Ion channels and disease: *Channelopathies*

Dr Stefan Trapp

Channelopathies

William Harvey
1657

"…nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual form of nature, by careful investigation of the rarer forms of disease."

Channelopathies

Prime examples to aid our understanding of the role of ion channels in health and disease

Congenital Channelopathies

-mutation in gene encoding ion channel

Acquired Channelopathies

-autoimmune disease (e.g. Myasthenia gravis)

-dysregulation of protein expression

Congenital channelopathies can produce a major insight into the structure-function relationship in ion channels

Channelopathies

Major advances over the past 25 years

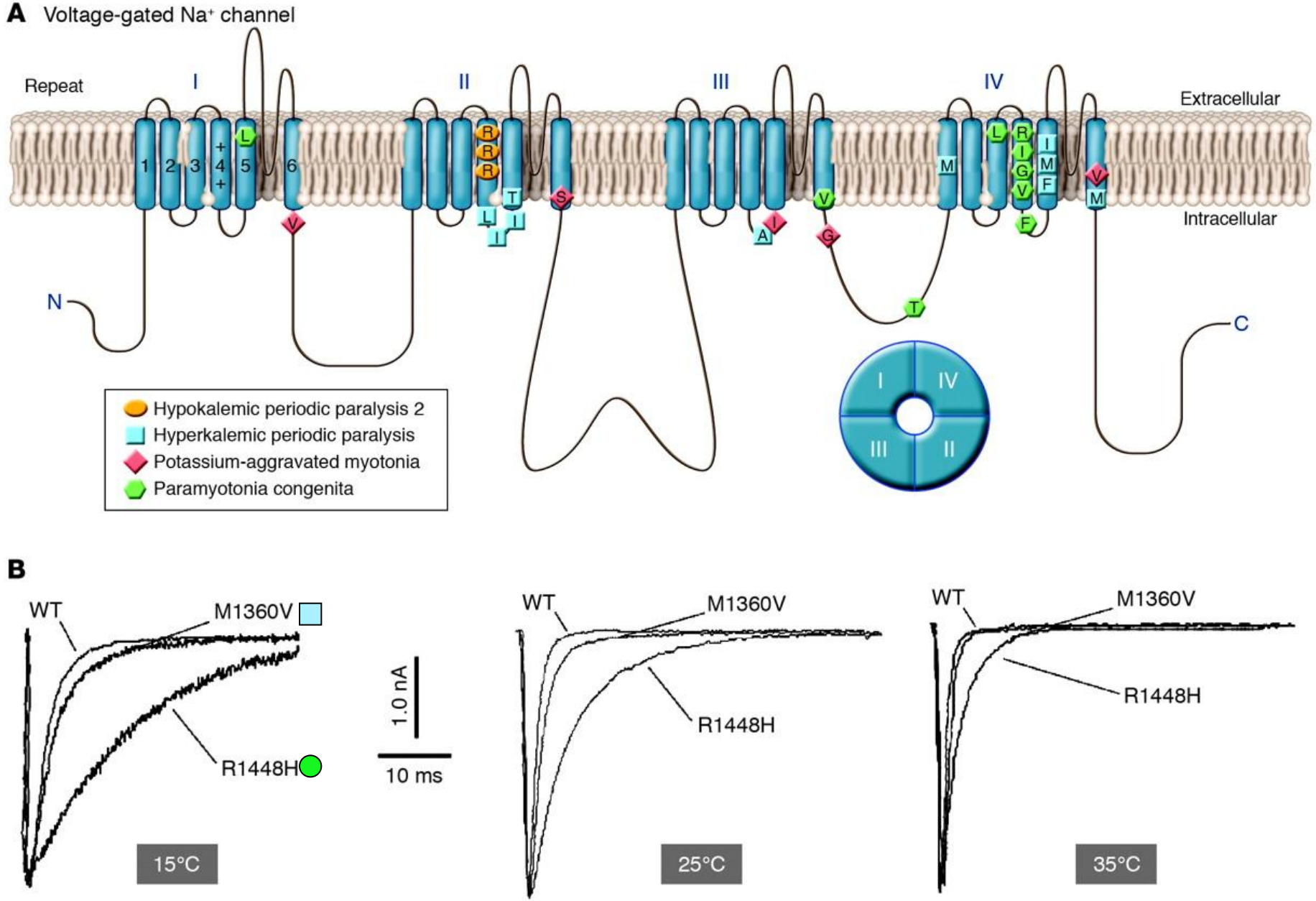
Facilitated by advances in:

- Genetics
  - Cloning techniques
  - Automated sequencing
  - Linkage analysis
- Molecular biology
  - Recombinant expression systems
  - Site-directed mutagenesis
  - Transgenic animal models
- Electrophysiology
  - Patch-clamp technique

Four examples

- Congenital indifference to pain and related syndromes
- ‘stiff legged goats’ and related myotonias
- Startle Disease
- Long QT syndrome

