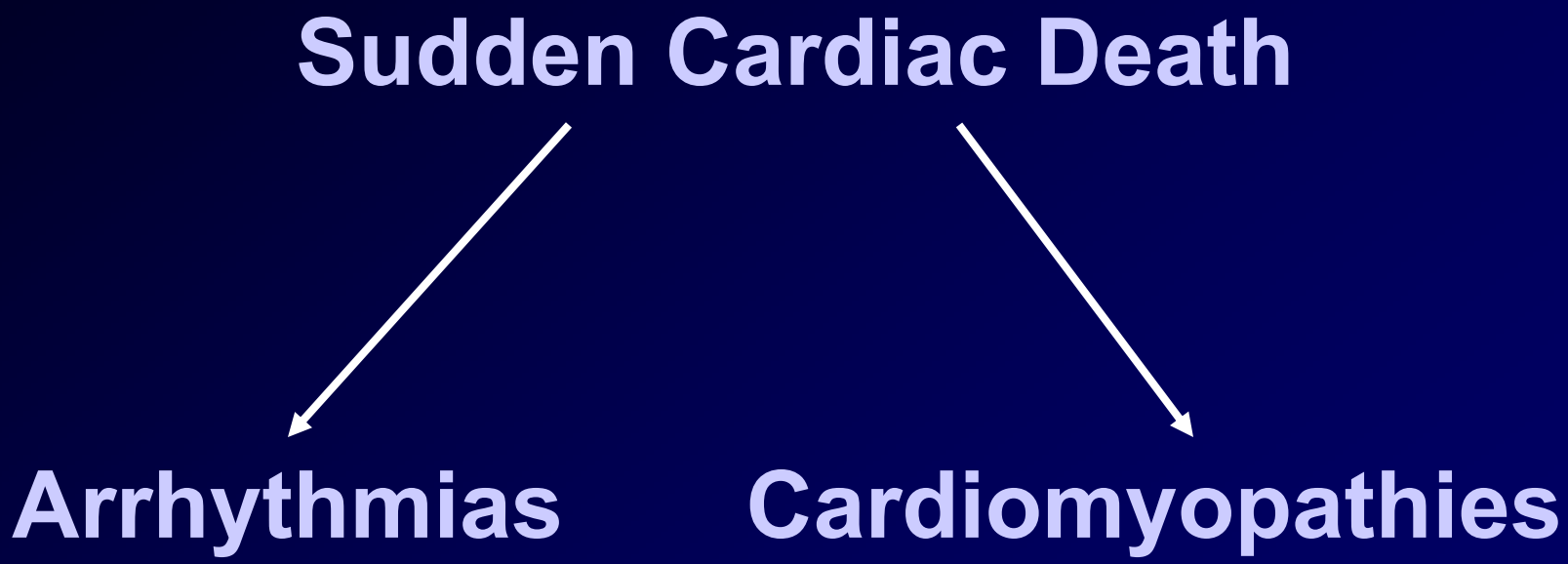
Texas Children's Hospital

HEART DISEASE IN CHILDREN

SUDDEN CARDIAC DEATH

- >300,000 sudden deaths yearly in U.S.
- Commonly occurs in young, healthy individuals
- May occur in 2 or more family members
- Typically no prior symptoms

CAUSES OF SUDDEN DEATH



ETIOLOGIES OF SUDDEN CARDIAC DEATH

ARRHYTHMIAS

- Long QT Syndrome
- Brugada Syndrome
- Sudden Infant Death Syndrome (SIDS)
- Conduction System Disease
- Short QT Syndrome

INHERITED LONG QT SYNDROME

- Autosomal Dominant Romano-Ward Syndrome
- Autosomal Recessive Jervell and Lange-Nielsen Syndrome
- Autosomal Dominant Andersen Syndrome

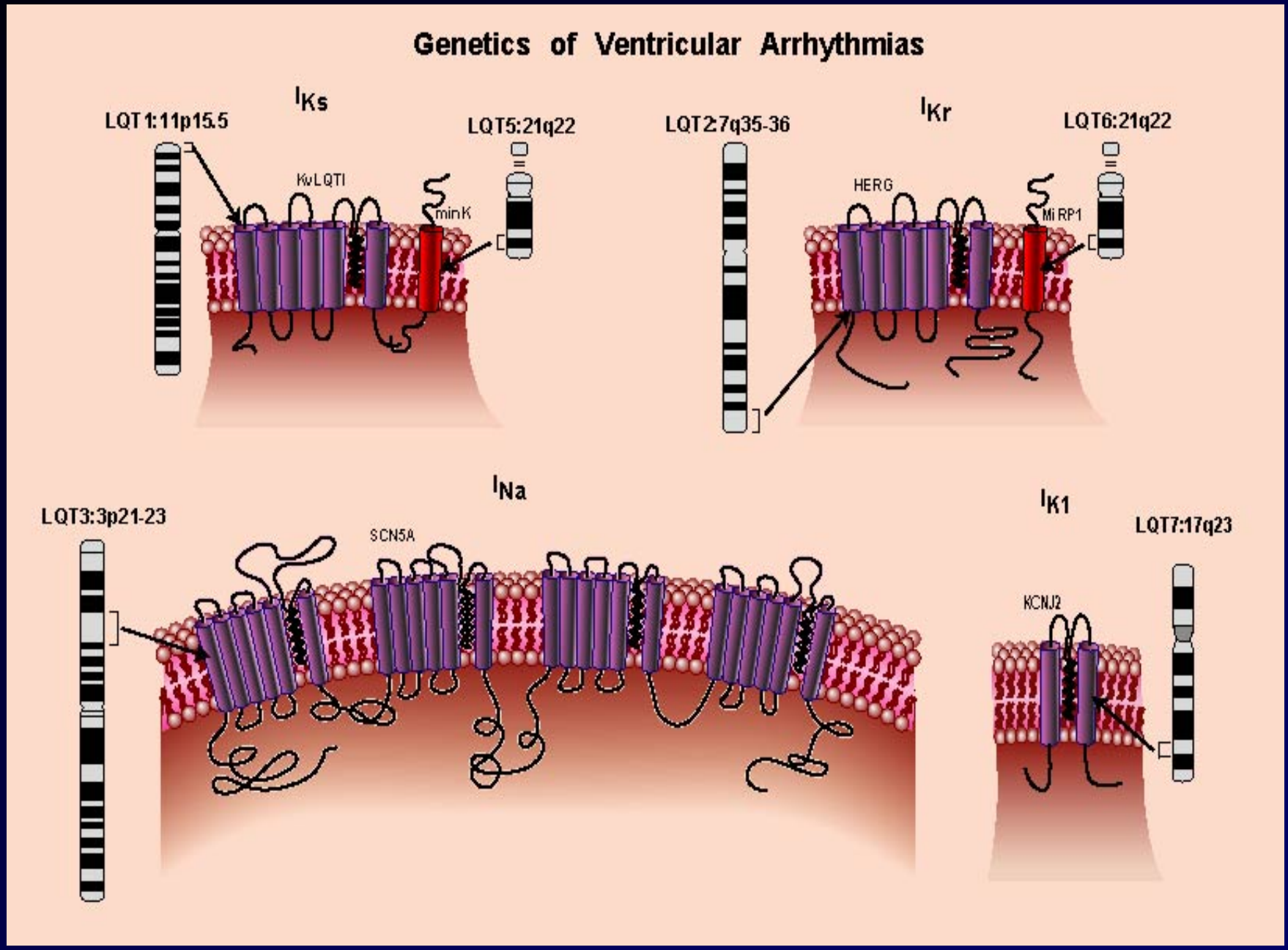
ROMANO-WARD SYNDROME

- Autosomal dominant inheritance
- Most common form of LQTS, 1:5000 live births
- Characterized by QT prolongation, VT or VF, T wave abnormalities, AV block
- Moderate sudden death rate
- Triggered events (emotion, exercise, auditory, sleep)

LQT Syndrome

Romano-Ward Syndrome

Locus Name	Location	Gene Name	Gene Product
LQT1	11p15.5	KCNQ1	K^+-Channel (I_{KS})
LQT2	7q35	HERG	K^+-Channel (I_{Kr})
LQT3	3p21-23	SCN5A	Na^+-Channel (I_{Na})
LQT4	4q25-27	Ankyrin-B	Ankyrin-B
LQT5	21q22	KCNE1	K^+-Channel (I_{KS})
LQT6	21q22	KCNE2	K^+-Channel (I_{Kr})
LQT7	17q23.1-24.2	KCNJ2	K^+-Channel ($I_{Kir2.1}$)

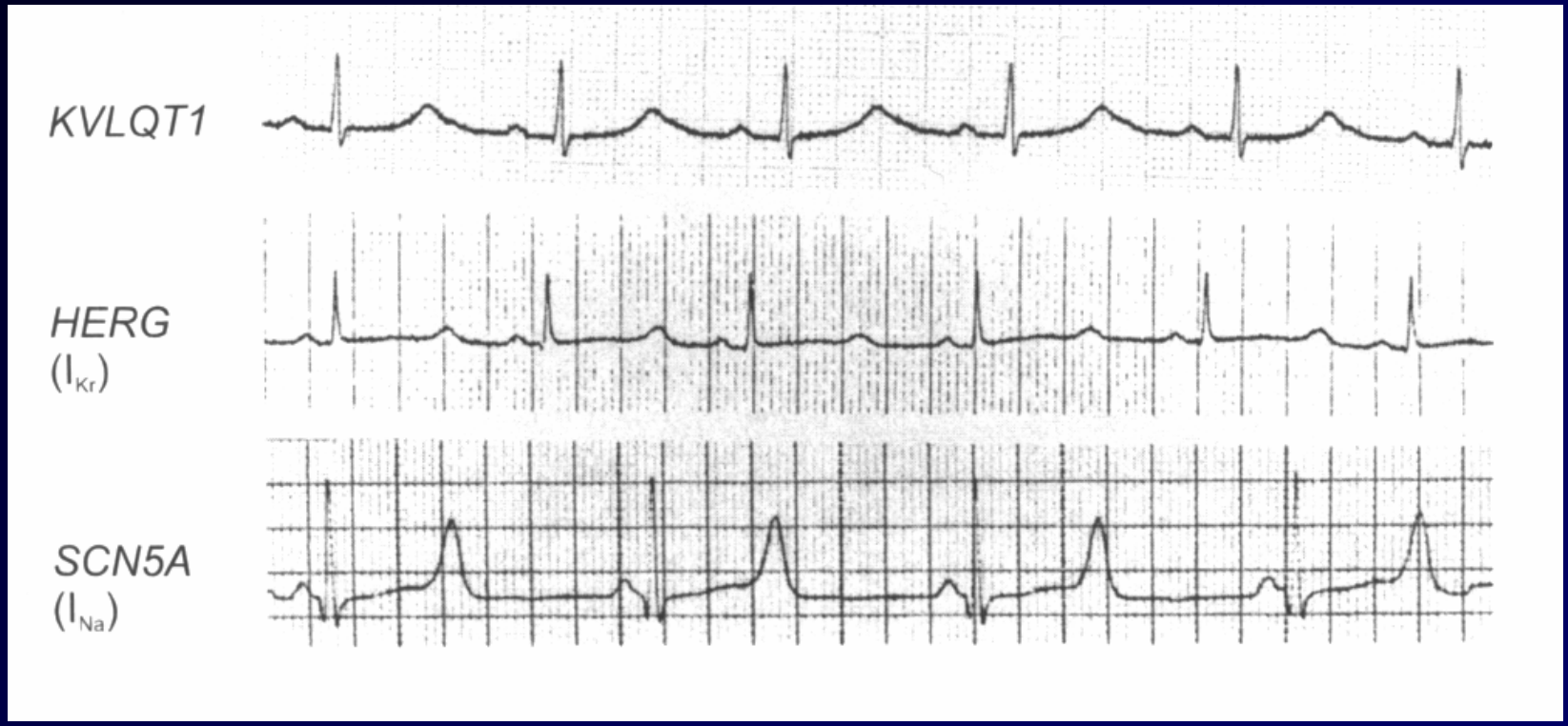

Genetics of Ventricular Arrhythmias
I_{Ks}
LQT1:11p15.5
KvLQTI
minK
LQT5:21q22
I_{Kr}
LQT2:7q35-36
HERG
MiRP1
LQT6:21q22
I_{Na}
LQT3:3p21-23
SCN5A
I_{K1}
KCNJ2
LQT7:17q23

LONG QT SYNDROME

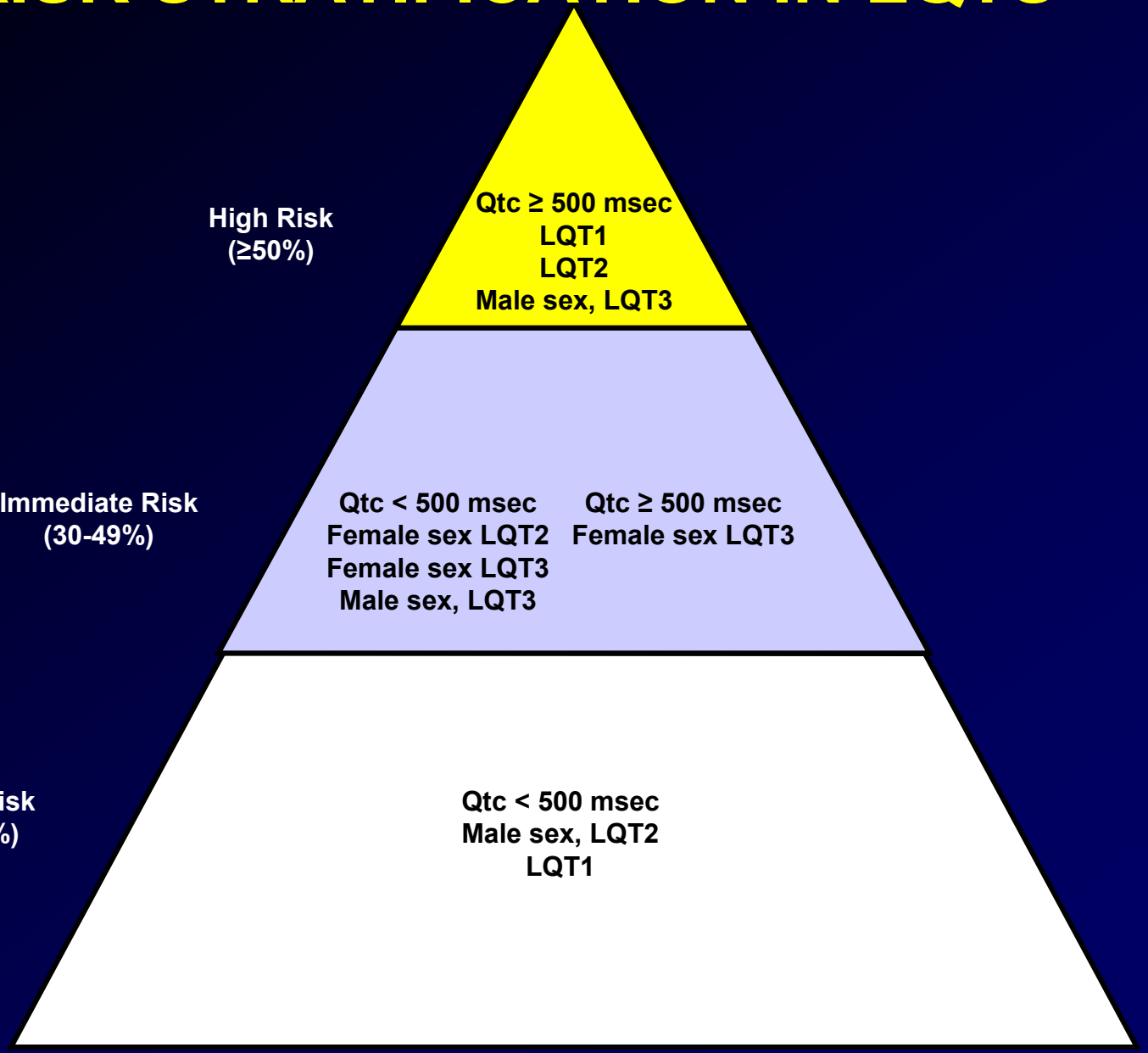
Triggers and Outcomes

LQT TYPE	TRIGGER	OUTCOME
LQT1	Emotion, Exercise, Swimming	Many syncopes, SCD
LQT2	Emotion, Exercise, Auditory	Many syncopes, SCD
LQT3	Sleep	Few syncopes, SCD first event

