Ion Channels: Nano-mediators of Health, Disease and Therapy

Nanomedicine Roadmap Initiative, Project Launch

May, 2004

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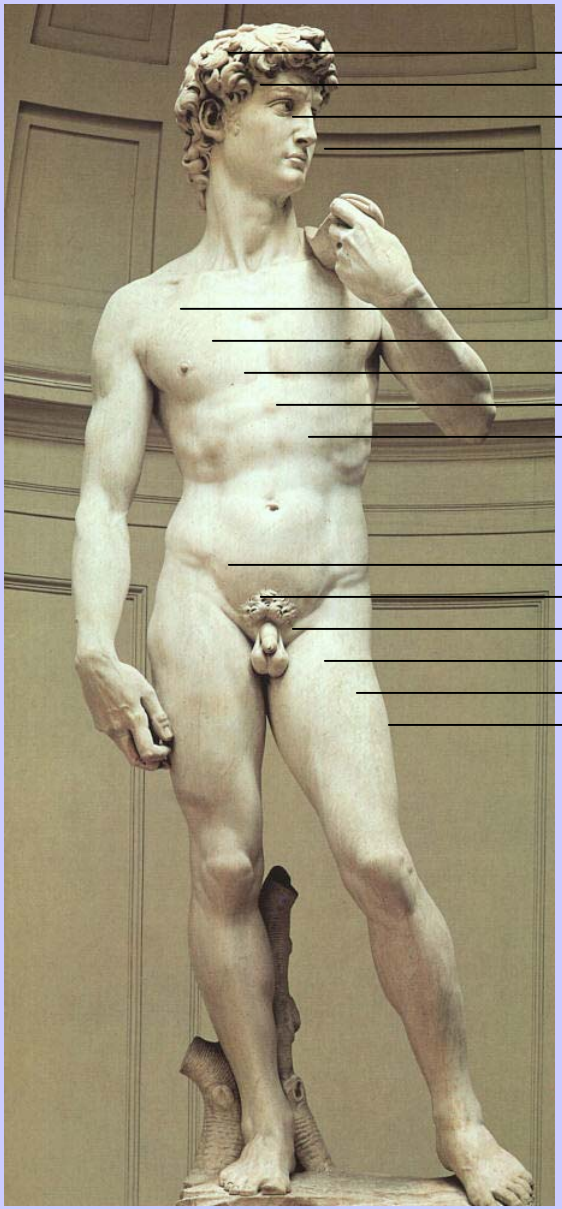
Yale University

Ion channels: sight, sound, thoughts, movement…

Ion Channels and disease



Epilepsy
Deafness
Blindness
Anxiety

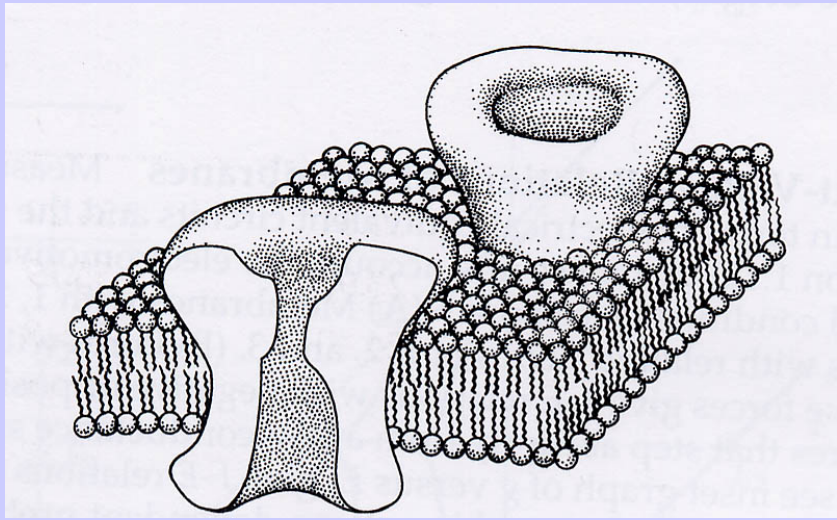
Arrhythmia
Cystic Fibrosis
Hypertension
Malignant Hyperthermia

Myotonias
Osteoporosis
Sterility
Incontinence

Cancer
Diabetes
Asthma/Allergy
Stroke
Autoimmune
Inflammation
Drug sensitivity

MICHELANGELO di Lodovico Buonarroti Simoni (b. 1475, Caprese, d. 1564, Roma); with thanks to

Ion channel principles I



- Membrane-spanning, water-filled pores in all cell membranes
- Two tasks under strict control: “gate” & “conduct”
  - Gate: open and close to a specific stimulus with exact timing
  - Conduct: pass specific ions (K, Na, Cl) with marked efficiency
  - Control as to where, number, activity

Ion channel principles II

$$I = N\ i\ Po$$

I, total current
N, number of channels
i, single channel current
Po, open probability
(environment)

Case

CC: Newborn female with LQTS

HPI: NSVD at 38 weeks.
With first feeding developed cyanosis and hypotonia.

PE: WNL except for bradycardia.

Labs: ECG - SR @ 82, QTc 0.61 (nl<0.44).
Audiograms: bilateral sensory deafness.

F/U On propranolol without syncope, seizures or arrhythmia.