Na^+ channel-related muscle channelopathies

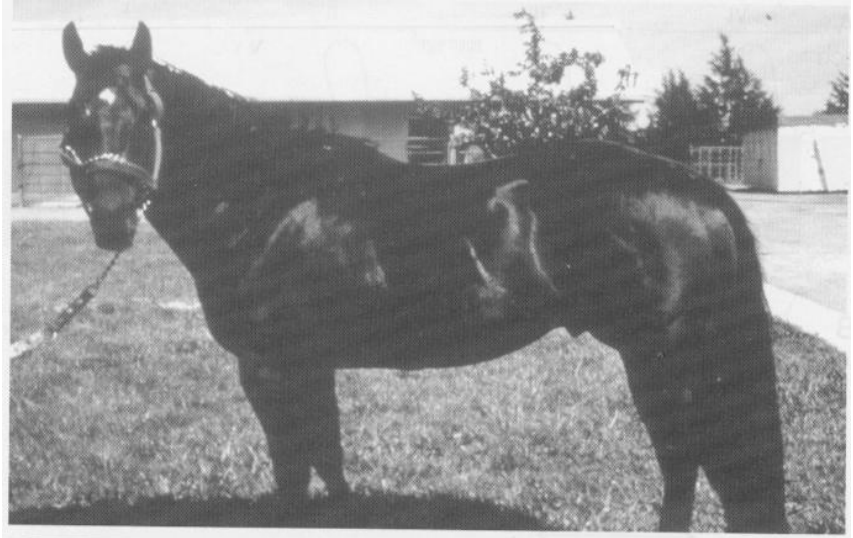


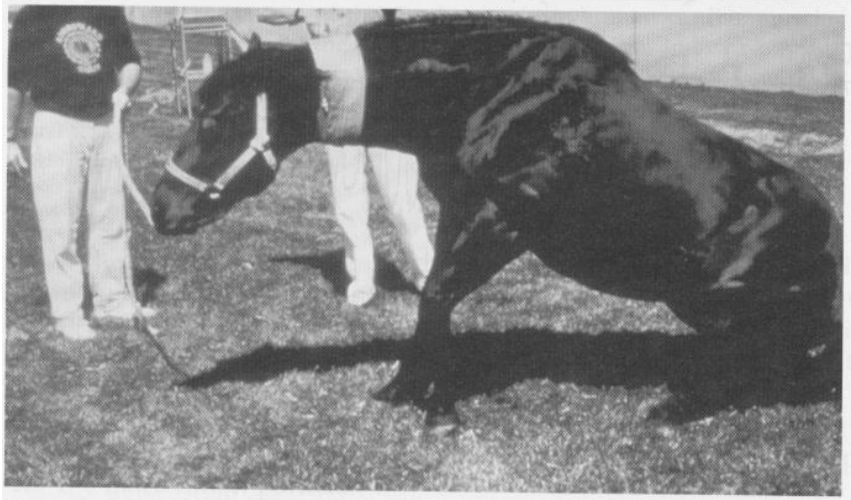
Jurkat-Rott, K. et al. J. Clin. Invest. 2005;115:2000-2009

equine hyperkalaemic periodic paralysis

‘common’ in American thoroughbred quarter horses

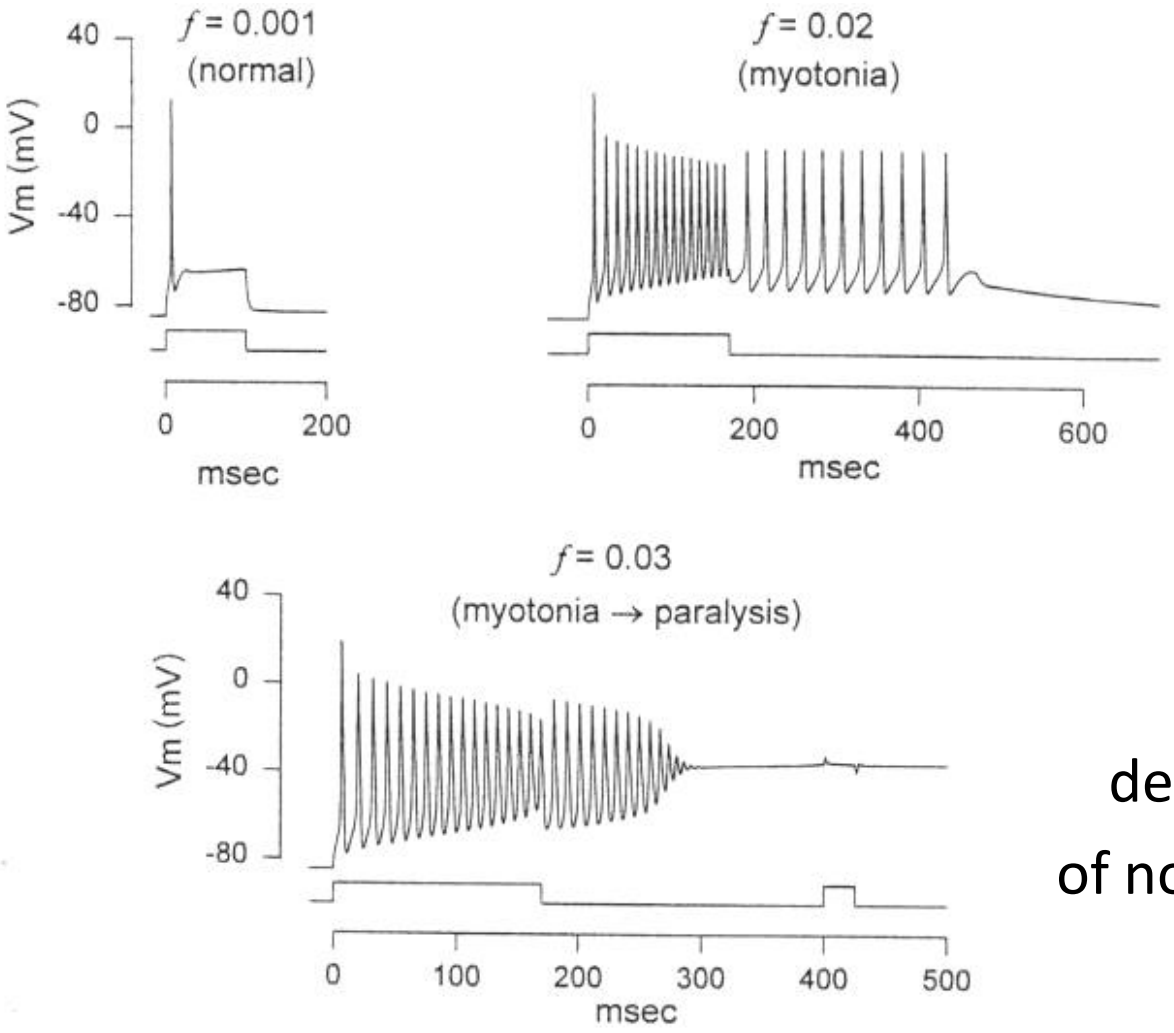
Very well developed musculature
Most famous stallion ‘Impressive’