Long QT Syndrome: Electrocardiograms



RISK STRATIFICATION IN LQTS



Priori et al., NEJM 2003

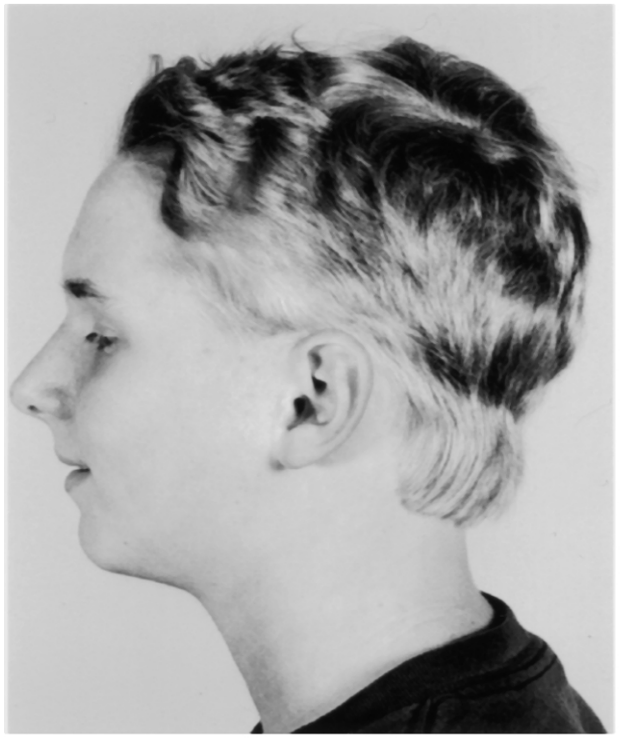
COMPLEX FORMS OF LONG QT SYNDROME

ANDERSEN SYNDROME

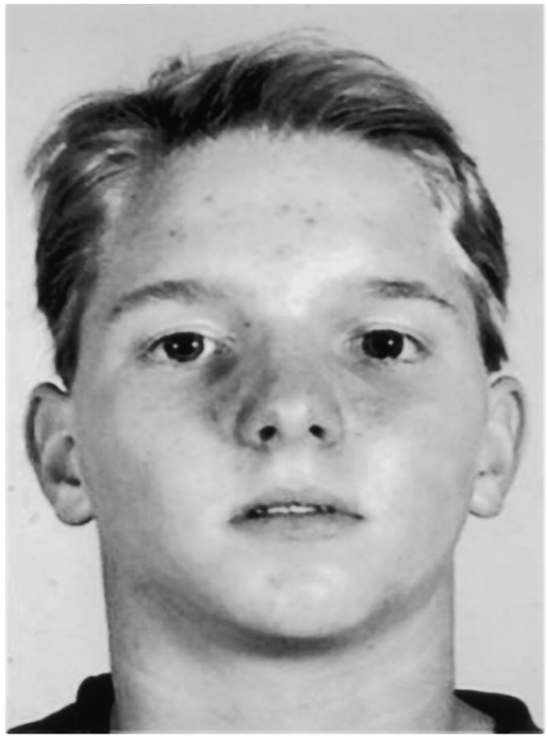
- Autosomal dominant inheritance
- Complex disorder
  - Hypokalemic periodic paralysis
  - Skeletal abnormalities
  - Dysmorphic features
  - QT prolongation, VT or VF
- Low sudden death rate

ANDERSEN SYNDROME

- Short stature
- Scoliosis (curvature of the spine)
- Clinodactyly (permanent lateral or medial curve of a finger or toe)
- Hypertelorism (wide-set eyes)
- Small or prominent ears that are low set or slanted
- Micrognathia (small chin)
- Broad forehead



(A)



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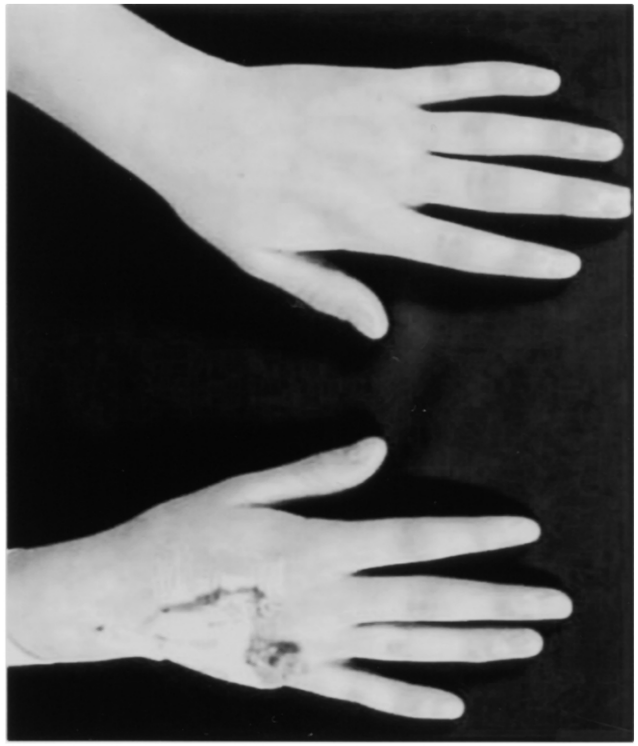

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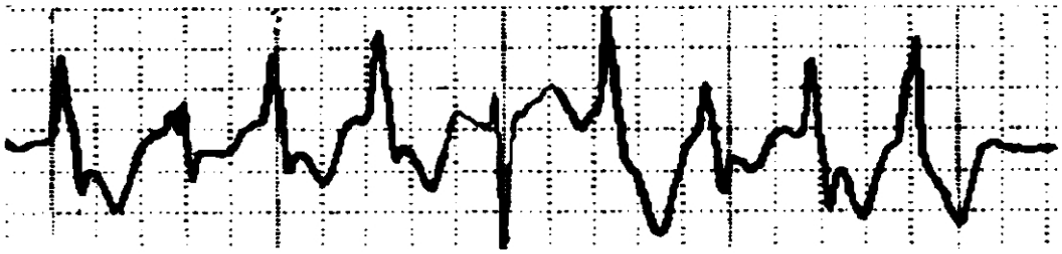
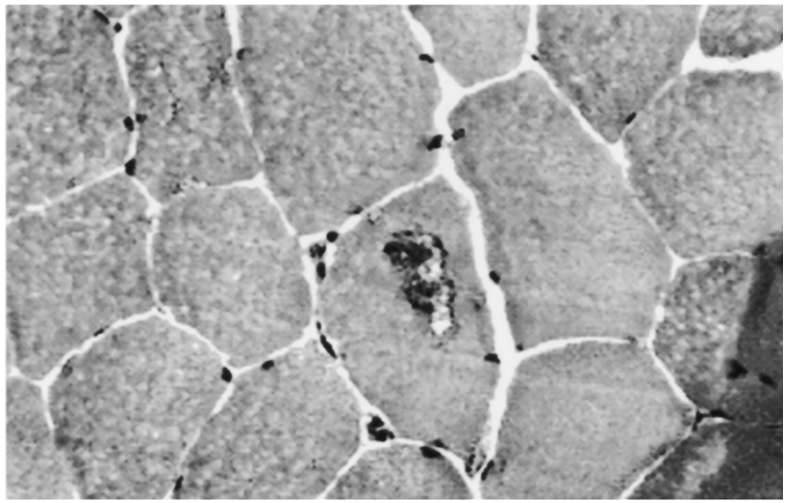


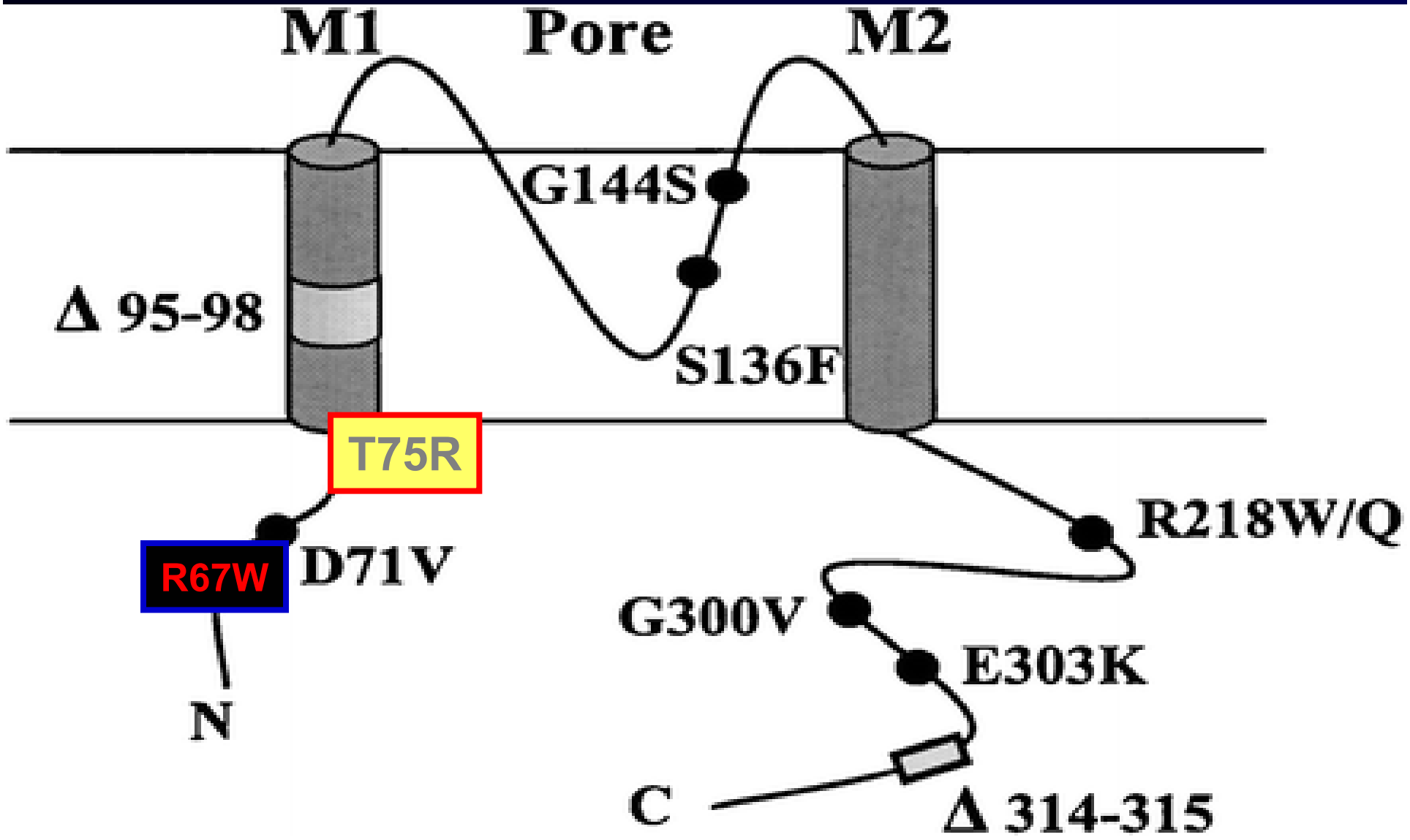
(D)



(E)

Cell 2001

KIR2.1


M1
Pore
M2
G144S
Δ 95-98
S136F
T75R
R218W/Q
R67W
D71V
G300V
E303K
N
C
Δ 314-315

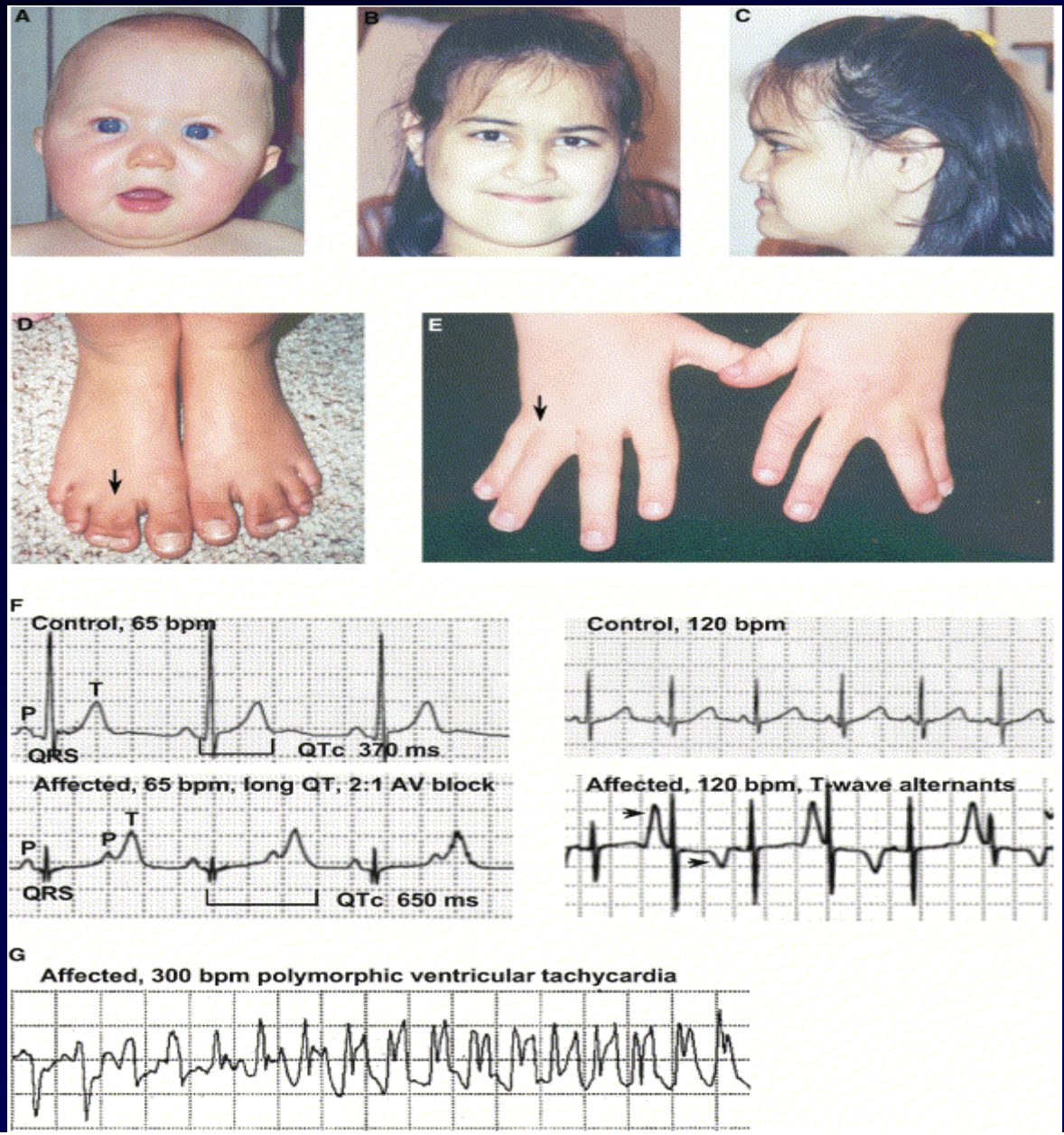
JERVELL & LANGE-NIELSEN SYNDROME

- Homozygous mutations most common
  - KvLQT1 (KCNQ1)
  - minK
- Loss of Iks in heart, inner ear
- Sensorineural hearing loss due to lack of production of K^+-rich endolymph in inner ear which bathes Organ of Corti
- Severe QT prolongation, bad outcome