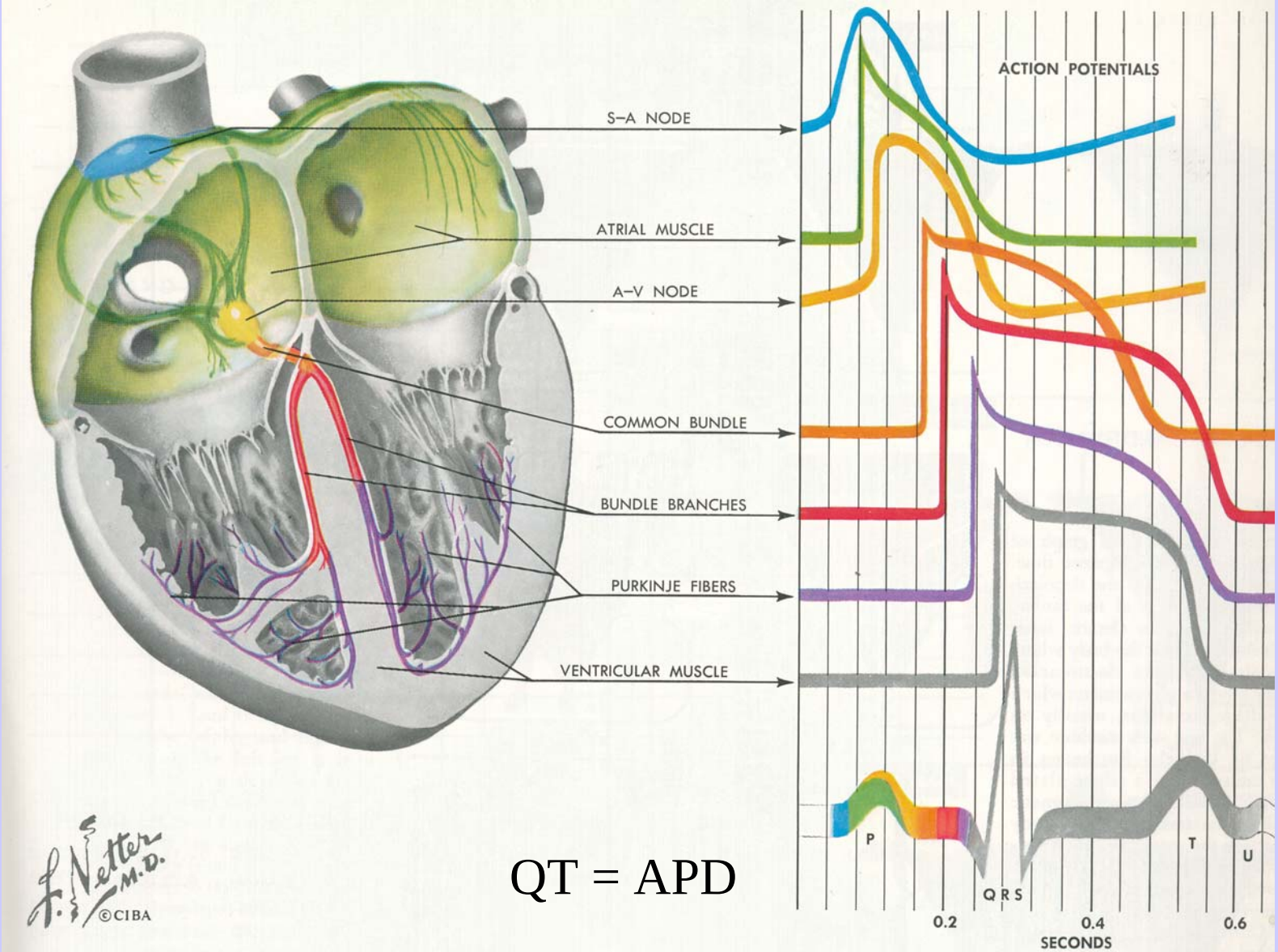
Seven months after delivery mother had a cardiac arrest and died.

The family was referred for genetic evaluation (~50).

25% had significantly prolonged QTc
55% had borderline elevations in QTc
11% had syncope

DNA analysis: a common mutation in one allele of a gene encoding an ion channel subunit in all affected family members.

Only the patient had a hearing deficit and two mutant alleles.


S–A NODE
ATRIAL MUSCLE
A–V NODE
COMMON BUNDLE
BUNDLE BRANCHES
PURKINJE FIBERS
VENTRICULAR MUSCLE
ACTION POTENTIALS
P
Q R S
T
U
0.2
0.4
0.6
SECONDS
F. Netter M.D.
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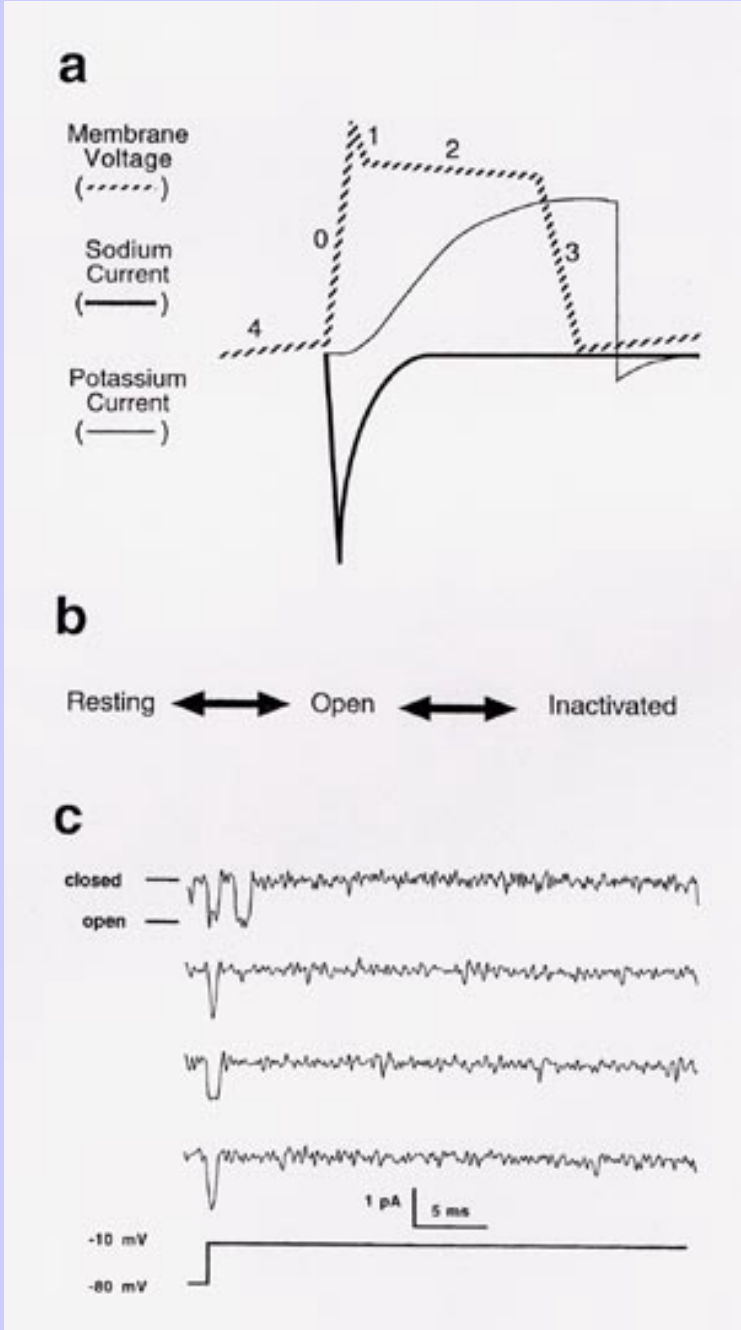
QT = APD