Injection of KCl induces attack of paralysis

Computer simulations of electrical activity in a model muscle cell

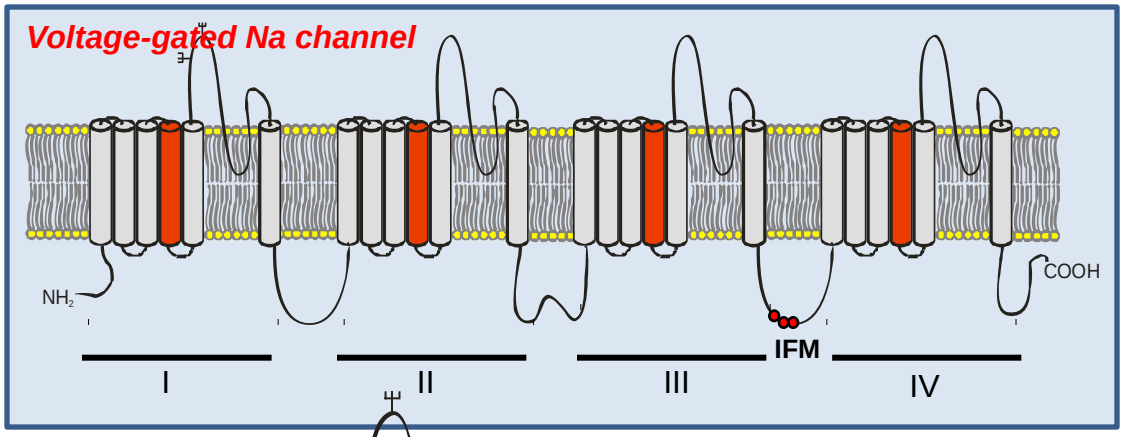


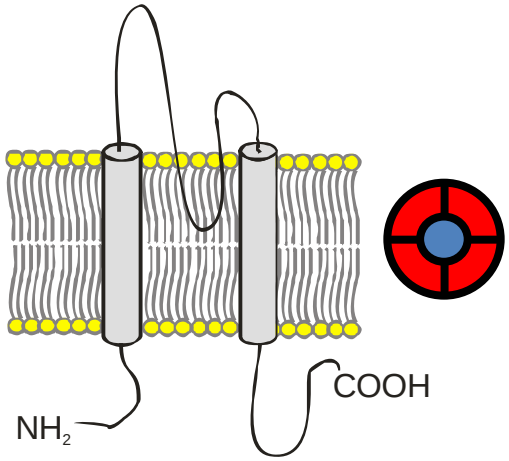
The phenotype
(myotonia v paralysis)
depends on the fraction (f)
of non-inactivating Na^+ current

‘Francis Crick … said that in the pioneering days of structure determination researchers were driven by the conviction that once they had solved a biological structure, its function or mechanism would become immediately obvious. …

Fersht, AR (1995). Curr.Opin.Struct.Biol.5-79-84.

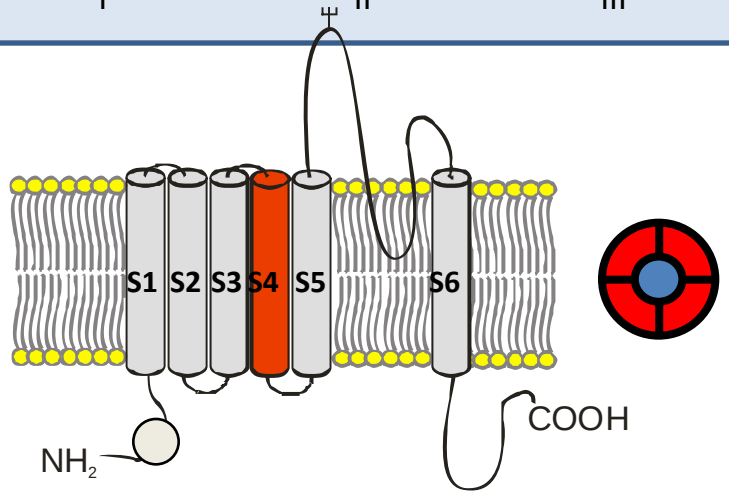
K channels are the most diverse ion channel family





Inward rectifiers (K_{ir})

KCNH



Voltage-gated K channels (K_v)