TIMOTHY SYNDROME

- **Multiorgan Dysfunction Syndrome**
  - Lethal arrhythmias
  - Webbing of fingers, toes
  - Congenital heart disease
  - Immune deficiency
  - Intermittent hypoglycemia
  - Cognitive abnormalties
  - Autism

TIMOTHY SYNDROME



TIMOTHY SYNDROME

- Cardiac L-type Calcium Channel (Ca(V)1.2) Mutations
  - de novo missense mutation (G406R)
  - Expressed in all affected tissues
  - Functional analysis reveals mutant channel produces maintained inward calcium currents by causing nearly complete loss of voltage-dependent channel inactivation
    - Causes intracellular calcium overload, delayed cardiomyocyte repolarization and increased risk of arrhythmia

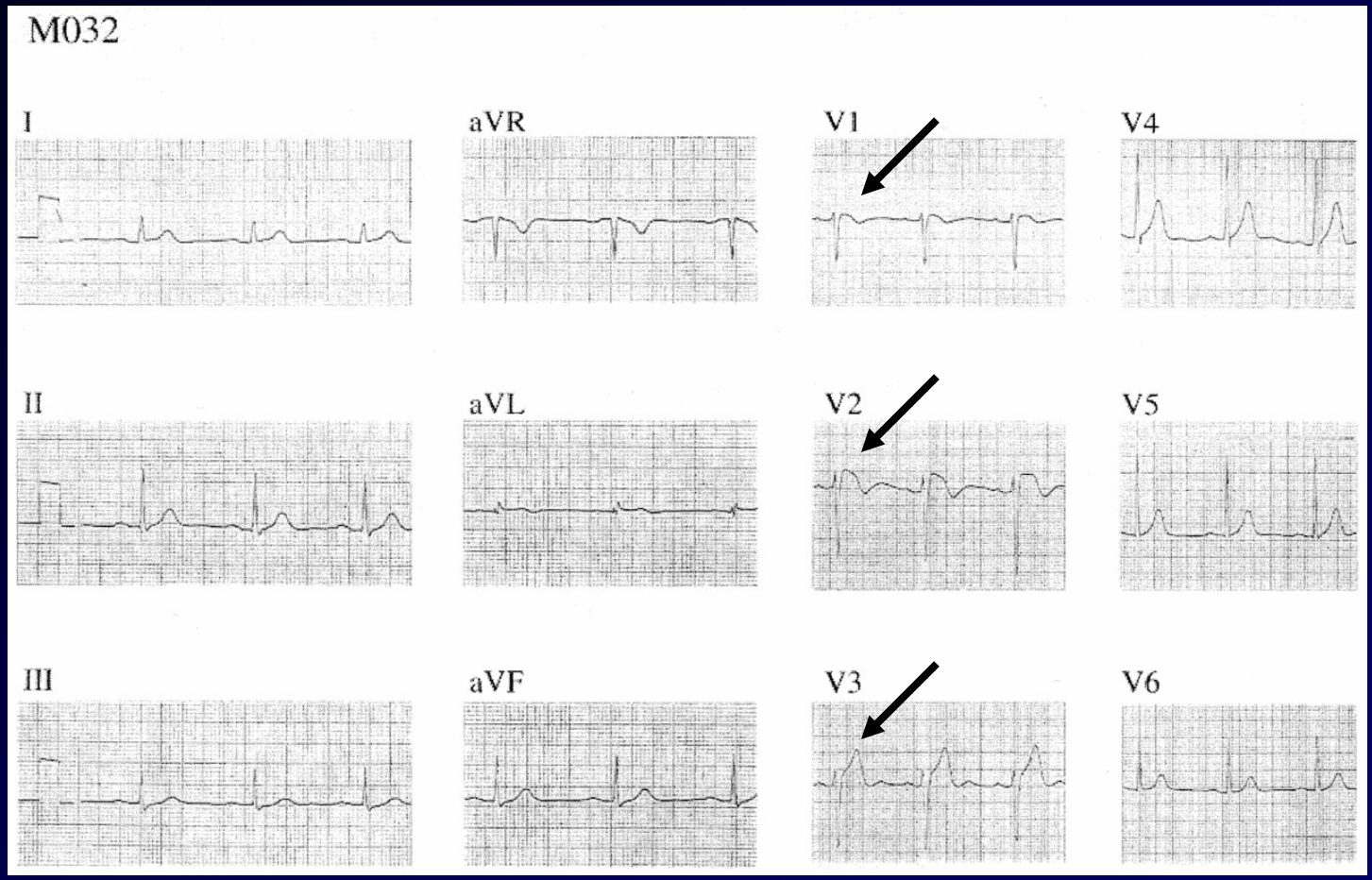
BRUGADA SYNDROME

- Autosomal dominant disorder
- Characterized by sudden death during sleep
- Sudden death most common in males
- Not usually associated by triggered event

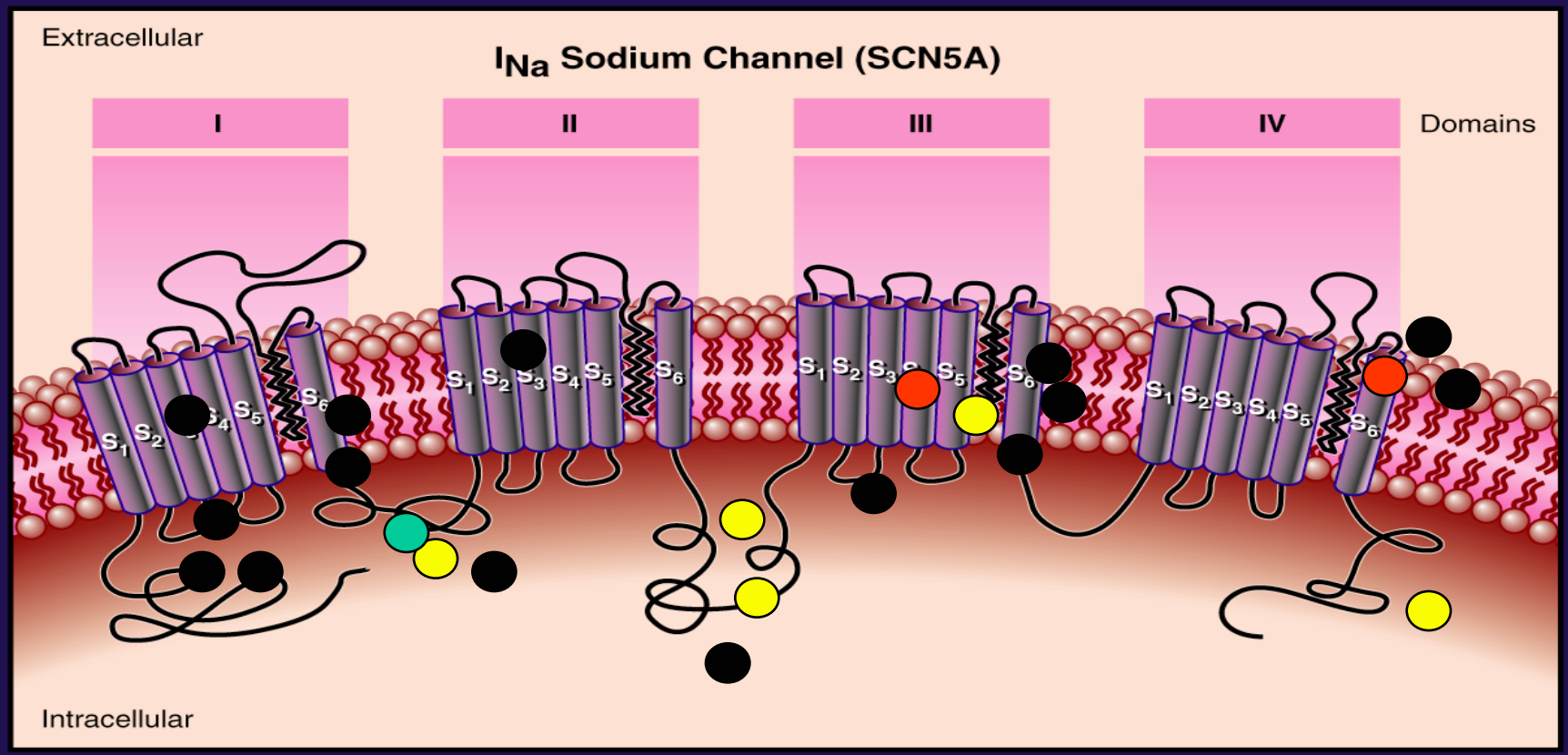
BRUGADA SYNDROME

- Characteristic ECG
  - ST-segment elevation, V_1-V_3
  - Right bundle branch block
  - Episodic VF
  - Normal QT interval
- May appear normal, provoked by ajmaline, flecainide

BRUGADA SYNDROME

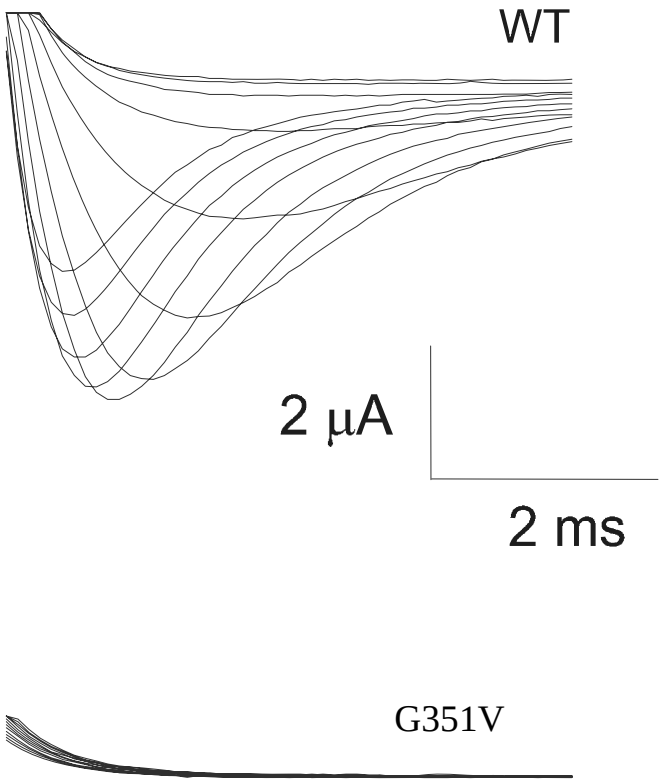


BRUGADA SYNDROME



SIDS Lev syndrome Isolated conduction disease Brugada

BRUGADA SYNDROME



BRUGADA SYNDROME

- CLINICAL HETEROGENEITY OF SCN5A MUTATIONS
  - Long QT Syndrome (LQT3)
  - Brugada Syndrome/SUDS
  - SIDS
  - PCCD/Lenegre/Lev Syndrome
  - Isolated Cardiac Conduction Defect Syndrome

SHORT QT SYNDROME

CLINICAL ASPECTS

- Described by Gussak et al (2000)
- Short QTc (<300 msec)
- Paroxysmal atrial fibrillation
- Short refractory periods
- Sudden death common
- Young age of onset (<1 year of age)

SHORT QT SYNDROME

GENETIC ASPECTS

- Autosomal dominant
- 3 genes identified to date
  - SQT1: HERG/KCNH2
  - SQT2: KVLQT1/KCNQ1
  - SQT3: Kir2.1/KCNJ2
- Gain of function abnormalities