Action potentials: ion channel function

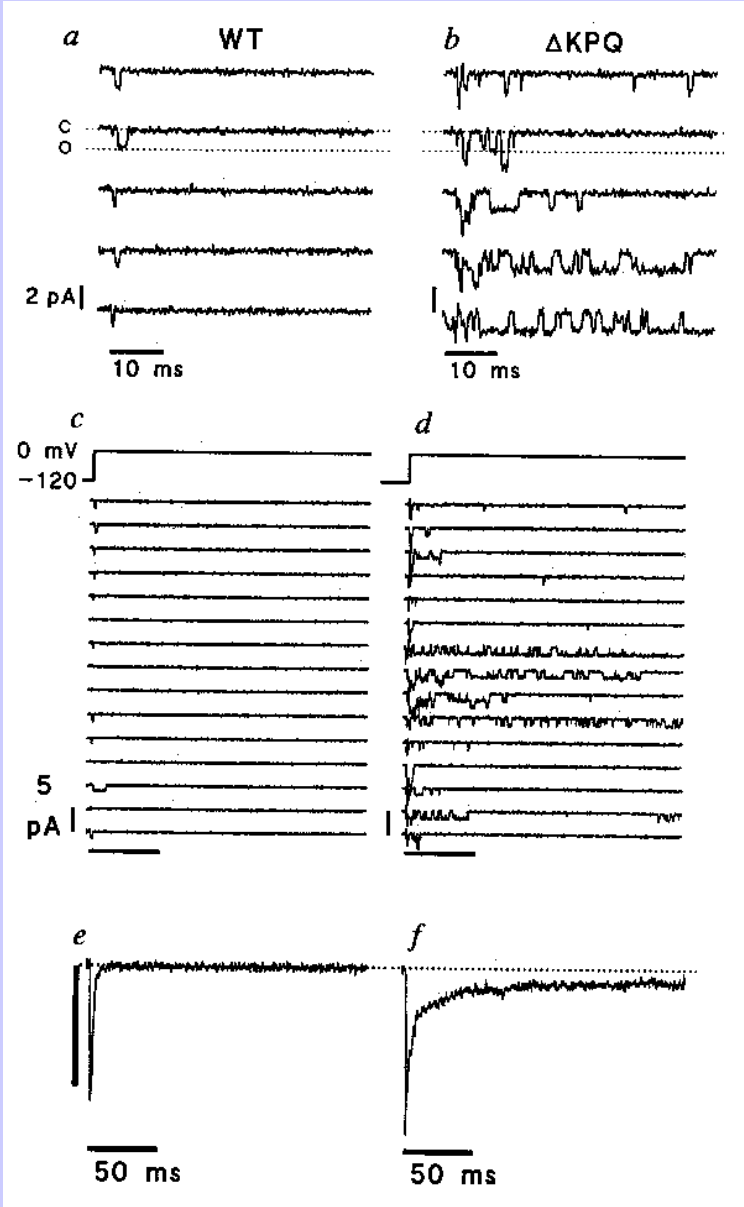
$$I = N\ i\ Po$$

LQTS?