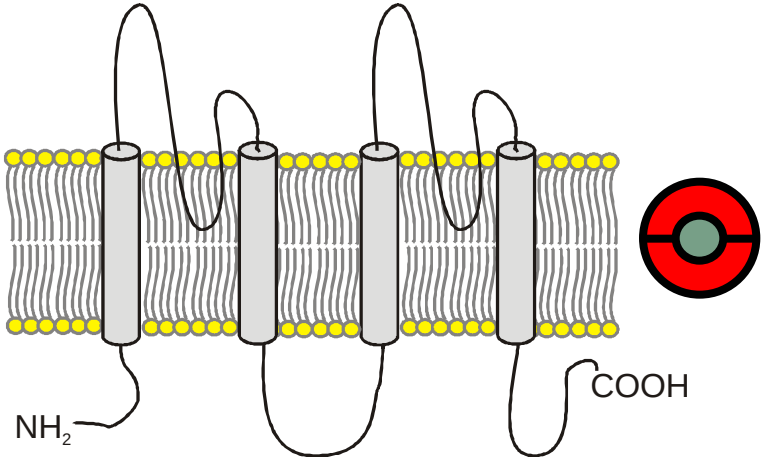
(Delayed rectifiers)

KCNQ channels

(slow delayed rectifiers)

Ca-activated K channels (BK, SK)

(KCNM, KCNN)



2 pore domain K channels

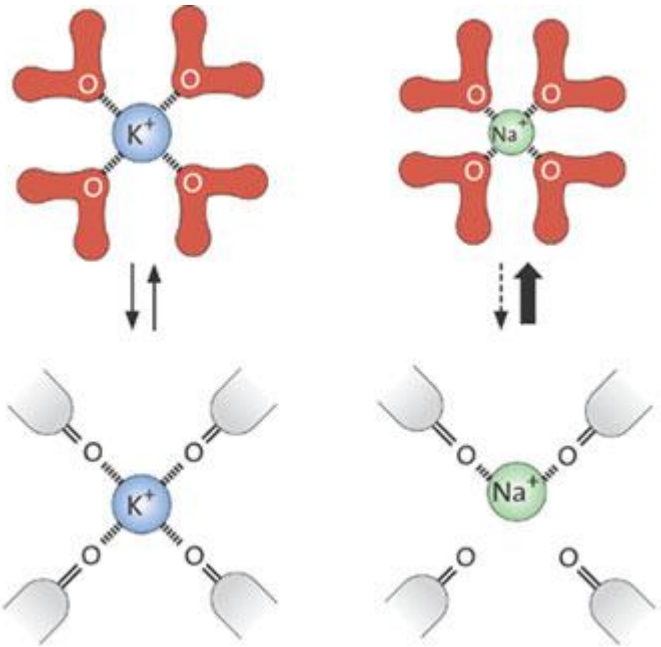
KCNK

Modular design allows heteromultimers!