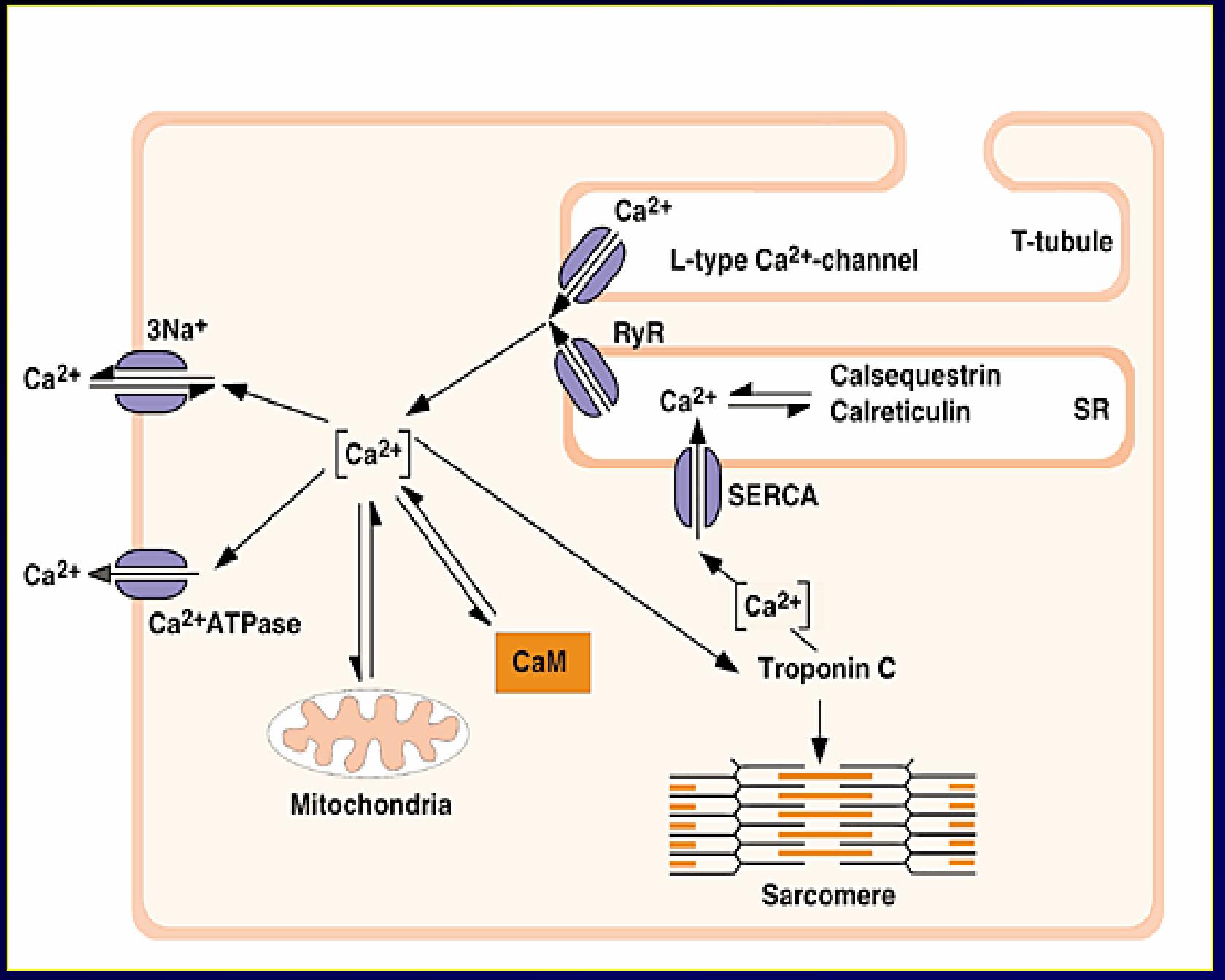
SQT Syndrome

Short QT Syndrome

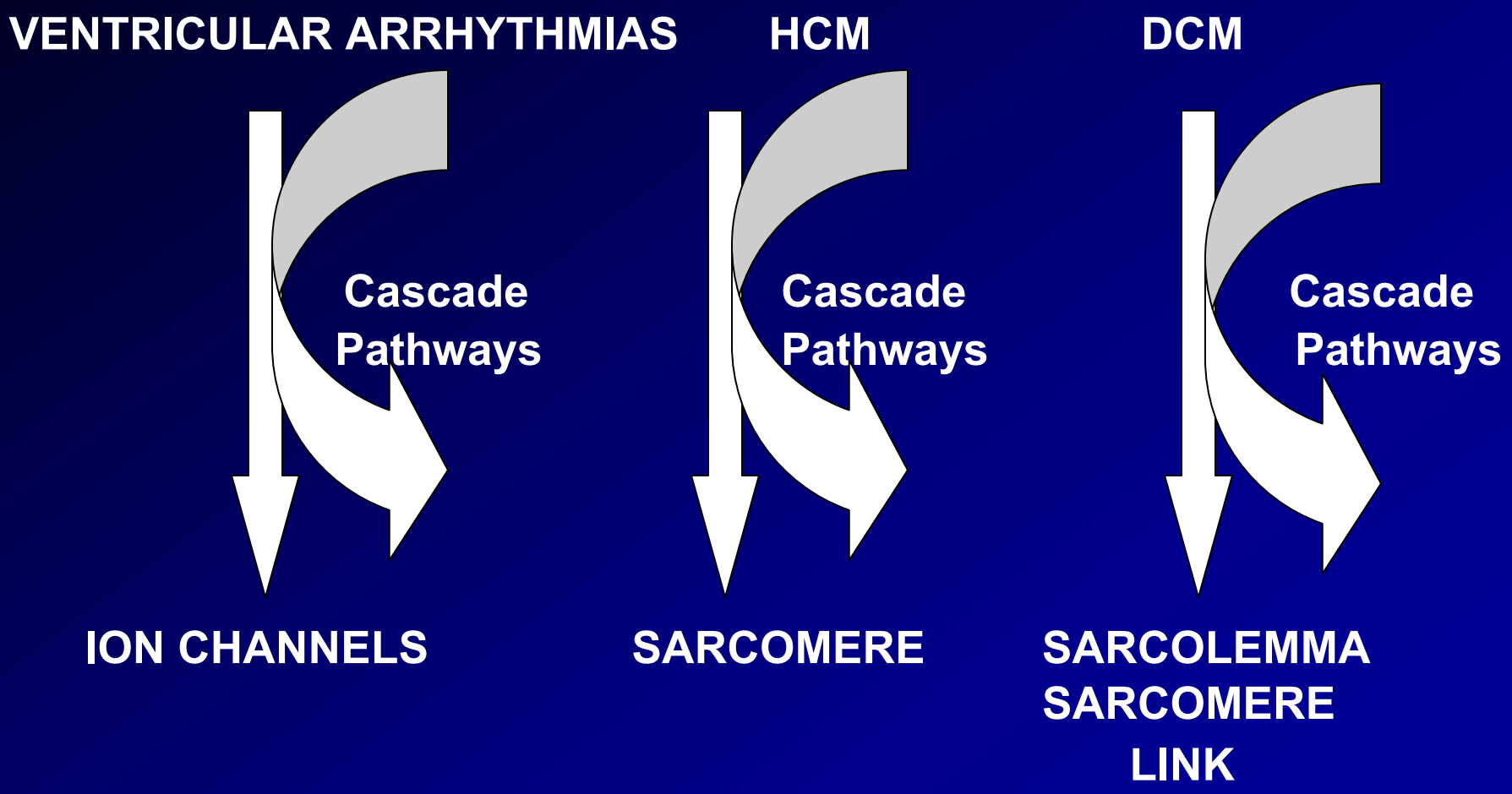
Locus Name	Location	Gene Name	Gene Product
SQT2	11p15.5	KCNQ1	K^+-Channel (I_{KS})
SQT1	7q35	HERG	K^+-Channel (I_{Kr})
LQT3	3p21-23	SCN5A	Na^+-Channel (I_{Na})
LQT4	4q25-27	Ankyrin-B	Ankyrin-B
LQT5	21q22	KCNE1	K^+-Channel (I_{KS})
LQT6	21q22	KCNE2	K^+-Channel (I_{Kr})
SQT3	17q23.1-24.2	KCNJ2	K^+-Channel ($I_{Kir2.1}$)

CATECHOLAMINERGIC POLYMORPHIC VT

- Initially described by Coumel et al (1978)
- Characterized by stress-induced, bidirectional VT that may degenerate into cardiac arrest or sudden death
- No structural heart disease or QT interval prolongation
- VT pattern resembles the arrhythmias associated with calcium overload and the delayed after depolarizations seen with digitalis toxicity


Ca2+
L-type Ca2+-channel
T-tubule
3Na+
Ca2+
RyR
Ca2+
Calsequestrin
Calreticulin
SR
[Ca2+]
SERCA
Ca2+
Ca2+ATPase
[Ca2+]
CaM
Troponin C
Mitochondria
Sarcomere

FINAL COMMON PATHWAYS



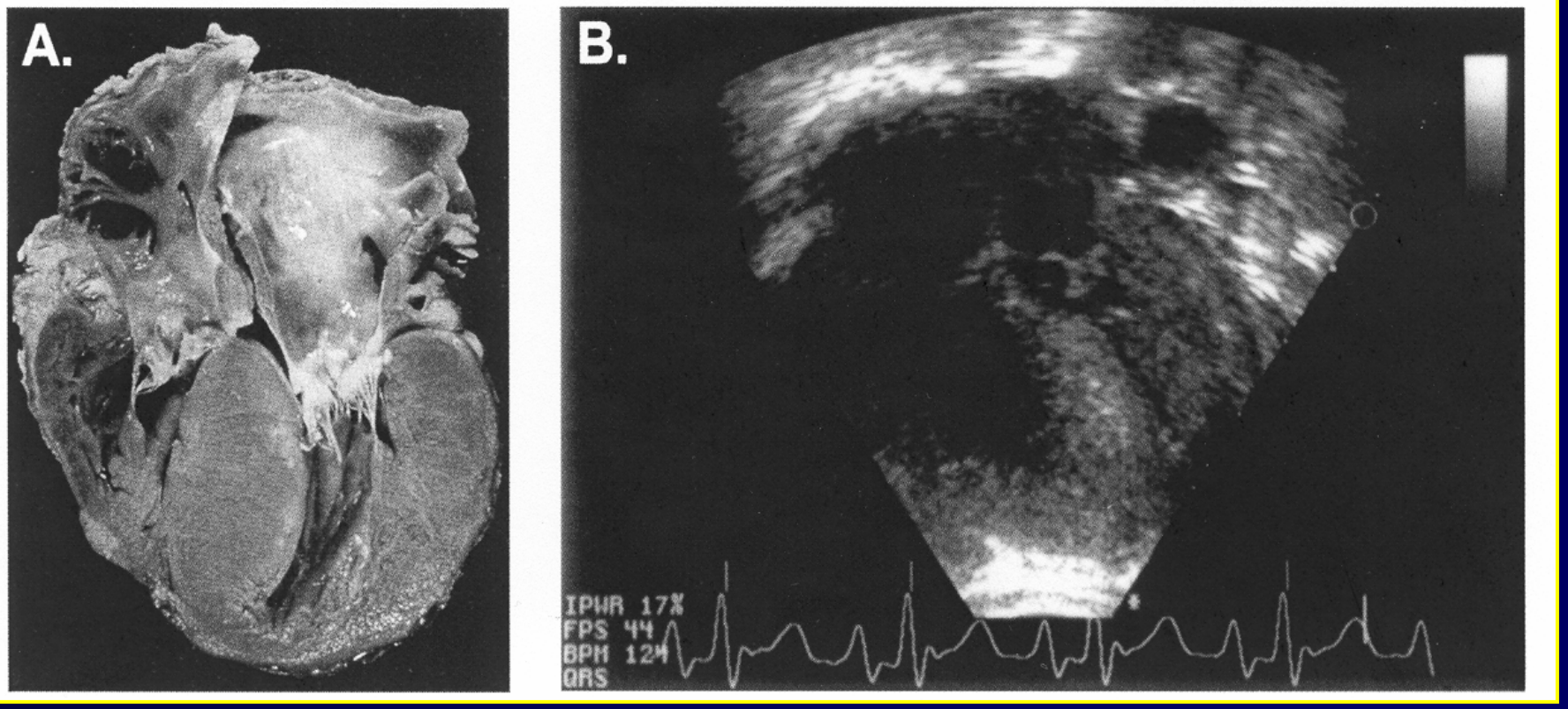
ETIOLOGIES OF SUDDEN CARDIAC DEATH

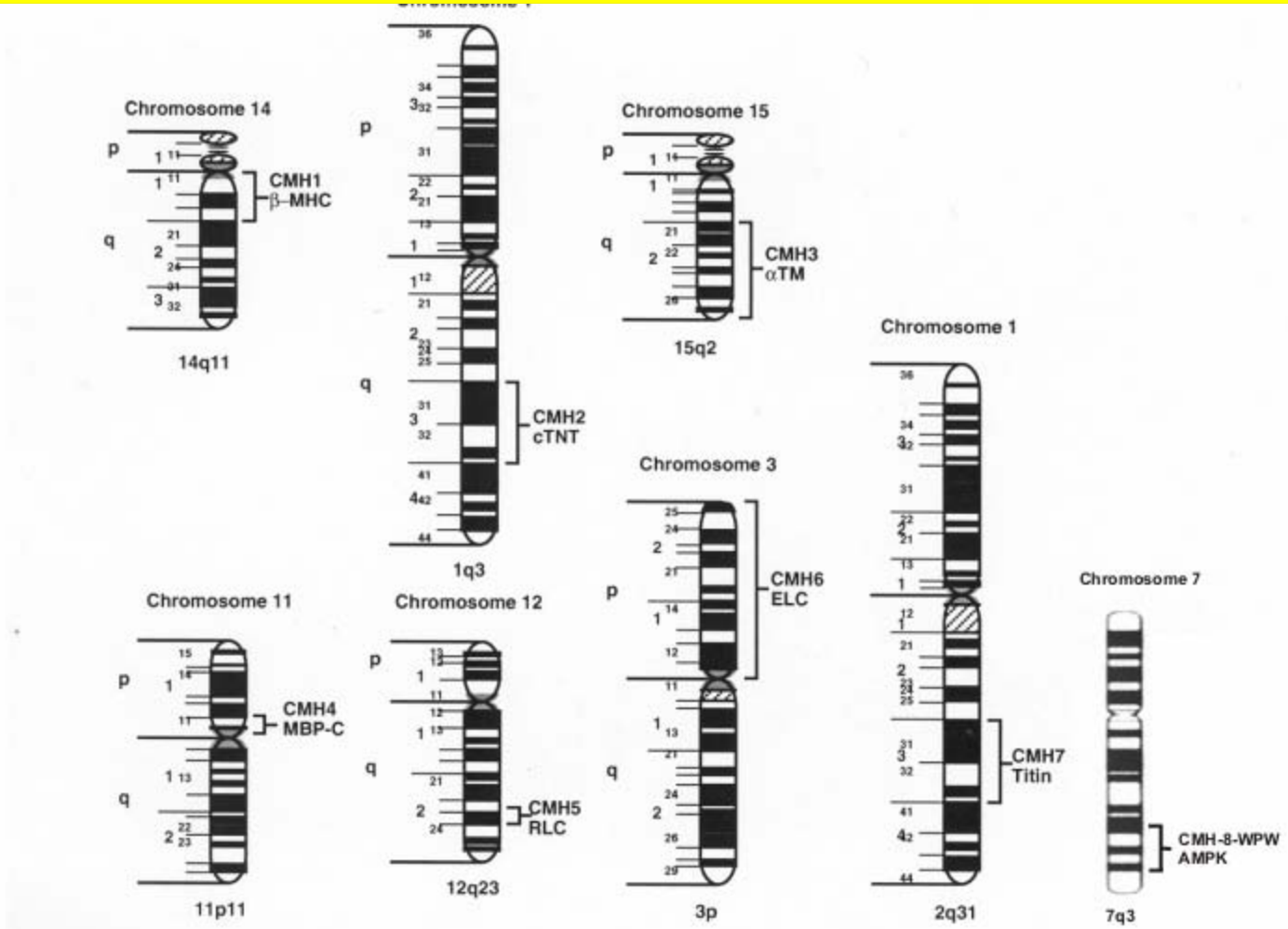
CARDIOMYOPATHIES

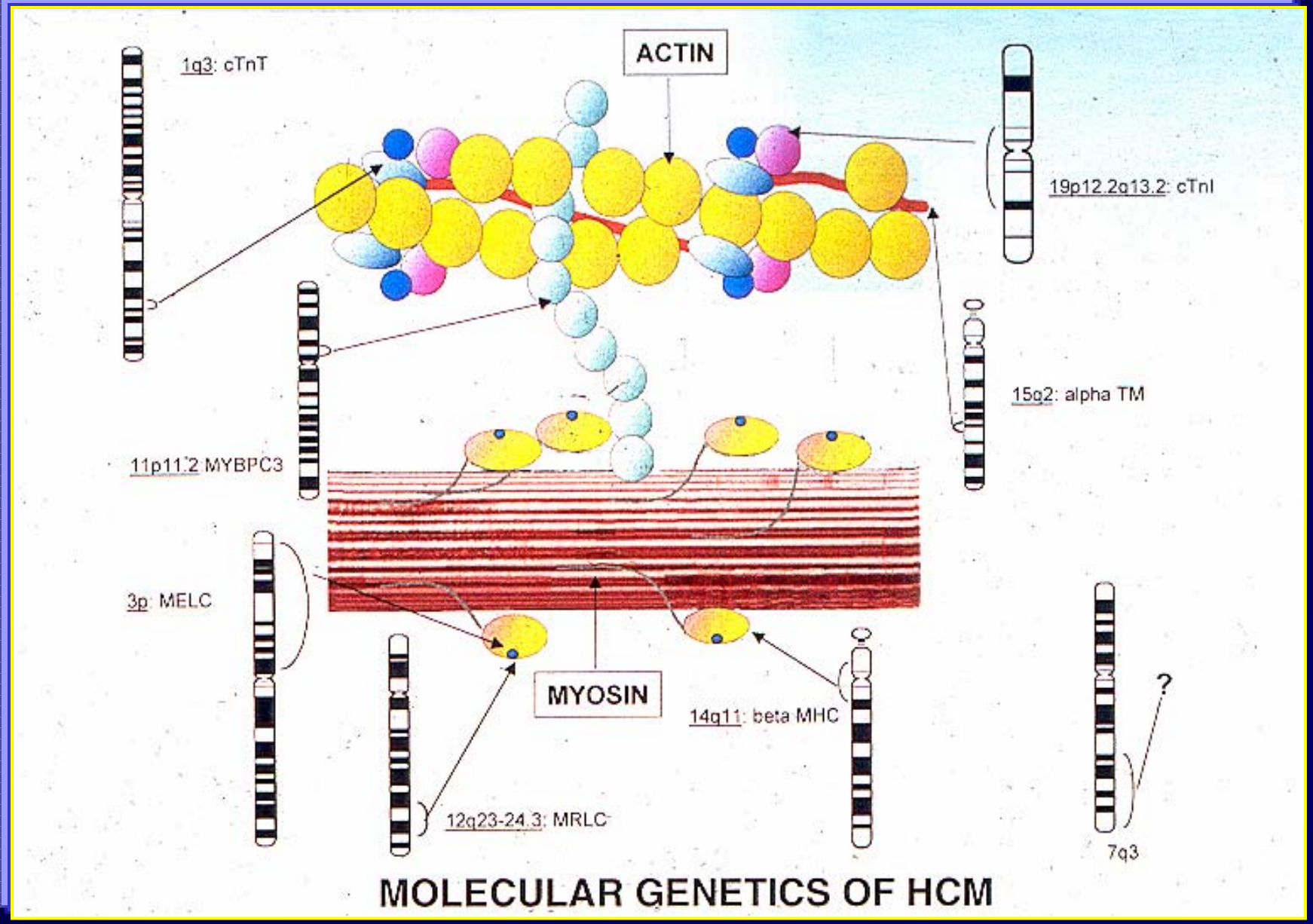
- Hypertrophic Cardiomyopathy
- Dilated Cardiomyopathy/Myocarditis
- Restrictive Cardiomyopathy
- Arrhythmogenic RV Dysplasia/ Cardiomyopathy