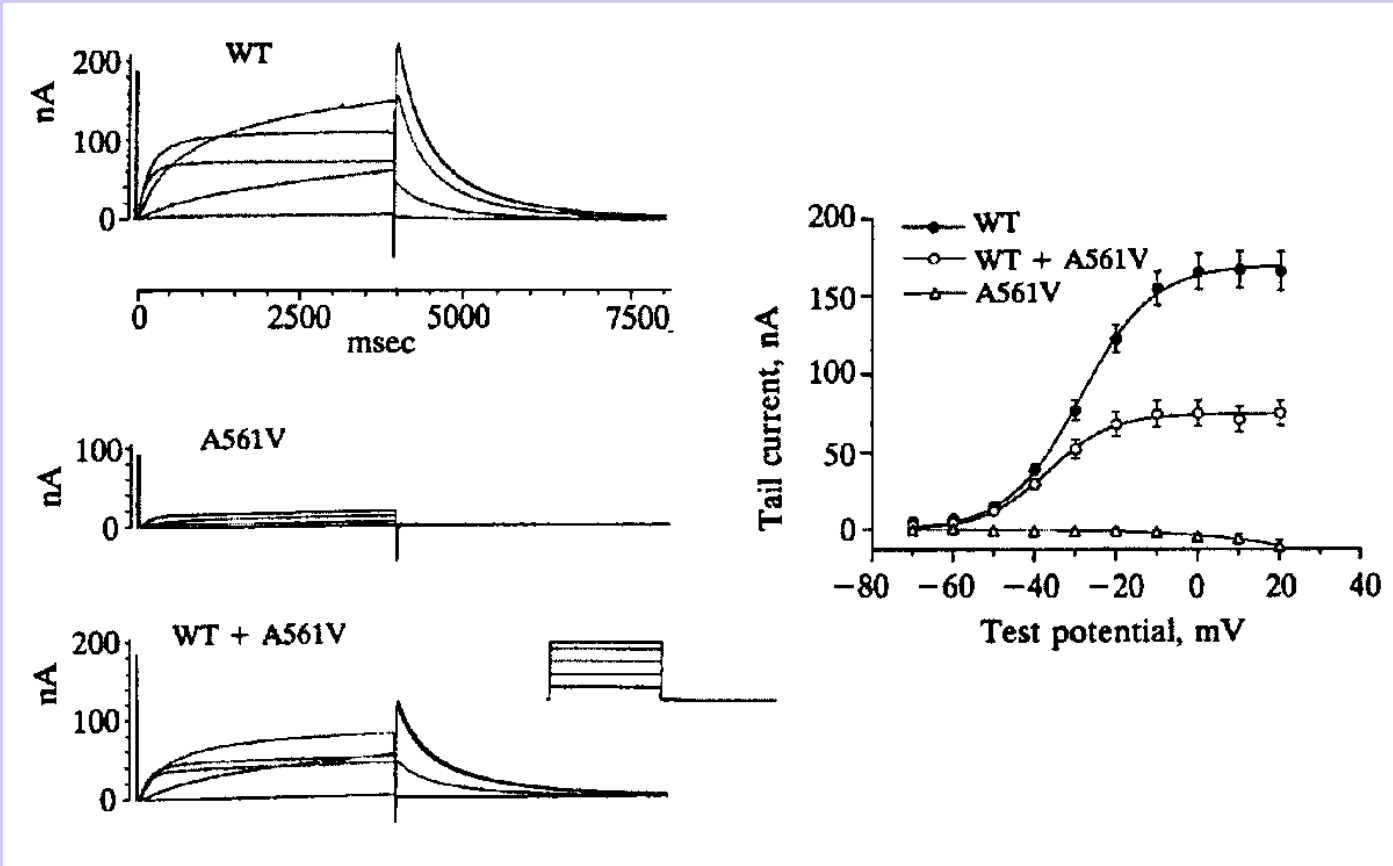
Increased sodium channel activity

SCN5a



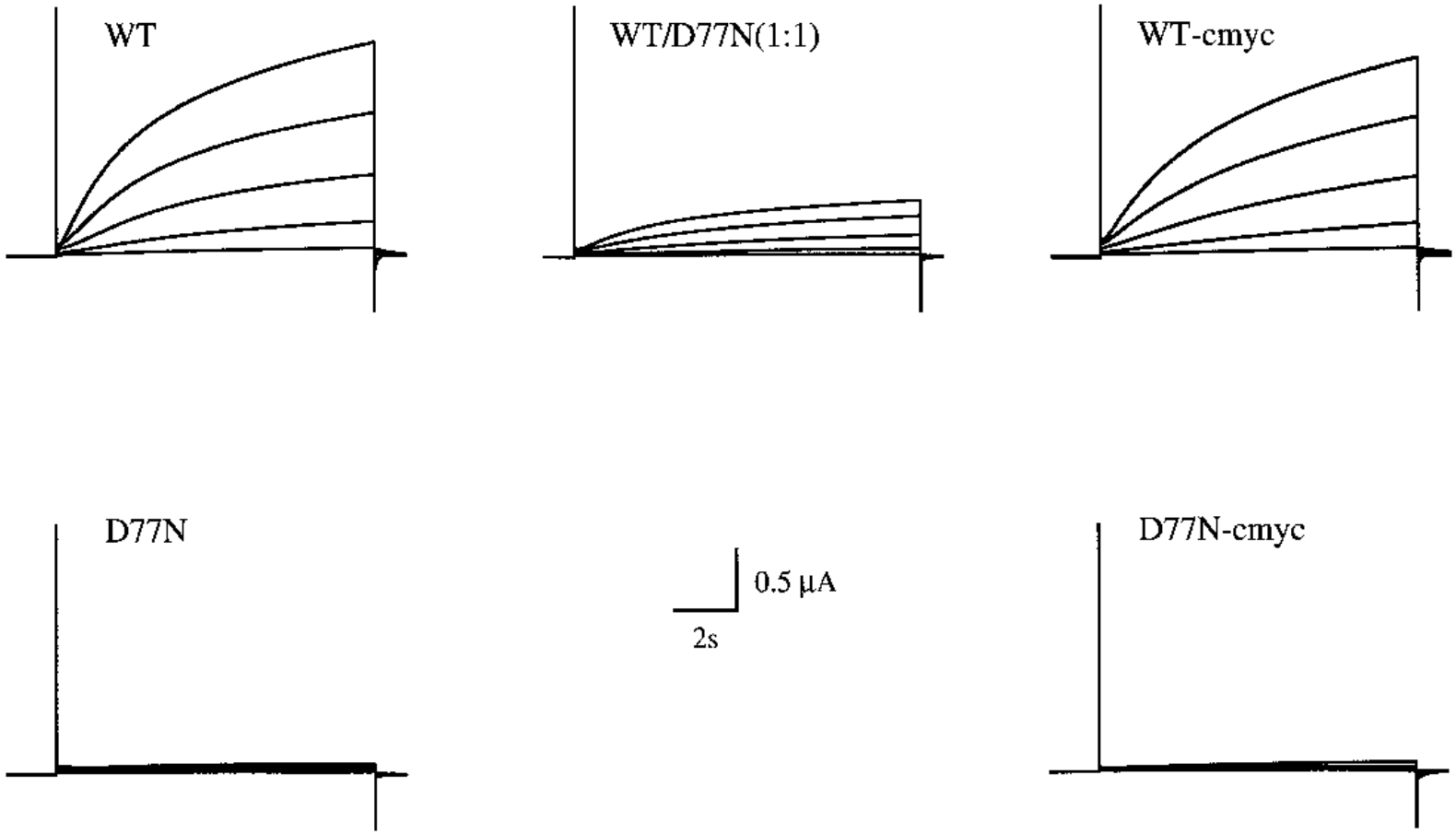
Bennett et al. 1995. Nature. 376:683-685