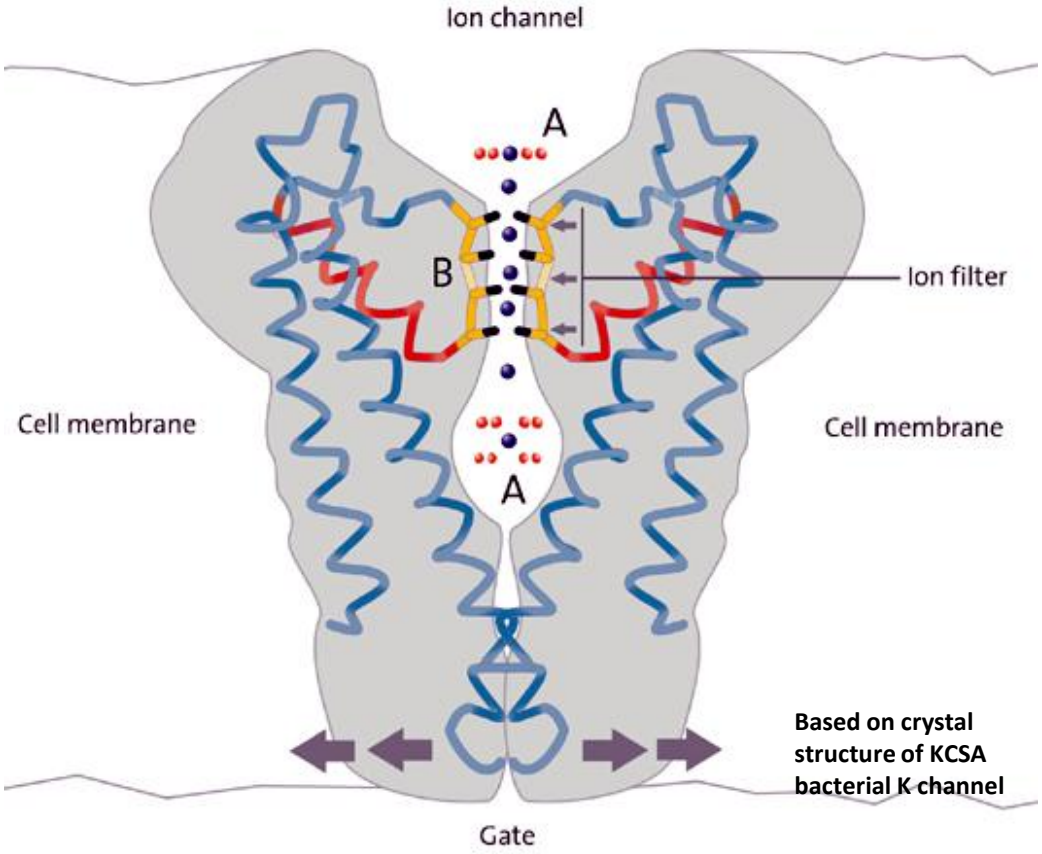
K channel structural features

2 essential functional features

- High throughput
- **High selectivity**

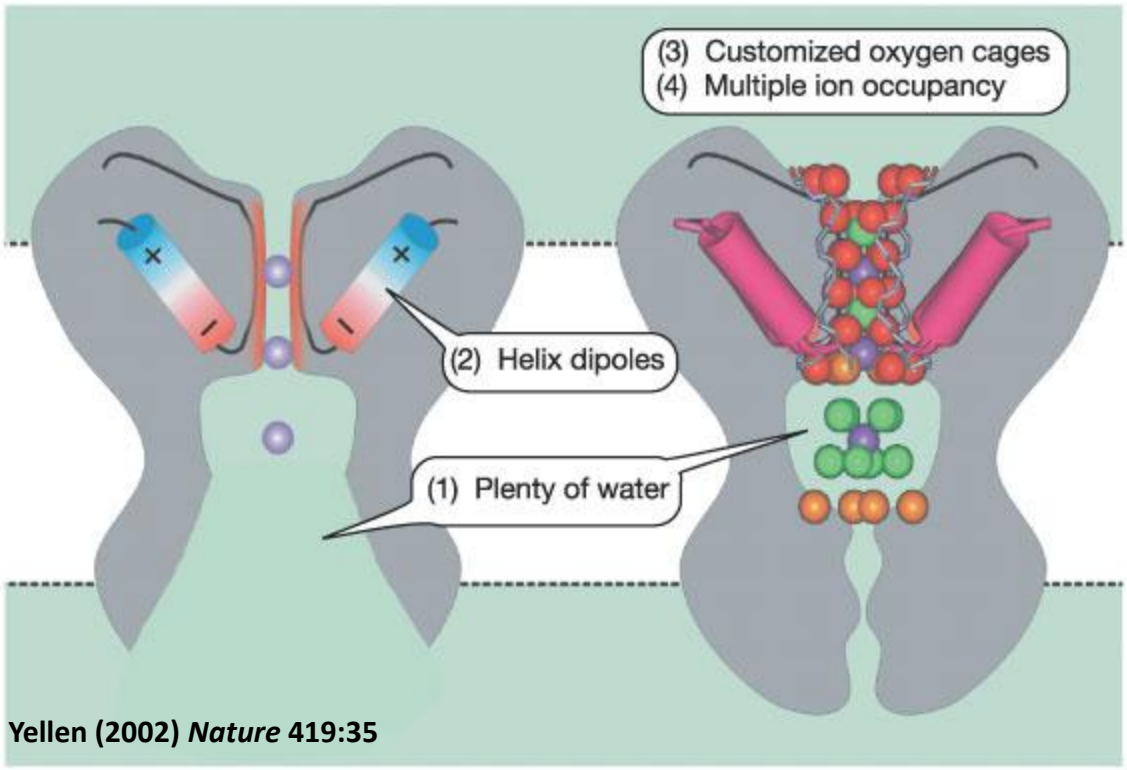


Selectivity filter



Consensus sequence in pore loop:TXXTXGYGD
K channel signature sequence

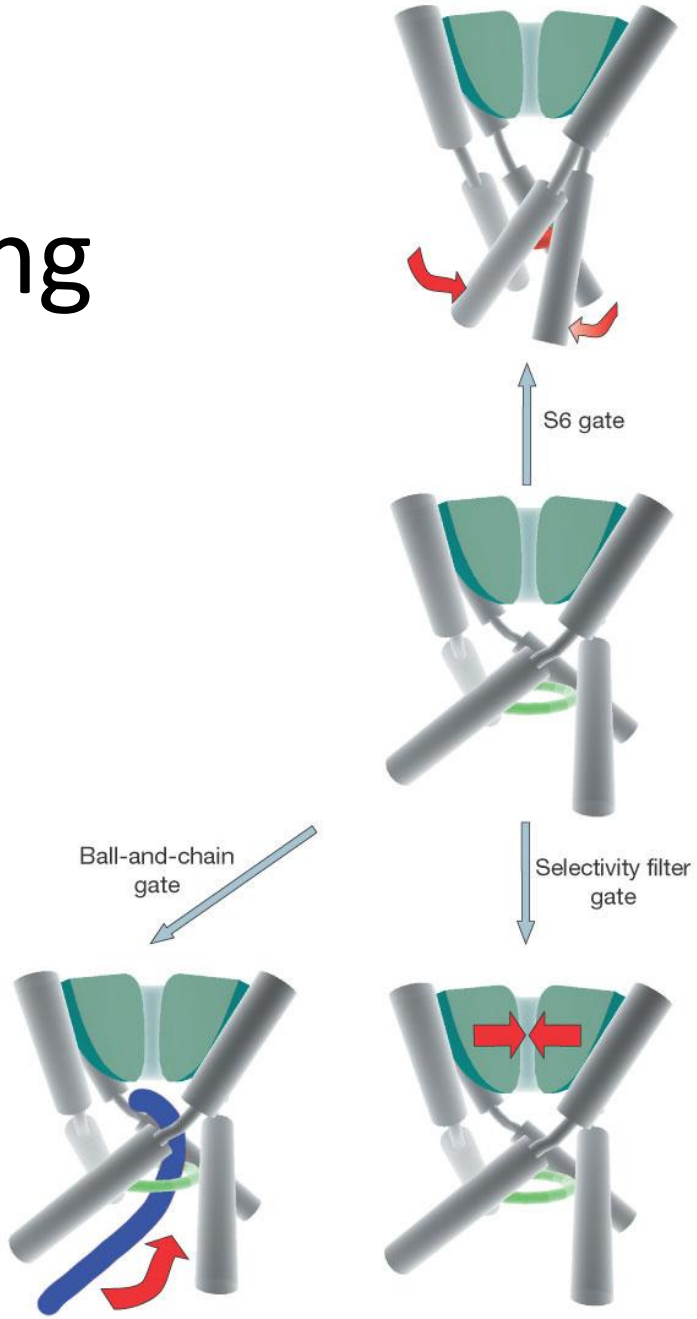
High throughput



Yellen (2002) *Nature* 419:35

No ionic charges involved!