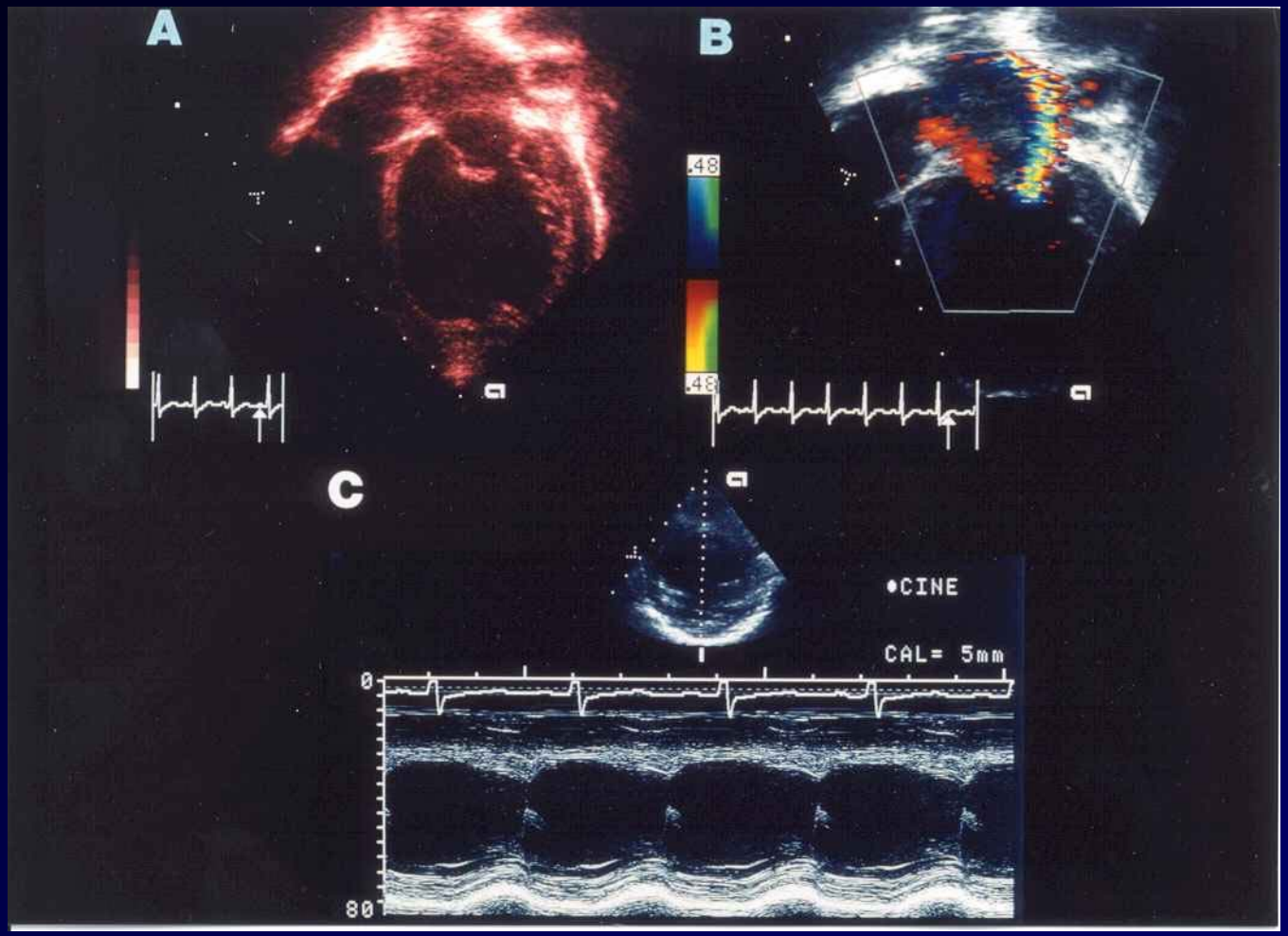
HYPERTROPHIC CARDIOMYOPATHY

- Most common cause of sudden death in young healthy individuals
- Characterized by hypertrophy of interventricular septum, posterior wall and hypercontractile systolic function with diastolic dysfunction
- Inherited usually as autosomal dominant


A.
B.
IPWR 17%
FPS 44
BPM 124
QRS


Chromosome 14
p
q
CMH1
β–MHC
14q11
1q3
CMH2
cTNT
Chromosome 15
CMH3
αTM
15q2
Chromosome 1
Chromosome 3
CMH6
ELC
3p
CMH7
Titin
2q31
Chromosome 7
CMH-8-WPW
AMPK
7q3
Chromosome 11
CMH4
MBP-C
11p11
Chromosome 12
CMH5
RLC
12q23


ACTIN
1q3: cTnT
19p12.2q13.2: cTnI
15q2: alpha TM
11p11.2 MYBPC3
3p: MELC
MYOSIN
14q11: beta MHC
12q23-24.3: MRLC
?
7q3
MOLECULAR GENETICS OF HCM


A
B
.48
.48
C
•CINE
CAL= 5mm
0
80

Left Ventricular Dysfunction

- Associated with heart failure, sudden death
- Multiple etiologies
  - Ischemia
  - Genetic
  - Viral / Inflammatory
  - Toxic

Left Ventricular Dysfunction

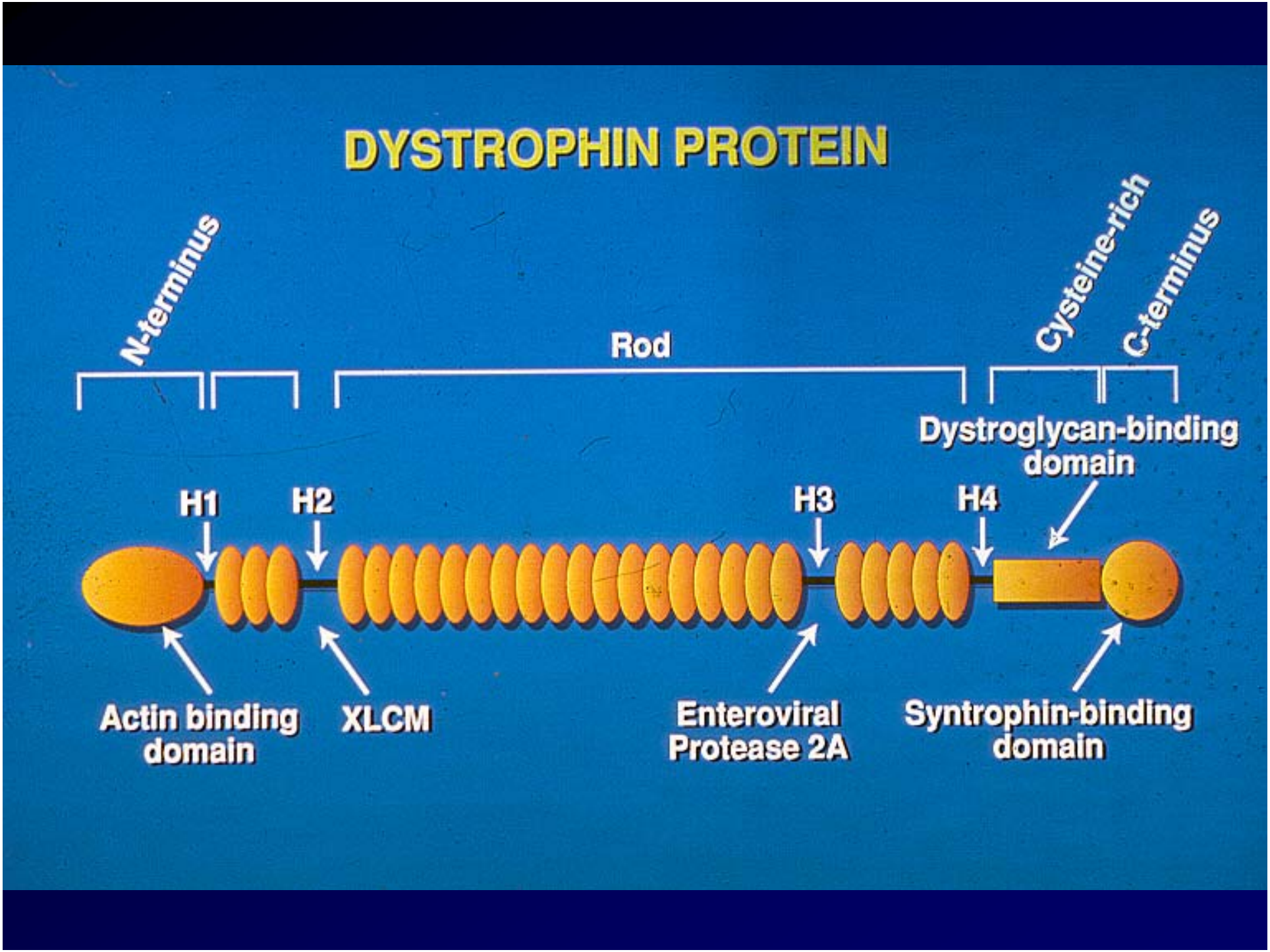
- 50% 5-year Survival
- Death due to
  - Pump failure
  - Ventricular arrhythmias
  - Bradycardia / AV conduction

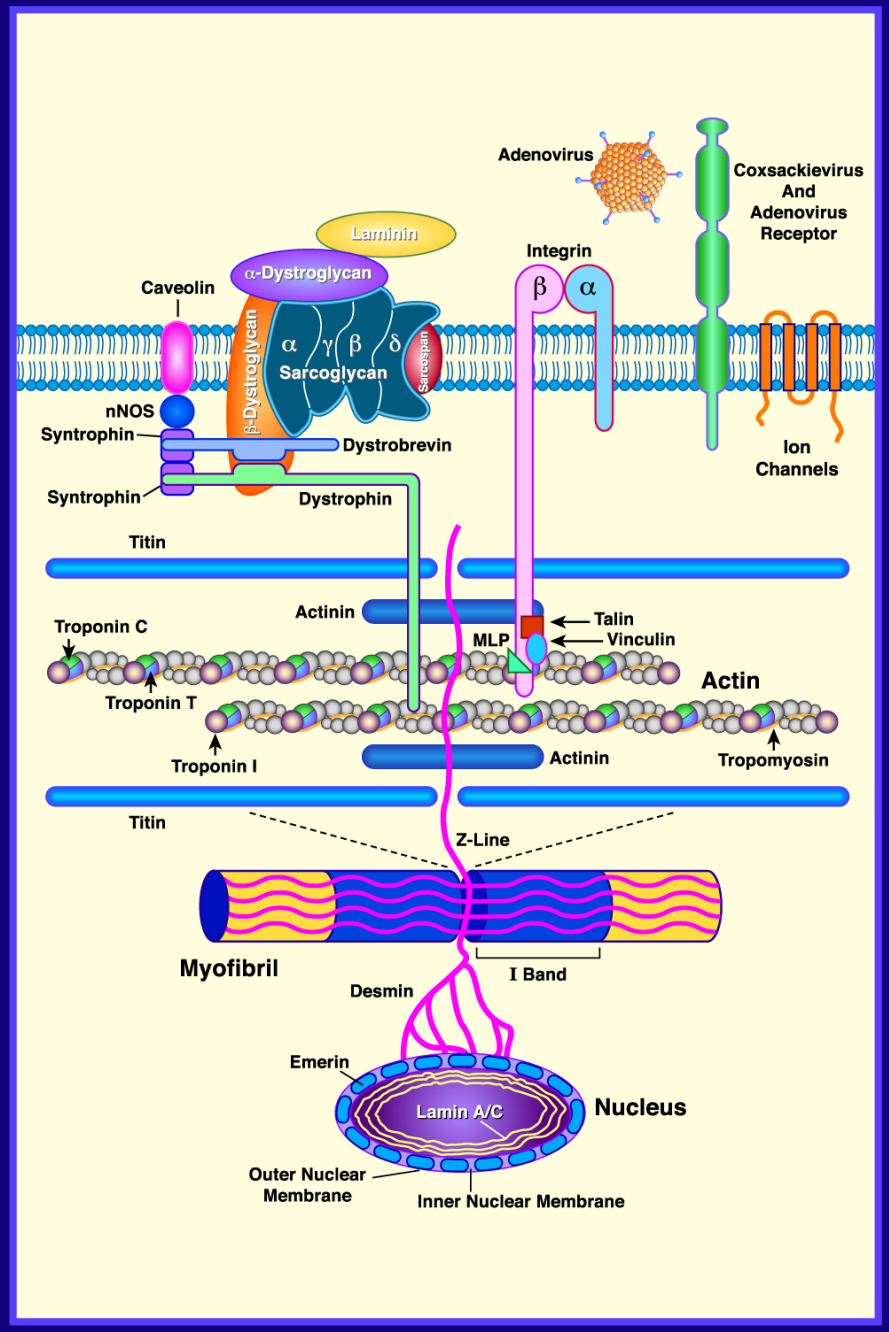
X-LINKED DILATED CARDIOMYOPATHY (XLCM)

- Initially described in 1987 (Berko & Swift, NEJM)
- X-linked recessive inheritance
- Presentation in males in teens, early twenties with heart failure
- Manifesting female carriers symptomatic in fourth decade
- Elevated CK-MM

X-LINKED DILATED CARDIOMYOPATHY (XLCM)

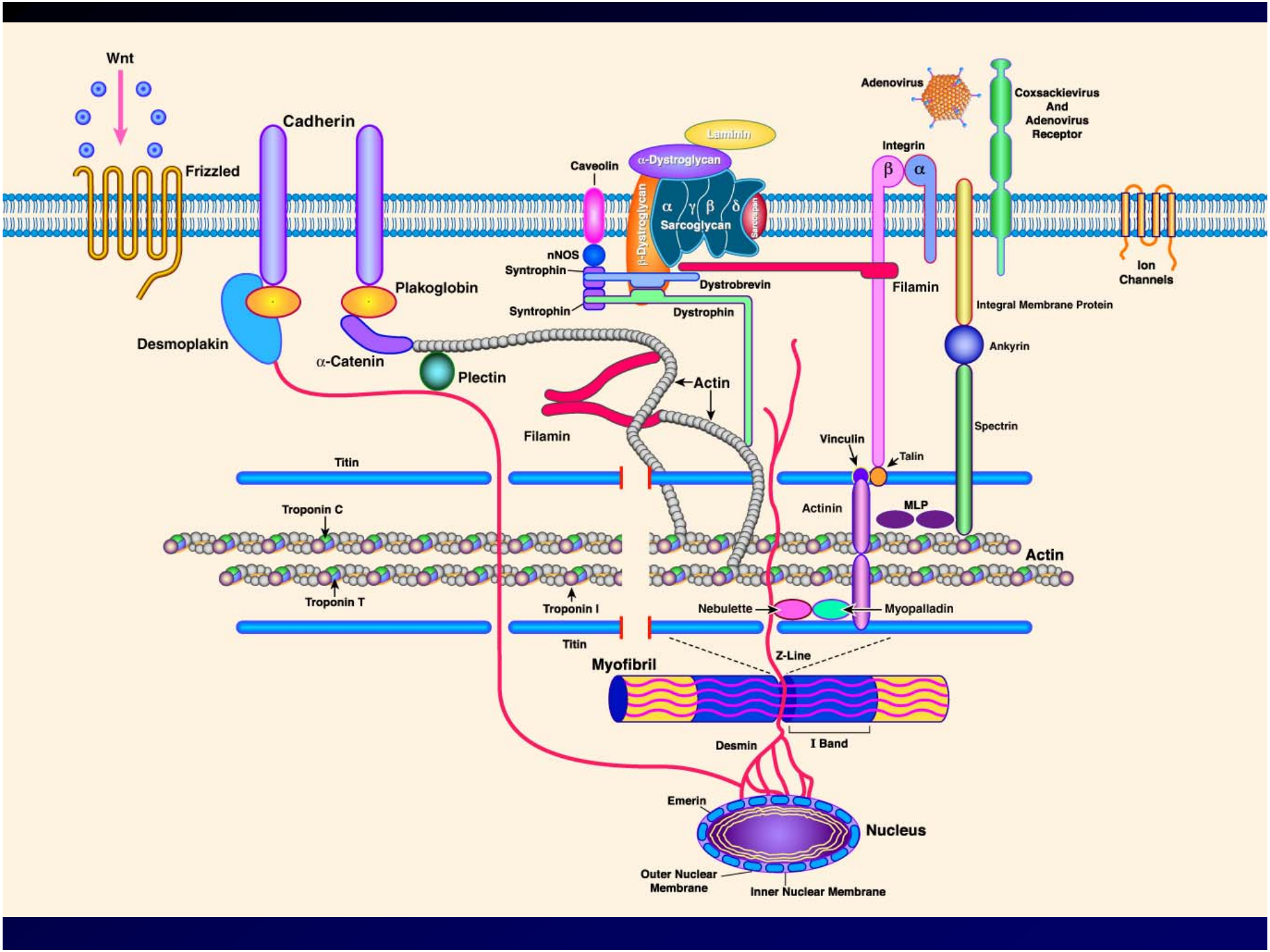
- Dystrophin
  - Cytoskeletal protein expressed in skeletal, cardiac, smooth muscle, brain
  - Interacts with actin (N-terminus) and dystrophin-associated protein complex in sarcolemma (C-terminus)
  - Contributes to intracellular organization, force transduction, and membrane stability
  - Mechanical stress thought to contribute to dysfunction