Decreased potassium channel flux



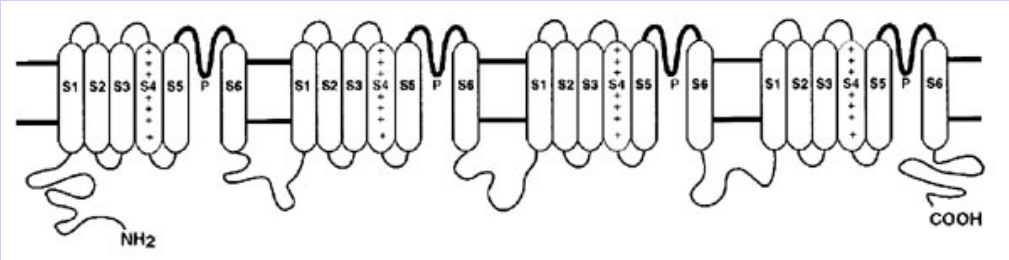
HERG

Sanguinetti et al. 1996. PNAS. 93:2208-2212

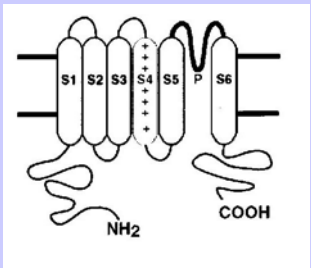

WT
WT/D77N(1:1)
WT-cmyc
D77N
0.5 μA
2s
D77N-cmyc

Topology of subunits in these cardiac ion channels

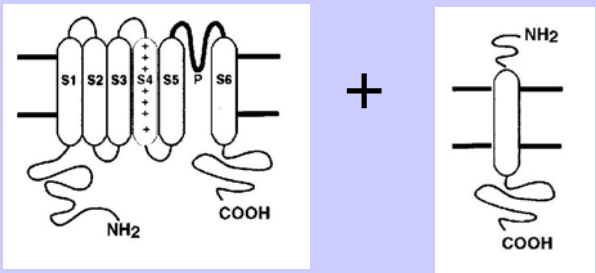
SCN5A



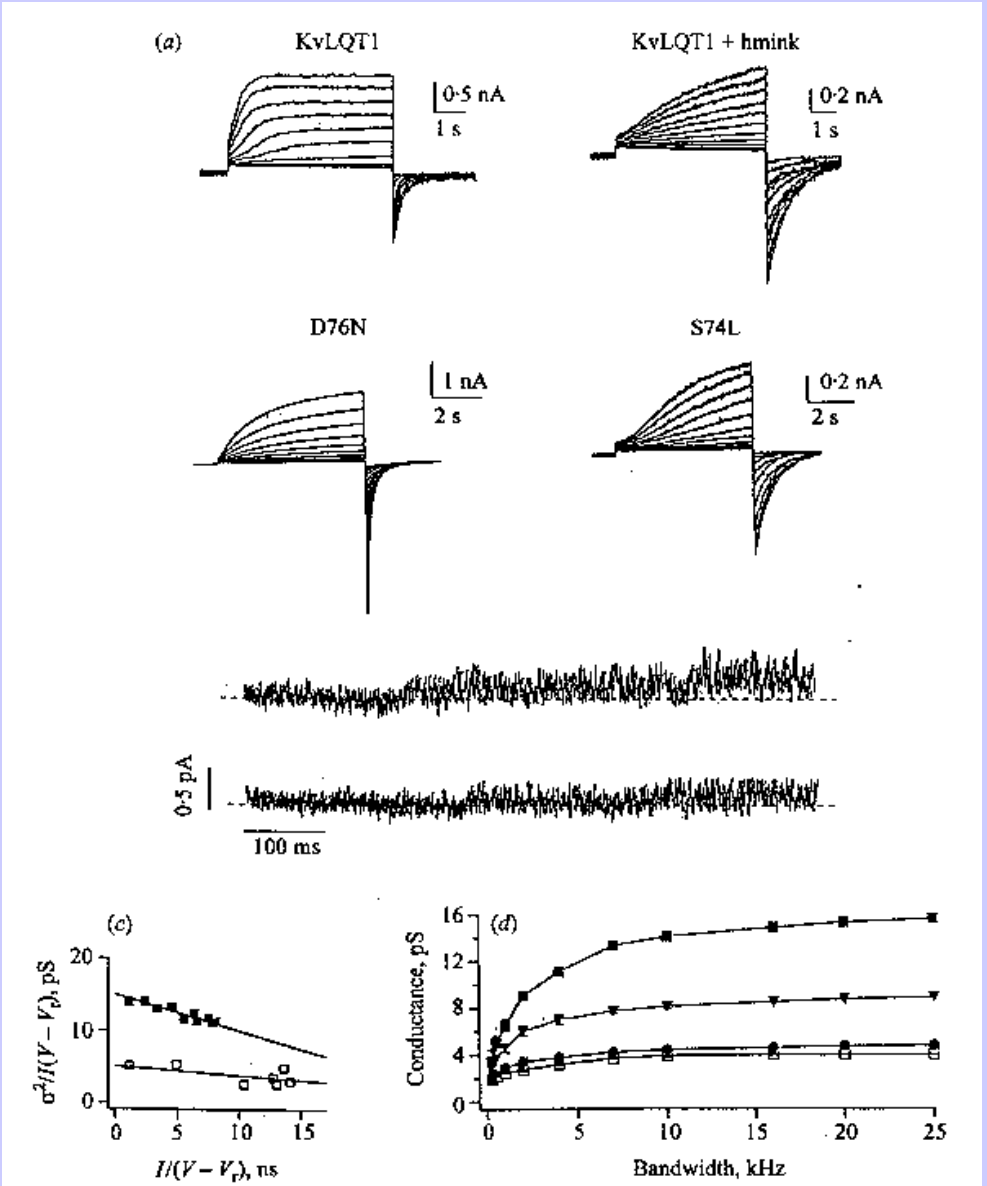
HERG



KvLQT1 + MinK



KvLQT1 + MinK forms I_{Ks}



$I = N\ i\ Po$

MinK is in an accessory subunit family

```
KCNE1 (MinK)  ---------- ------MILS NTTAVTPFLT KLWQETVQQG GNMSGLARRS PRSSDGKLEA LYVLMVLGFF GFFTLGIMLS YIRSKKLEHS NDPFNVYIES
KCNE2 (MiRP1) ---------- MSTLSNFTQT LEDVFRRIFI TYMDNWRQNT TAEQEALQAK VDAENFYYVI LYLMVMIGMF SFIIVAILVS TVKSKRREHS NDPYHQYIVE
KCNE3 (MiRP2) --METTNGTE TWYESLHAVL KALNATLHSN LLCRPGPGLG PDNQTEERRA SLPGRDDNSY MYILFVMFLF AVTVGSLILG YTRSRKVDKR SDPYHVYIKN
KCNE4 (MiRP3) ---------- ---------- ----MLKMEP LNSTHPGTAA SSSPLESRAA GGGSGNGNEY FYILVVMSFY GIFLIGIMLG YMKSKRREKK SSLLLLYKDE
KCNE5 (MiRP4) MNCSESQRLR TLLSRLLLEL HHRGNASGLG AGPRPSMGMG VVPDPFVGRE VTSAKGDDAY LYILLIMIFY ACLAGGLILA YTRSRKLVEA KDEPSQACAE

KCNE1 (MinK)  DAWQEKDKAY VQARVLESYR SCYVVENHLA IEQPNTHLPE TKPSP
KCNE2 (MiRP1) DWQEKYKSQI LNLEESKATI HENIGAAGFK MSP
KCNE3 (MiRP2) RVSMI
KCNE4 (MiRP3) ERLWGEAMKP LPVVSGLRSV QVPLMLNMLQ ESVAPALSCT LCSMEGDSVS SESSSPDVHL TIQEEGADDE LEETSETPLN ESSEGSSENI HQNS
KCNE5 (MiRP4) HEWAPGGALT ADAEAAAGSQ AEGRRQLASE GLPALAQGAE RV