K channel gating



‘Stiff-legged’ Myotonic goats

“a herd of goats in Texas that fell over every time the train went past their field”

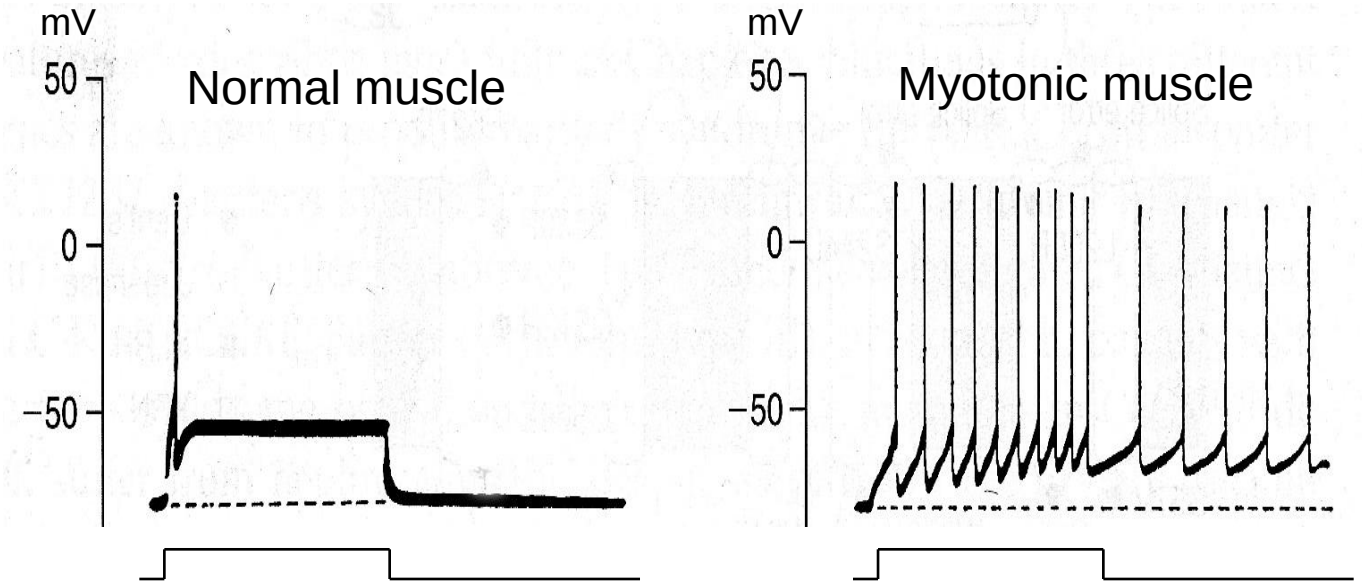
Congenital myotonia

- Muscle stiffness, alleviated by warm-up
- Continuous muscle activity produces well-developed muscles (attractive for breeders)

First channelopathy identified

(Bryant 1969)

Myotonic muscle shows enhanced excitability (due to reduced chloride conductance)



Adrian & Bryant (1974) J Physiol 240, 505

CLC-1 channel mutations cause myotonia

- Results from a loss-of-function mutation in *CLCN1*, the gene encoding the skeletal muscle Cl^- channel.

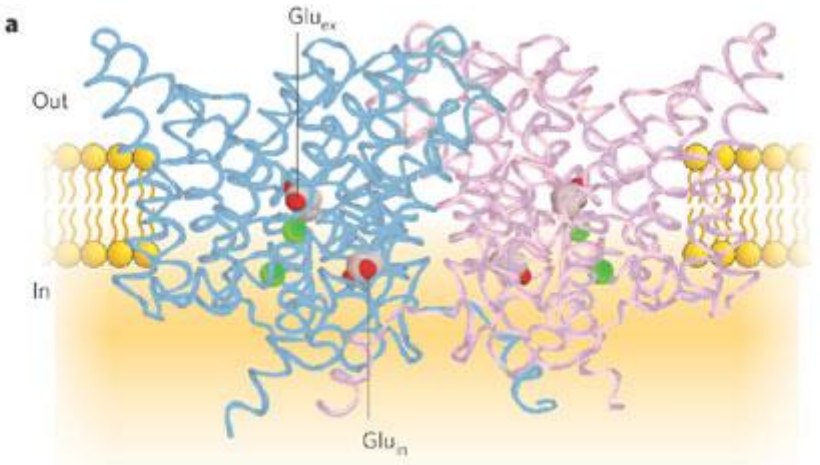
- Mutations in the human *CLCN1* gene cause *Myotonia congenita* (Thomsen's disease; 1876) and *Generalised myotonia* (Becker's Disease; 1957)