DYSTROPHIN PROTEIN
N-terminus
Rod
Cysteine-rich
C-terminus
Dystroglycan-binding domain
H1
H2
H3
H4
Actin binding domain
XLCM
Enteroviral Protease 2A
Syntrophin-binding domain


Adenovirus
Coxsackievirus And Adenovirus Receptor
Laminin
Integrin
α-Dystroglycan
Caveolin
β-Dystroglycan
α γ β δ
Sarcoglycan
Sarcospan
nNOS
Syntrophin
Dystrobrevin
Ion Channels
Syntrophin
Dystrophin
Titin
Actinin
Talin
Troponin C
MLP
Vinculin
Actin
Troponin T
Troponin I
Actinin
Tropomyosin
Titin
Z-Line
Myofibril
I Band
Desmin
Emerin
Lamin A/C
Nucleus
Outer Nuclear Membrane
Inner Nuclear Membrane

DCM GENETICS: AUTOSOMAL DOMINANT "PURE"

CHR LOCUS	GENE	PROTEIN
1q32	TNNT2	Cardiac Troponin T
2q31	TTN	Titin
2q35	DES	Desmin
5q33	SGCD	δ-Sarcoglycan
6q12-q16	?	?
6q22.1	PLN	Phospholamban
9q13-q22	?	?
9q22-q31	?	?
10q21	?	?
10q22-q23	VCL	Metavinculin
10q22.3-23.2	ZASP	ZASP
11p11	MYBPC3	Myosin Binding Protein C
11p15.1	MLP	Muscle LIM Protein
14q12	MYH7	β-Myosin Heavy Chain
15q14	ACTC	Cardiac Actin
15q22	TPM1	α-Tropomyosin


Wnt
Frizzled
Cadherin
Plakoglobin
Desmoplakin
α-Catenin
Plectin
Caveolin
Laminin
α-Dystroglycan
β-Dystroglycan
α γ β δ
Sarcoglycan
Sarcospan
nNOS
Syntrophin
Syntrophin
Dystrobrevin
Dystrophin
Filamin
Actin
Filamin
Titin
Troponin C
Troponin T
Troponin I
Titin
Adenovirus
Coxsackievirus And Adenovirus Receptor
Integrin
β α
Filamin
Integral Membrane Protein
Ion Channels
Ankyrin
Spectrin
Vinculin
Talin
Actinin
MLP
Actin
Nebulette
Myopalladin
Myofibril
Z-Line
I Band
Desmin
Emerin
Nucleus
Outer Nuclear Membrane
Inner Nuclear Membrane

AUTOSOMAL DOMINANT DCM

- Conduction system disease with DCM (CDDC)
  - AV block in second/third decade
  - Atrial fibrillation, other arrhythmias
  - Late onset DCM in fourth/fifth decade

LAMIN A/C

- Intermediate filament proteins located at nucleoplasmic side of inner nuclear membrane
- Presumed to have structural role in maintaining integrity of nuclear membrane
- Mutations cause Emery-Dreifuss muscular dystrophy, autosomal dominant Limb Girdle muscular dystrophy 1B, familial partial lipodystrophy, and CDDC (cardiac conduction disease and dilated cardiomyopathy)

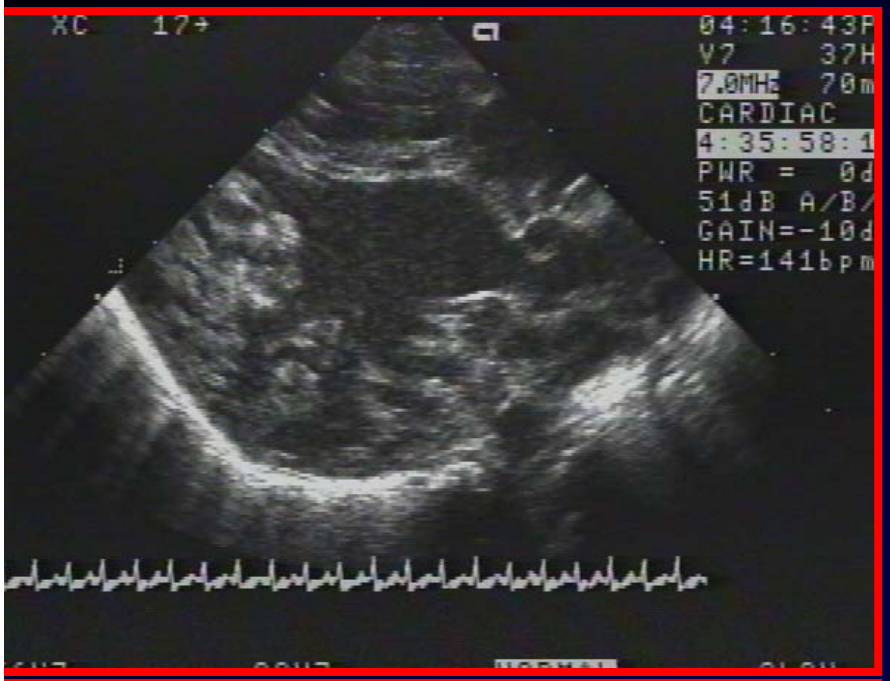
AUTOSOMAL DOMINANT DCM

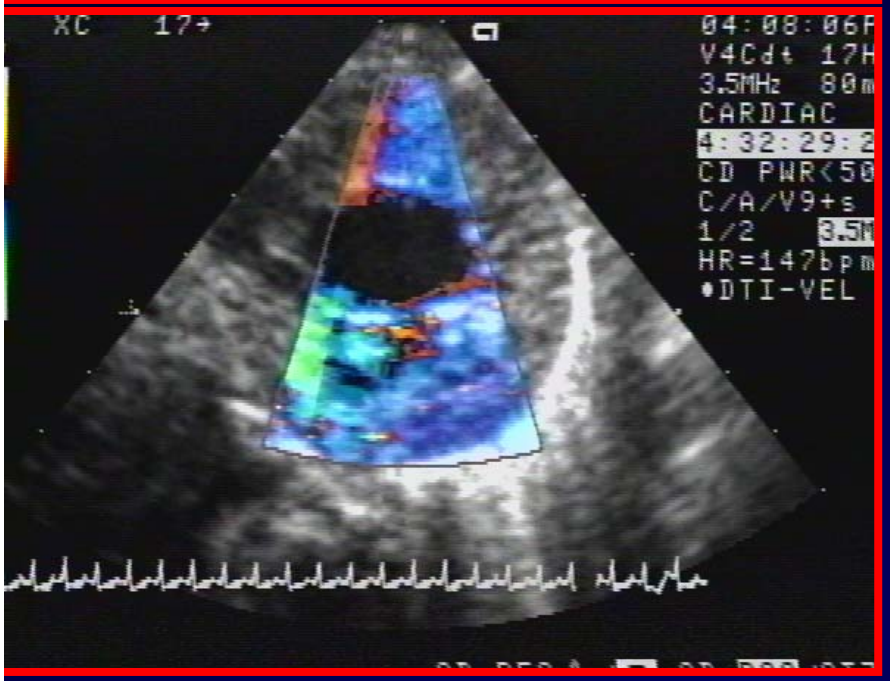
- Sarcomeric protein genes
  - Actin
  - β-Myosin heavy chain
  - Cardiac Troponin T
  - α-Tropomyosin
  - Titin

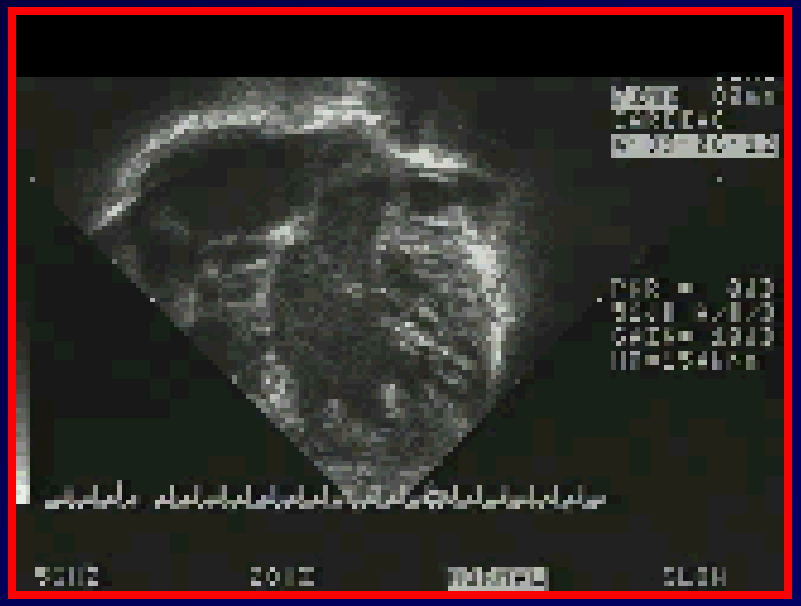
MUSCLE IS MUSCLE

DCM GENE	SKELETAL MYOPATHY
Dystrophin	Duchenne/Becker Muscular Dystrophy
G4.5 (Tafazzin)	Barth Syndrome
Emerin	X-Linked Emery-Dreifuss Muscular Dystrophy
Lamin A/C	AD Emery-Dreifuss Muscular Dystrophy
δ-Sarcoglycan	Limb Girdle Muscular Dystrophy 2F
β-Sarcoglycan	Limb Girdle Muscular Dystrophy
Desmin	Desmin Myopathy, Nemaline Rod Myopathy
Actin	Nemaline Rod Myopathy
α-Tropomyosin	Nemaline Rod Myopathy
ZASP	Myofibrillar Myopathy
Titin	Tibial Muscular Dystrophy

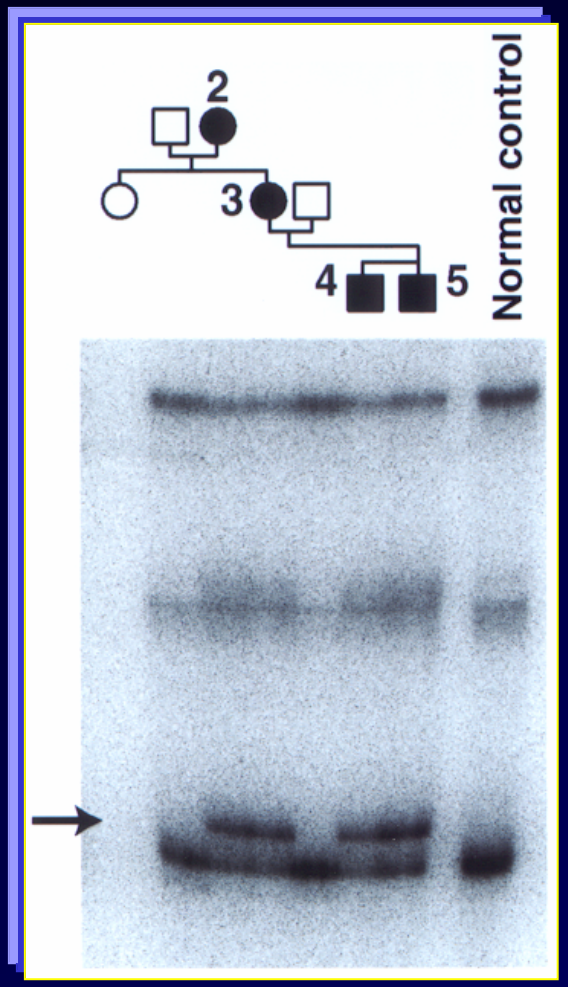
LV NONCOMPACTION

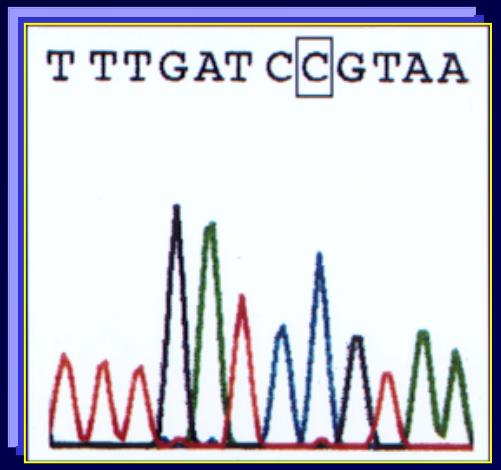




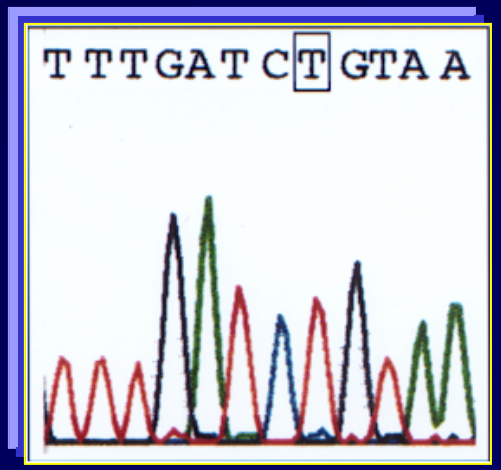


GENETIC ANALYSIS OF THE α-DYSTROBREVIN GENE (P121L)