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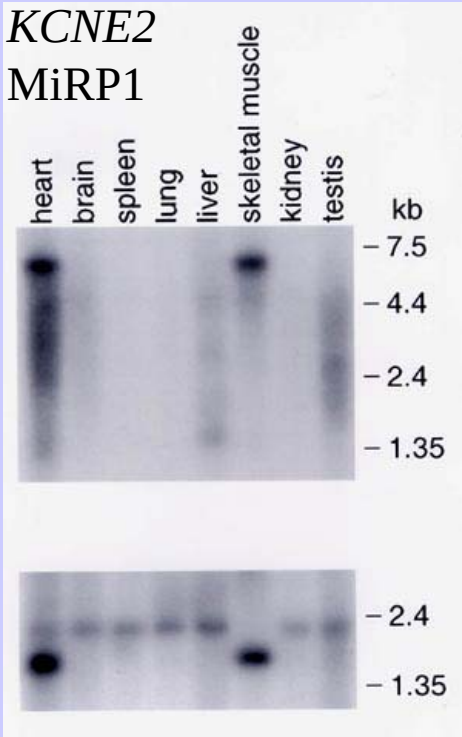
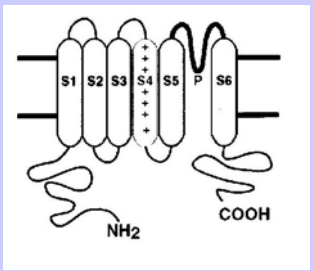
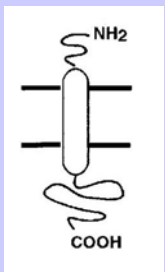
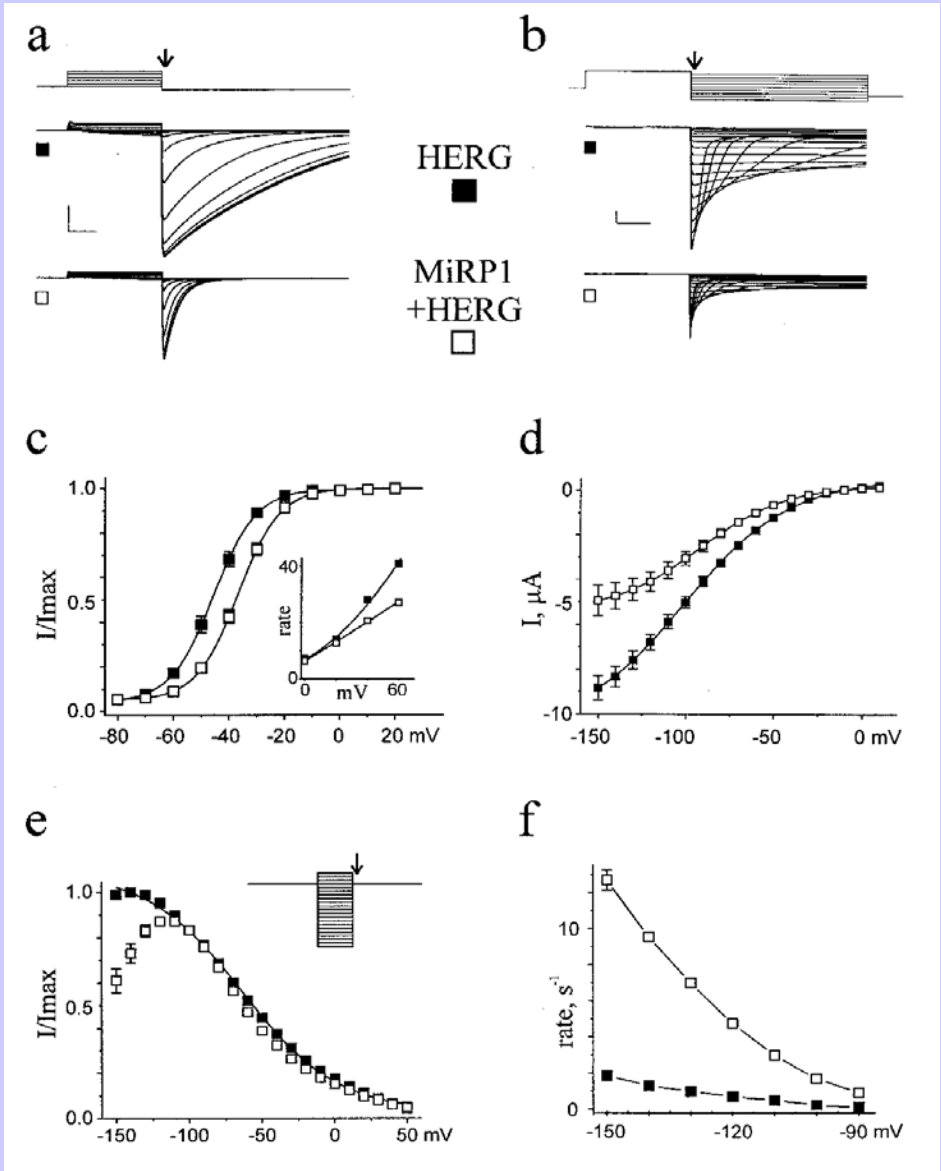


HERG + MiRP1 forms I_{Kr}

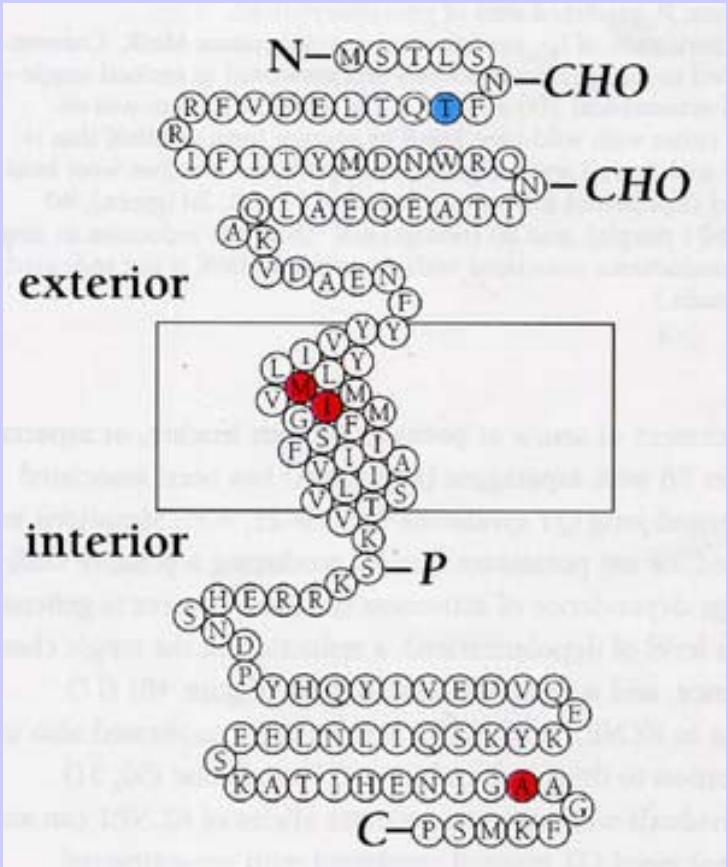


+





MiRP1 Mutations and Polymorphism Study II (Drug-induced LQTS)



T8A-MiRP1

M54T-MiRP1

I57T-MiRP1

A116V-MiRP1

$$I = N\ i\ Po$$

Ion channel principles III

- Ion channels make hearts beats (AP) by opening and closing in an orchestrated fashion to pass specific ions
- Normal function (I = N *i* Po)
- Altered N, *i*, or Po can cause (or predispose to) disease and be result from mutant subunits or 2^{o} events
- Ion channels are complexes of pore-forming & accessory (& regulatory) subunits

Ion channels and nanomedicine: the promise

1639

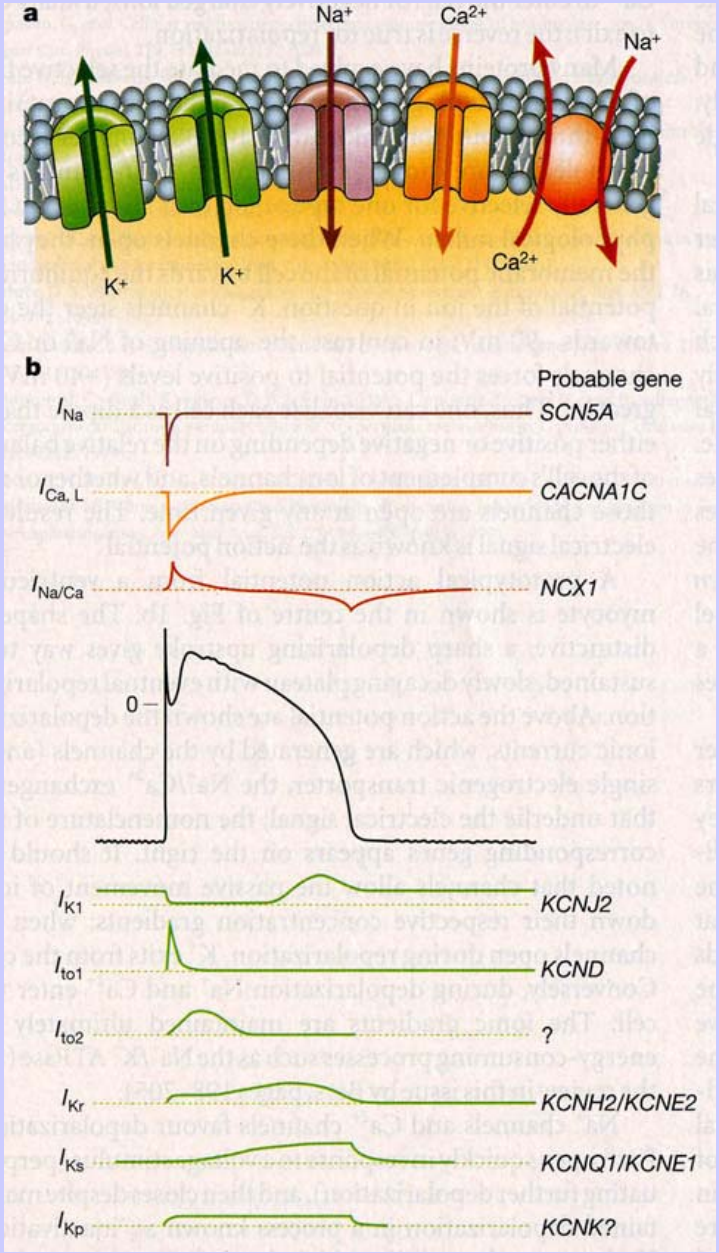
- Alter N, *i*, Po of native channels to treat or prevent disease (drugs)

- Gene therapy (alter function, replace absent or abnormal, or add new channels into normal or diseased tissue) with native or designed channel subunits

- Sensors (*in vitro* and *in vivo*)

Ion channels & nanomedicine: needs

1661



Marban. 2002. Nature. 415:213-218.

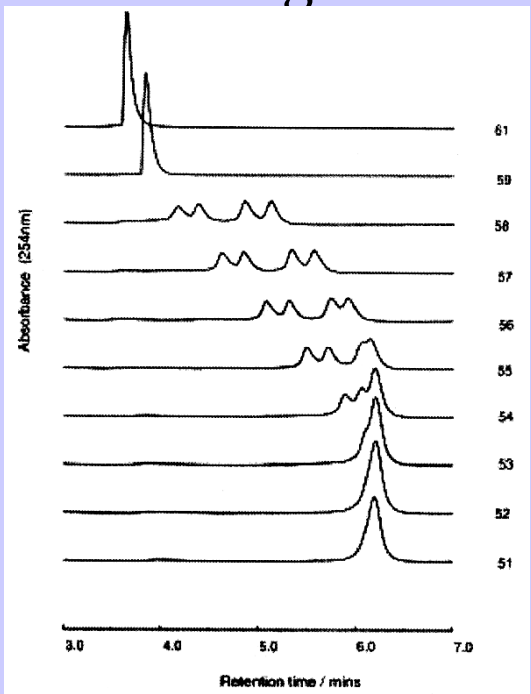
Identify and catalog the plethora of channels and modulators in human tissues (cell biology)

- Location (cellular & sub-cellular)