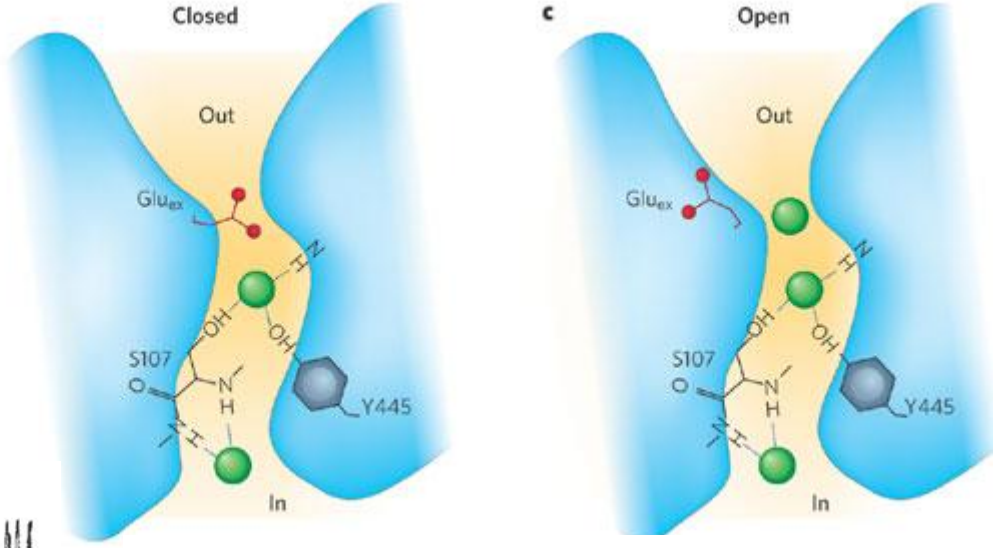
How can a loss of Cl^- conductance lead to myotonia?

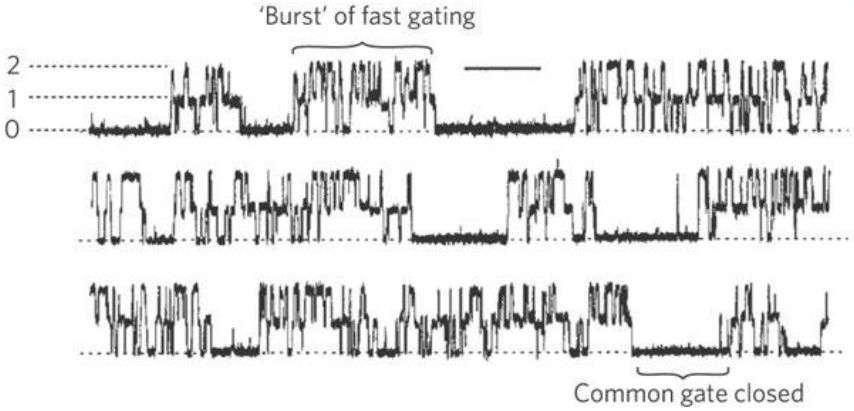
Isn't the K^+ conductance responsible for repolarisation after an action potential?

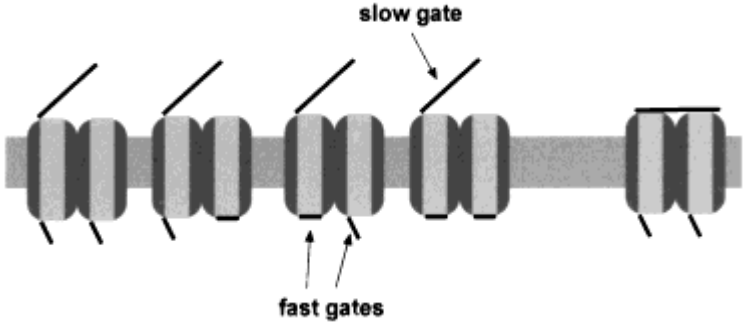
Extracellular potassium accumulation (in T-tubules)