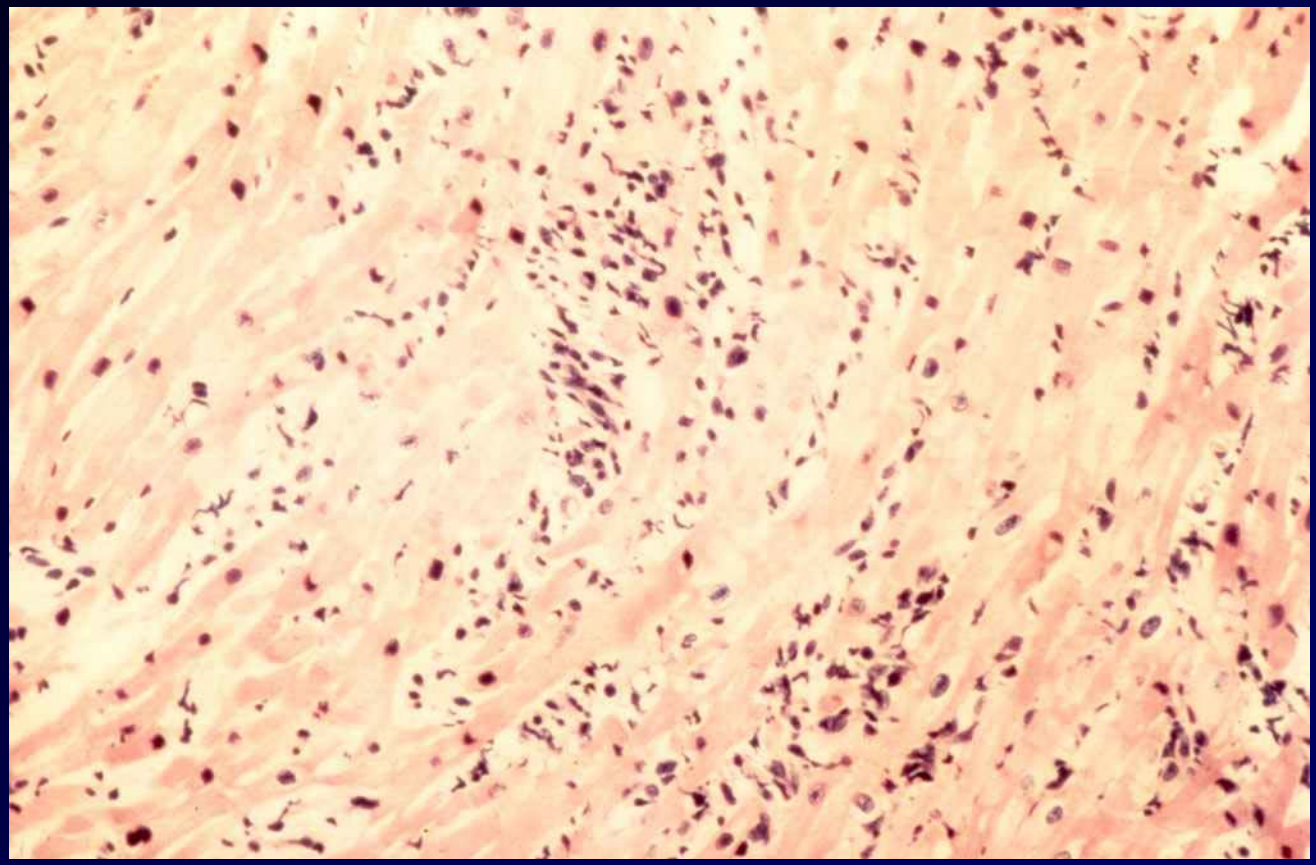
CONTROL



LVNC

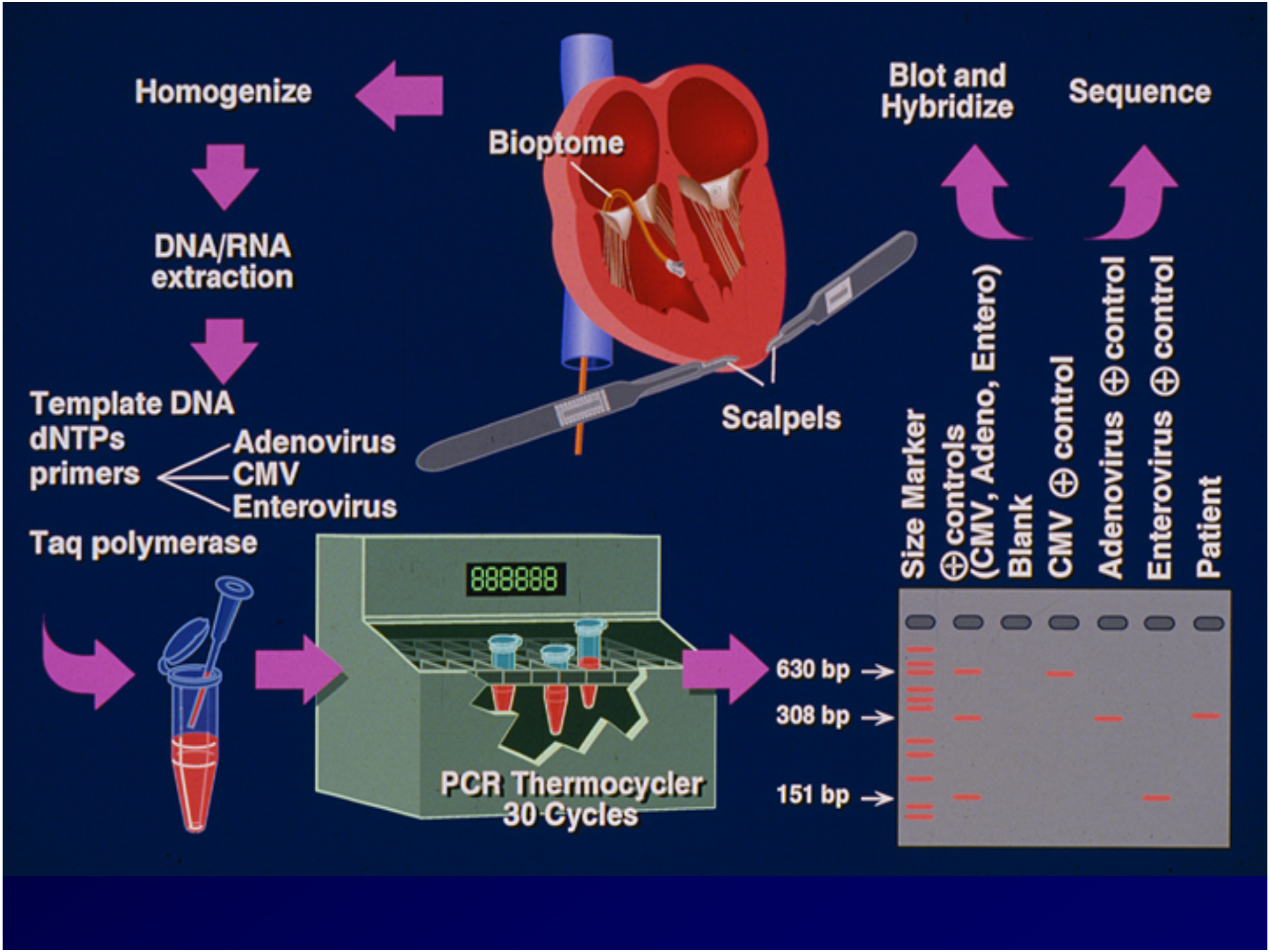
MYOCARDITIS

An inflammatory process affecting the heart and causing ventricular dysfunction. The inflammation may involve myocytes, interstitium, vascular elements, and/or pericardium and may be an acute or chronic process



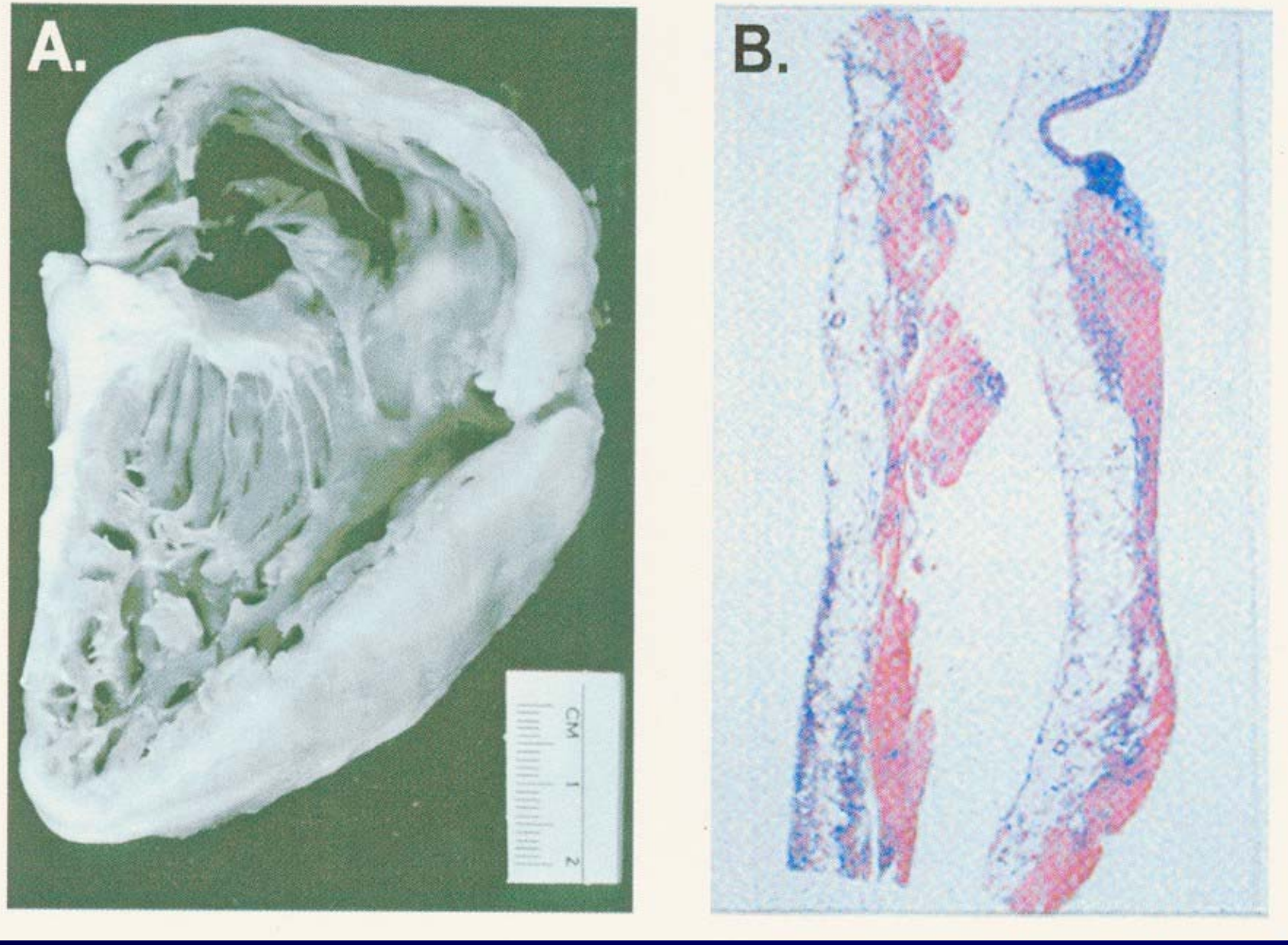
REPORTED VIRAL ETIOLOGIES OF MYOCARDITIS

- Enterovirus
  - Coxsackie B
  - Coxsackie A
  - Echo
- Adenovirus
- CMV
- Influenza A
- Parvovirus
- EBV
- Mumps
- RSV
- Hepatitis C


Homogenize
DNA/RNA extraction
Template DNA
dNTPs
primers
Adenovirus
CMV
Enterovirus
Taq polymerase
Bioptome
Scalpels
PCR Thermocycler
30 Cycles
Blot and Hybridize
Sequence
Size Marker
⊕ controls (CMV, Adeno, Entero)
Blank
CMV ⊕ control
Adenovirus ⊕ control
Enterovirus ⊕ control
Patient
630 bp
308 bp
151 bp

The Detection of Viruses by PCR in Myocardial Samples

DIAGNOSIS	# SAMPLES	# OF PCR+ SAMPLES	PCR AMPLIMER (#)
MYOCARDITIS	624	239 (38%)	Adenovirus 142 (23%) Enterovirus 85 (14%) CMV 18 (3%) Parvovirus 6 (<1%) Influenza A 5 (<1%) HSV 5 (<1%) EBV 3 (<1%) RSV 1 (<1%)
DCM	149	30 (20%)	Adenovirus 18 (12%) Enterovirus 12 (8%)
CONTROLS	215	3 (1.4%)	Enterovirus 1 (<1%) CMV 2 (<1%)