- Subtypes and splice variants
- Partners (accessory)
- Regulation of expression
- Modulation of activity

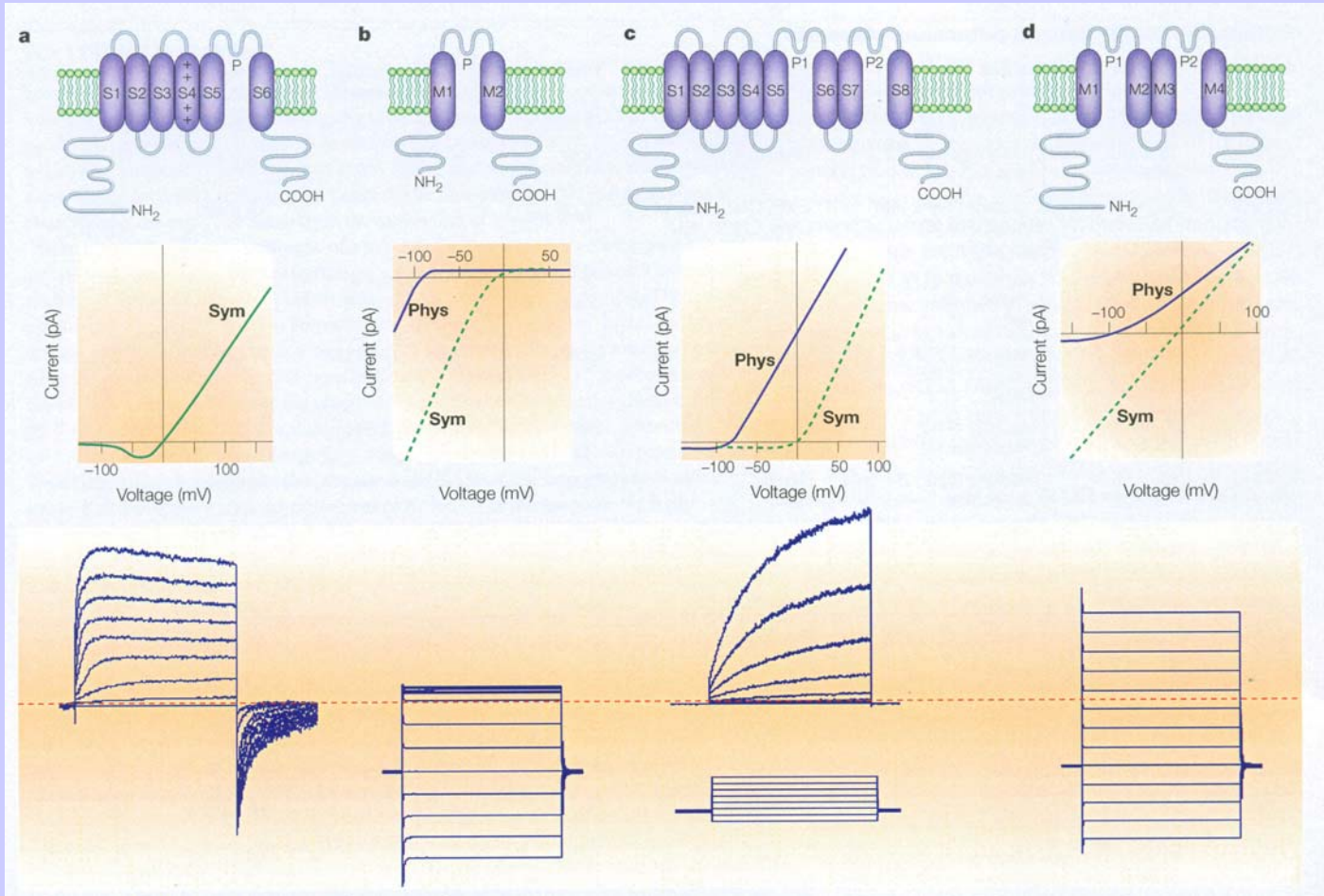
Identify genetic variations that alter function in populations and individuals (human genetics) in order to:

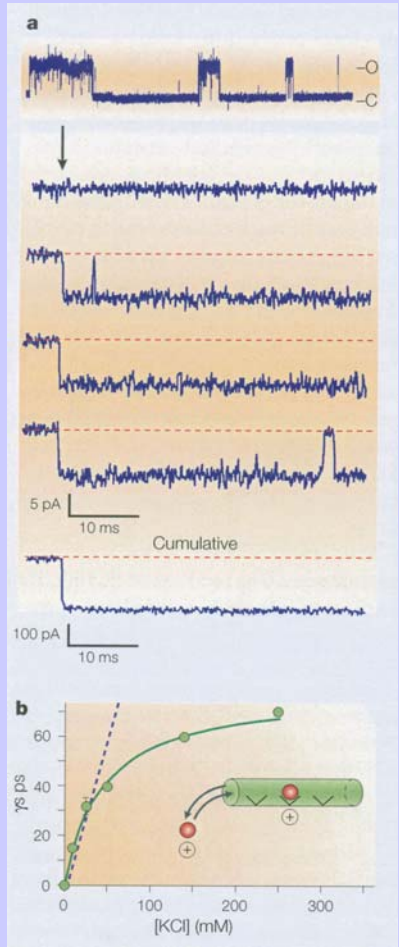
- Predict disease susceptibility (diagnosis and prevention)
- Predict drug response (optimize therapy)
- Avoid treatment side effects (adverse drug reactions)

denaturing HPLC



Delineate function for each complex (macroscopic & single biophysics)





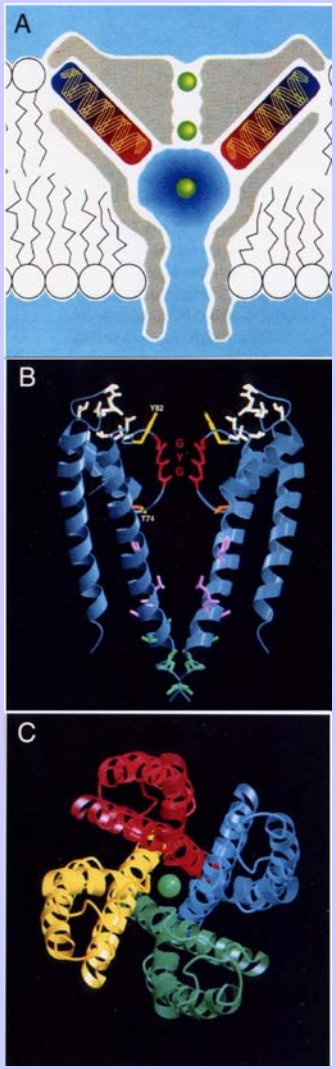
Kv Kir Tok K2P K2PØ

Identify and design agents to alter function (pharmacology & chemistry)

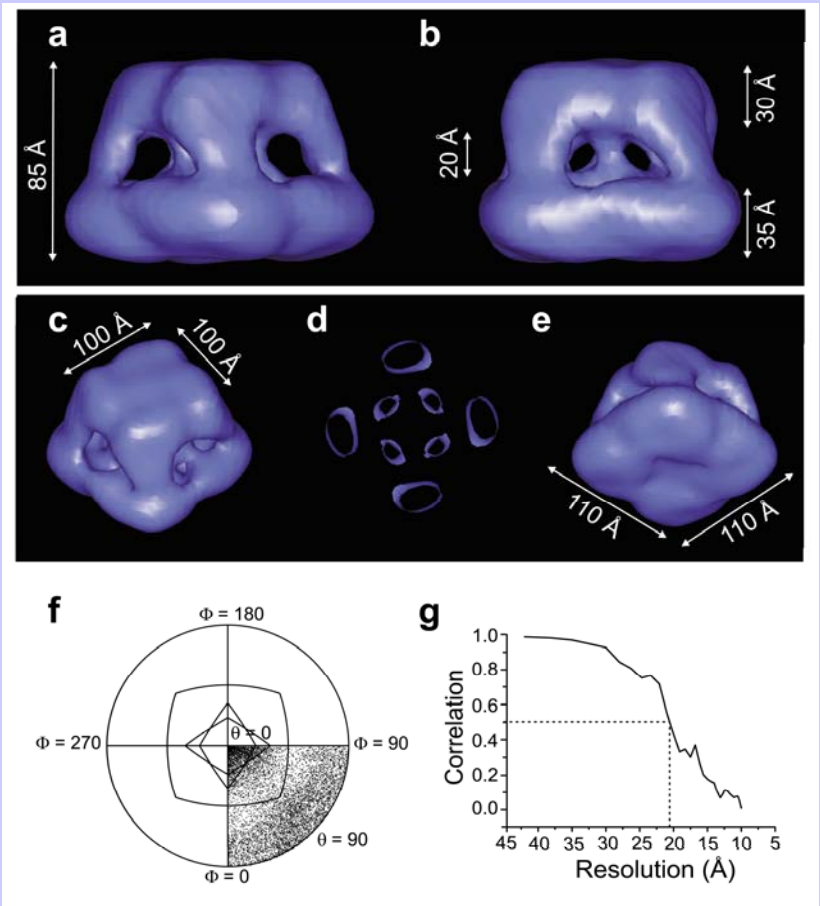
Table 1. K_d values of peptide and small-molecule modulators of Kv1.3 channels[a–c]

Inhibitor	K_d value	Inhibitor	K_d value
Stichodactyla helianthus toxin	11 pM	*Parabuthus* toxin 3	492 nM
Heterometrus spinnifer toxin 1	12 pM	*Parabuthus* toxin 1	800 nM
ShK–F6CA	48 pM	Resiniferatoxin	3 μM
Pandinus imperator toxin 2	50 pM	Nifedipine (3)	5 μM
ShK–Dap22	52 pM	Nitrendipine (11)	5 μM
Hongotoxin	86 pM	Ibu8	5 μM
Margatoxin	110 pM	Phencyclidine	5 μM
Agiotoxin-2	200 pM	Verapamil (2)	6 μM
Pandinus imperator toxin 3	500 pM	H37	10 μM
Kaliotoxin	650 pM	Hg^{2+}	10 μM
Noxiustoxin	1 nM	Quinine	14 μM
Psora4 (10)	3 nM	Cicutotoxin	18 μM
Charybdotoxin	3 nM	La^{3+}	20 μM