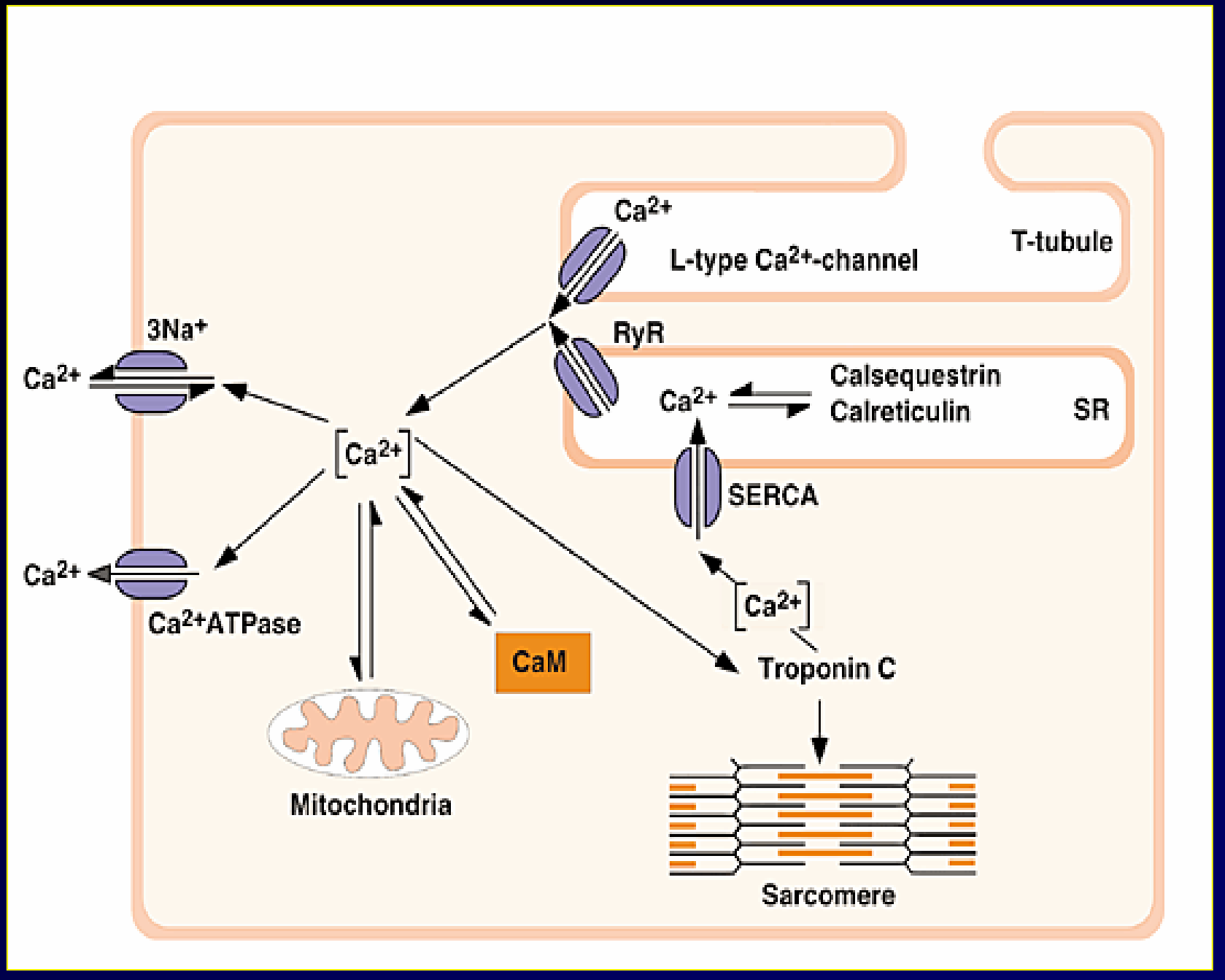

A.
B.
CM
1
2

FINAL COMMON PATHWAY HYPOTHESIS

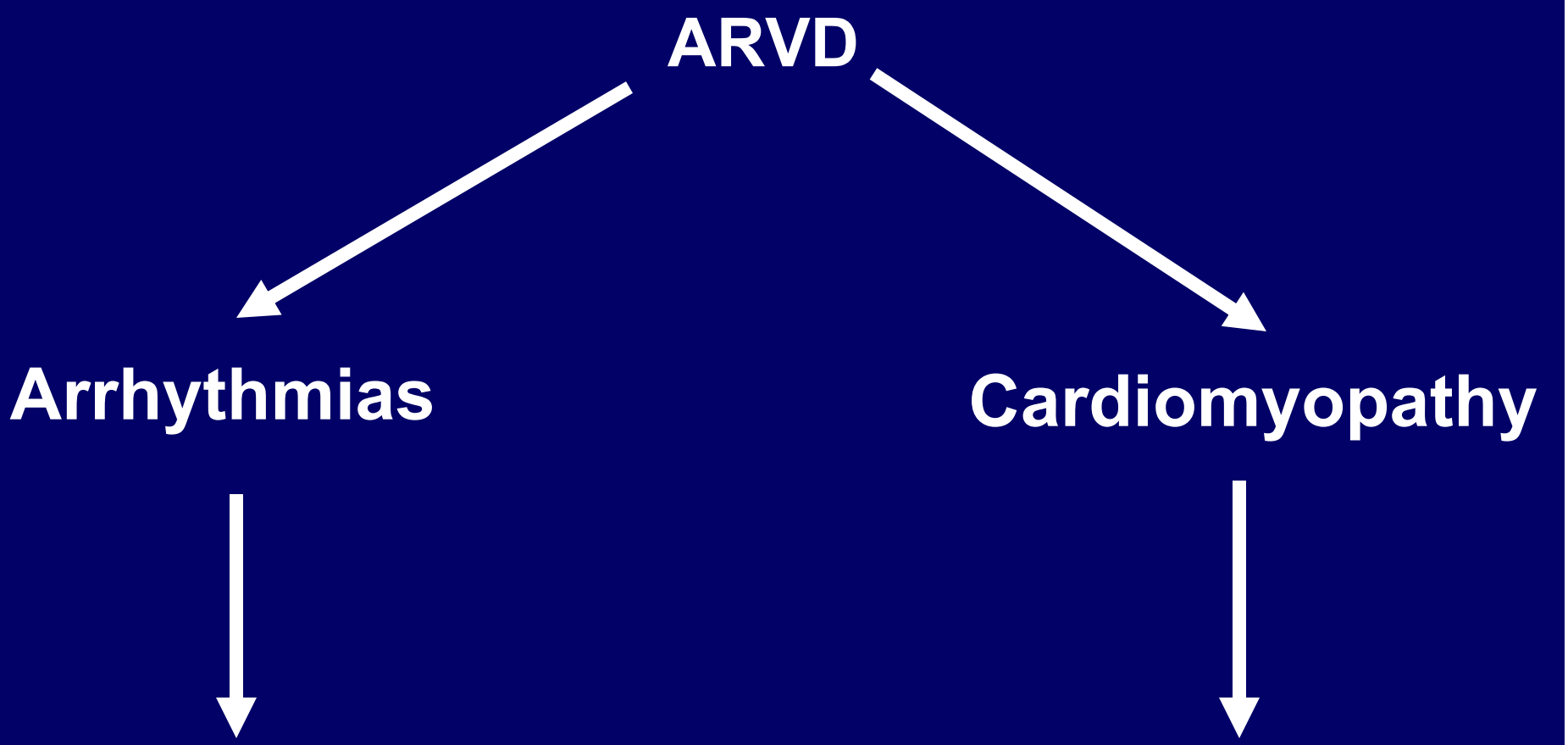
- Hypothesized that ARVD/C is a disease of adherens junctions primarily with secondary arrhythmias
- Screened ion channel genes and cell-cell junction genes

ARVD

Locus Name	*Inheritance*	*Map Position*	*Gene*
ARVD1	AD	14q23	?
ARVD2	AD	1q42-q43	?
ARVD3	AD	14q12	?
ARVD4	AD	2q32	?
ARVD5	AD	3p23	?
ARVD6	AD	10p12	?
ARVD7	AD	10p22	?
ARVD8	AD	6p24	?
Naxos	AR	17q21	?
Carvajal	AR	6p24	?


Ca2+
L-type Ca2+-channel
T-tubule
3Na+
Ca2+
RyR
Ca2+
Calsequestrin
Calreticulin
SR
[Ca2+]
SERCA
Ca2+
Ca2+ATPase
[Ca2+]
CaM
Troponin C
Mitochondria
Sarcomere

ORIGINAL GRANT PROPOSAL: FINAL COMMON PATHWAY

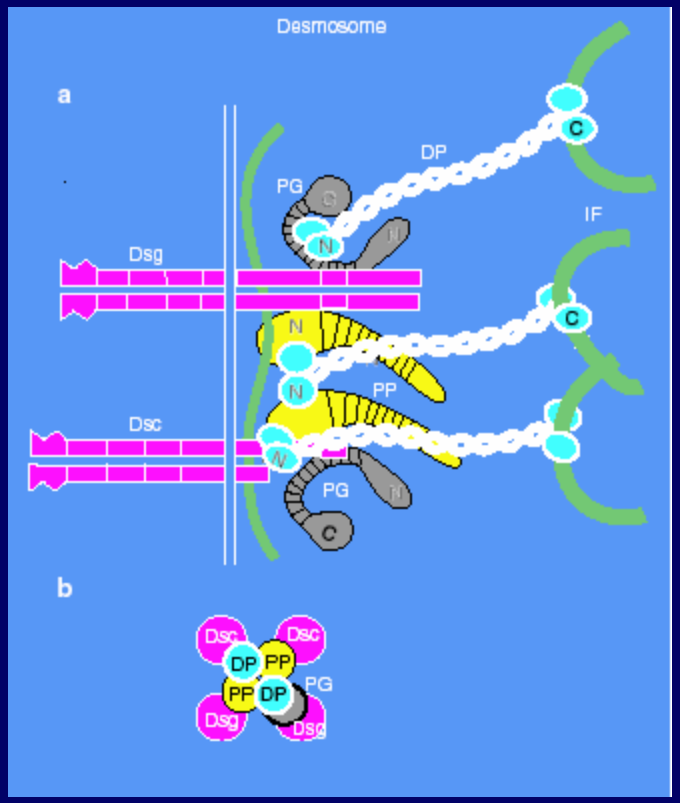


ARVD

Locus Name	*Inheritance*	*Map Position*	*Gene*
ARVD1	AD	14q23	?
ARVD2	AD	1q42-q43	RyR2
ARVD3	AD	14q12	?
ARVD4	AD	2q32	?
ARVD5	AD	3p23	?
ARVD6	AD	10p12	?
ARVD7	AD	10p22	?
ARVD8	AD	6p24	?
Naxos	AR	17q21	Plakoglobin
Carvajal	AR	6p24	Desmoplakin

DESMOSOMES

- The N-terminus of DPI can bind to PG, PKPs and desmocollin 1a, while the C-terminus of DPI binds to IFs.



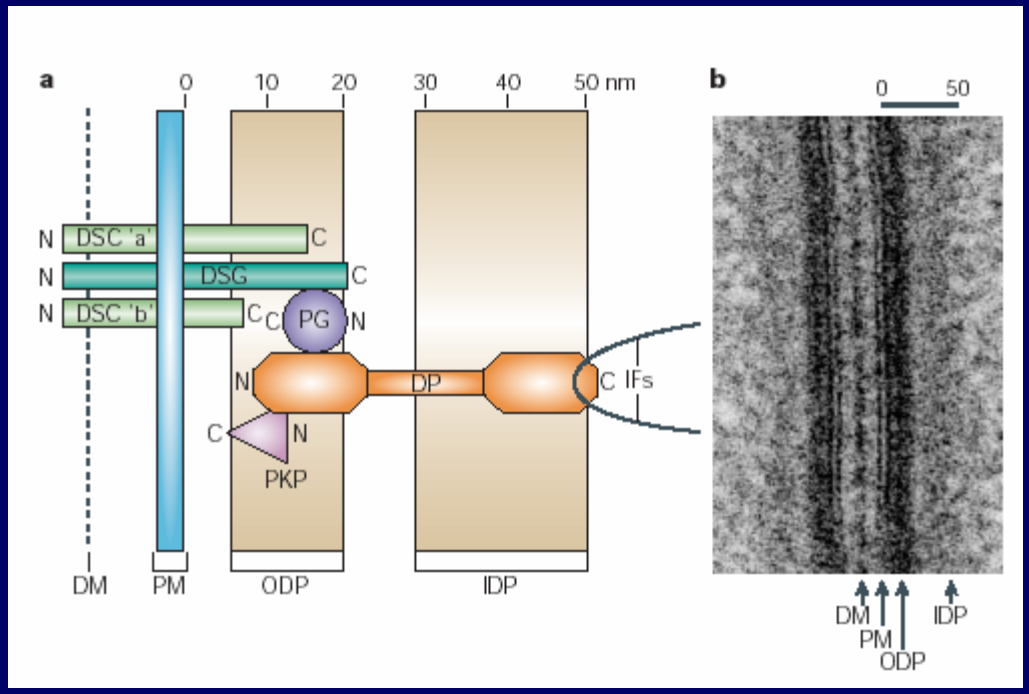
(S. Hatsell and P. Cowin, 2003)

ARVD

- Tiso et al (2001) identified ARVD2 gene at chromosome 1q42, the cardiac ryanodine receptor (RYR2)
  - 4 independent Italian families with mutations
  - Autosomal dominant inheritance
  - Missense mutations in all cases
- RYR2 gene large; 105 exons, encoding 565 kDa monomer of a tetrameric structure
- RYR2 protein interacts with 4 FK-506 binding proteins (FKBP12.6)

DESMOSOMES

- Specialized cell-cell adhesion junctions that connect the plasma membrane with the intermediate filament network
- Abundant in epithelial cells and intercalated disks of cardiomyocytes



(Gestios, S., et al. 2004)

ARVD

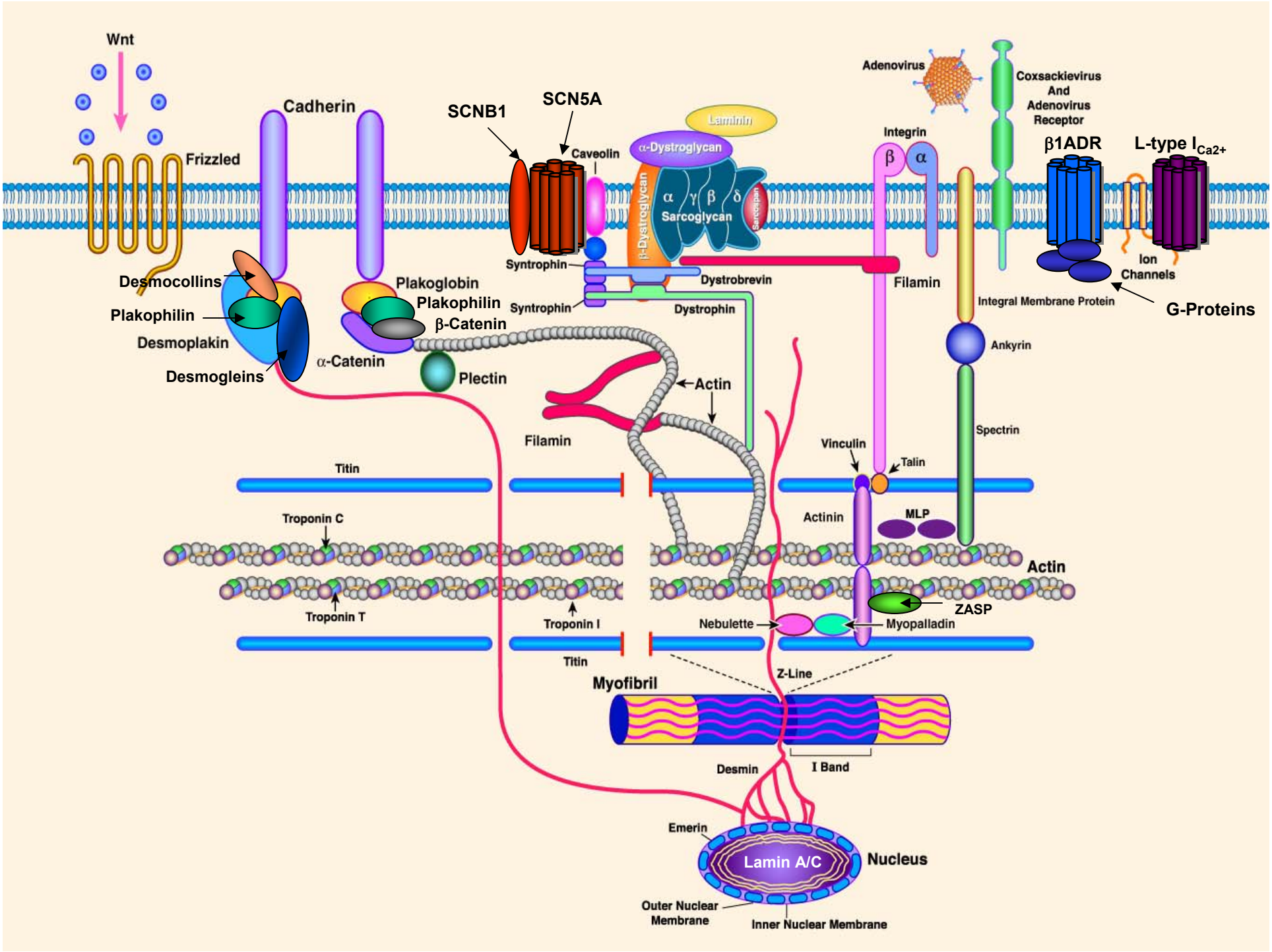
Locus Name	*Inheritance*	*Map Position*	*Gene*
ARVD1	AD	14q23	?
ARVD2	AD	1q42-q43	RyR2
ARVD3	AD	14q12	?
ARVD4	AD	2q32	?
ARVD5	AD	3p23	?
ARVD6	AD	10p12	?
ARVD7	AD	10p22	?
ARVD8	AD	6p24	Desmoplakin
ARVD9	AD	12p11	Plakophilin 2
Naxos	AR	17q21	Plakoglobin
Carvajal	AR	6p24	Desmoplakin

ARRHYTHMOGENIC RIGHT VENTRICULAR DYSPLASIA (ARVD)

Acquired Forms

- Proposed that ARVD may be a sequela of myocarditis (like dilated cardiomyopathy or DCM)
- Viruses associated with myocarditis and DCM considered potential etiologic agents:
  - Enteroviruses
    - Grumbach et al (1998) reported the detection of Coxsackievirus B3 in the myocardium of 3 of 8 patients with ARVD
  - Adenoviruses

DIAGNOSIS	SAMPLE #	VIRAL PCR +	PCR AMPLIMER (# POSITIVE)	%OF POSITIVES (% OF SAMPLES)
Myocarditis	624	262 (42%)	Adenovirus (142) Enterovirus (85) CMV (18) Parvovirus (6) HSV (5) Influenza A Virus (5) RSV (1)	54% (22%) 32% (14%) 7% (3%) 2% (<1%) 2% (<1%) 2% (<1%) <1% (<1%)
Dilated Cardiomyopathy	149	30 (20%)	Adenovirus (18) Enterovirus (12)	60% (12%) 40% (8%)
ARVD	12	7 (58%)	Enterovirus (5) Adenovirus (2)	71% (42%) 29% (17%)
Controls	215	3 (1.4%)	CMV (2) Enterovirus (1)	67% (<1%) 33% (<1%)


Wnt
Frizzled
Cadherin
SCNB1
SCN5A
Caveolin
Laminin
α-Dystroglycan
β-Dystroglycan
α γ β δ
Sarcoglycan
Sarcospan
Adenovirus
Coxsackievirus And Adenovirus Receptor
Integrin
β α
β1ADR
L-type I_{Ca2+}
Ion Channels
G-Proteins
Integral Membrane Protein
Filamin
Syntrophin
Dystrobrevin
Dystrophin
Desmocollins
Plakophilin
Desmoplakin
Desmogleins
Plakoglobin
Plakophilin
β-Catenin
α-Catenin
Plectin
Actin
Ankyrin
Spectrin
Vinculin
Talin
Titin
Troponin C
Troponin T
Troponin I
Actinin
MLP
ZASP
Nebulette
Myopalladin
Z-Line
Myofibril
Desmin
I Band
Emerin
Lamin A/C
Nucleus
Outer Nuclear Membrane
Inner Nuclear Membrane

FINAL COMMON PATHWAY HYPOTHESIS

Conclusions

1. ARVD/C is a disease of the desmosomes primarily
2. Arrhythmias in ARVD/C occur secondary to disruption cell junction interactions
3. Therapeutic approaches in the future should focus on normalizing the function of the affected pathways