Titystoxin-Kα	4 nM	Trifluoperazine	20 μM
Pandinus imperator toxin 1	11 nM	Capsaicin	26 μM
Tetraphenylporphyrin 3* (1)	20 nM	Diltiazem	27 μM
Bunodosoma granulifera toxin	39 nM	Progesterone	30 μM
trans-N-propyl-carbamoyloxy-PAC (7)	50 nM	κ-Hefutoxin	40 μM
Correolide (6)	90 nM	Luteolin	50 μM
Sulfamidbenzamidoindane (8)	100 nM	Flecainide	60 μM
Maurotoxin	150 nM	4-AP	190 μM
CP339818	150 nM	Zn^{2+}, Co^{2+}	200 μM
WIN173173 (4)	200 nM	Ba^{2+}, Cd^{2+}	2 mM
UK78282 (5)	200 nM	TEA	10 mM
Dendrotoxin	250 nM	Mn^{2+}	20 mM
PAC	270 nM		

Define composition & structure of human ion channels (biochemistry & structural biology)



KcsA by X-ray.
Doyle et al. 1998. Nature. 280:69-77.



Kv4.2/KChiP (cardiac Ito) by EM

Success will accrue from inter-disciplinary collaborations

Colleagues

Voltage-gated channels	Background channels	Fungal channels
Geoff Abbott*	Detlef Bockenhauer	Aamir Ahmed*
Peter Bowers	Nitza Ilan*	**Maggie Butler**
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Haijun Chen	Ann Kao*	Karen Ketchum*
Cyrus Komer*	Ita O'Kelly*	Natalia Nikoleva*
Leo Kim	Astrid Kollewe	Ted Shih
Dan Levy	Sindhu Rajan	Andrew Sellers*
Federico Sesti*	David Rosenthal*	Jing-mei Wang*
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D. Roden (Vanderbilt)		
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L. Ptacek (UCSF)		
G. Fishman (NYU)		
T. McDonald (Einstein)		
N. Grigorieff (Brandeis)		

NIH, DDCF