nature
Chloride channel structure


a
Glu$_{ex}$
Out
In
Glu$_{in}$

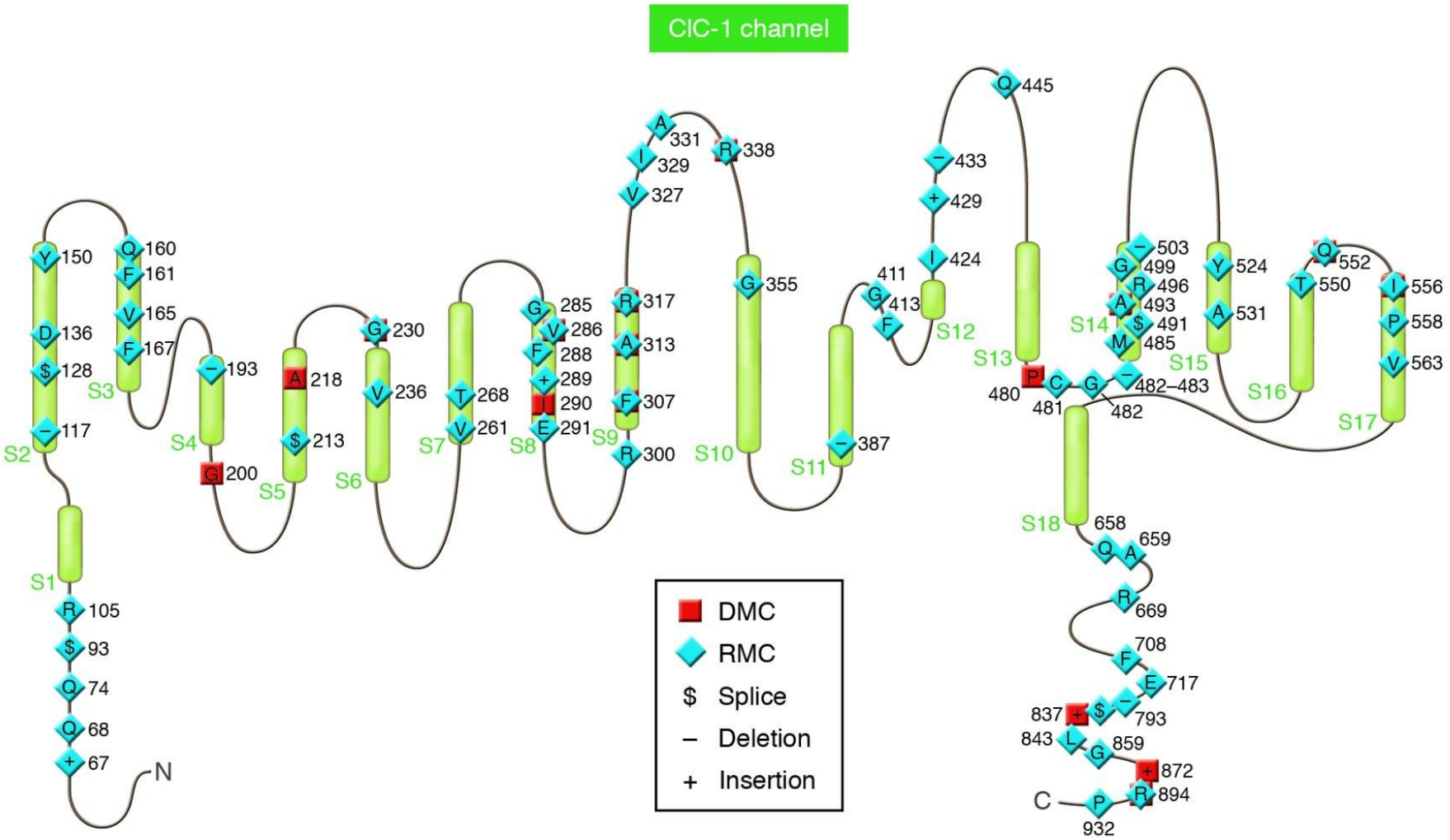

b
Closed
Out
Glu$_{ex}$
S107
Y445
In
c
Open
Out
Glu$_{ex}$
S107
Y445
In


'Burst' of fast gating
2
1
0
Common gate closed


slow gate
fast gates

‘Francis Crick … said that in the pioneering days of structure determination researchers were driven by the conviction that once they had solved a biological structure, its function or mechanism would become immediately obvious. It came as a shock when they found this was not necessarily so and that the opposite was more frequently true’

Fersht, AR (1995). Curr.Opin.Struct.Biol.5-79-84.



DMC: dominant myotonia congenita
RMC: recessive myotonia congenita

Jurkat-Rott, K. et al. J. Clin. Invest. 2005;115:2000-2009

Startle disease

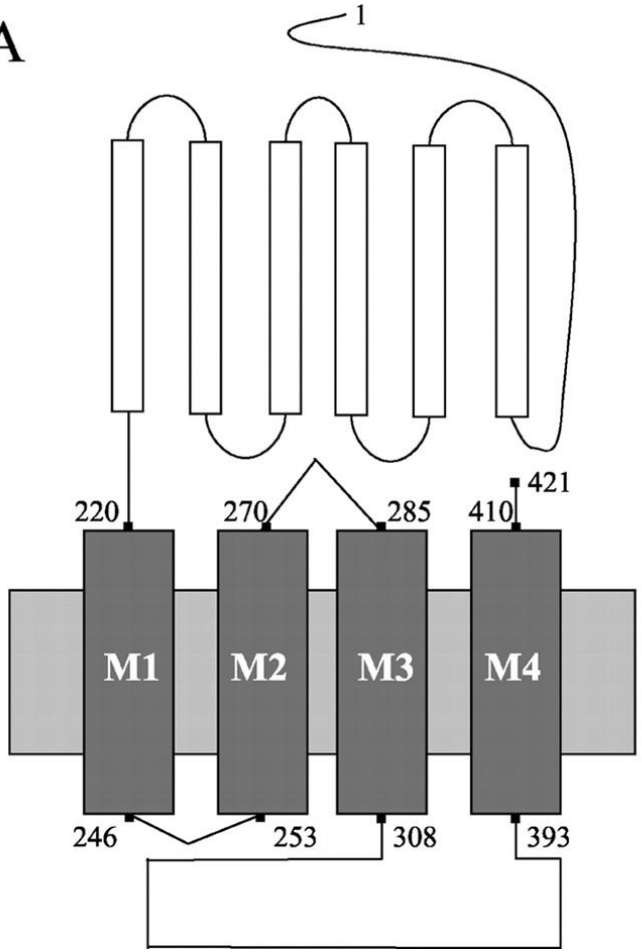
- Spasmodic mouse
- Cattle myoclonus
- Hyperekplexia

Glycine receptor mutations causing Startle Disease in human, mouse & cattle

Mutation and Subunit	Inheritance Mode	Effect on GlyR Function
α1 P250T	Autosomal dominant	Reduced single-channel conductance, reduced glycine sensitivity, increased desensitization rate
α1 V260M	Autosomal dominant	As yet unknown
α1 Q266H	Autosomal dominant	Reduced open probability, reduced glycine sensitivity
α1 S270T	Autosomal dominant	As yet unknown
α1 R271L/Q	Autosomal dominant	Reduced glycine sensitivity, reduced single-channel conductance
α1 K276E	Autosomal dominant	Reduced glycine sensitivity, reduced open probability
α1 Y279C	Autosomal dominant, variable penetrance	Reduced glycine sensitivity, reduced whole cell current magnitude
α1 I244N	Autosomal recessive	Reduced glycine sensitivity, reduced whole cell current magnitude, increased desensitization rate
α1 Deletion of exons 1-6	Autosomal recessive	Presumed nonfunctional
α1 S231R	Autosomal recessive	Reduced membrane insertion
α1 Stop codon at Y202	Autosomal recessive	Reduced surface expression, possible heterozygosity with α1 V147M
α1 G342S	Compound heterozygous?	No effect of individual mutation, possible heterozygosity with other mutations
α1 R252H + α1 R392H	Compound heterozygous	Reduced membrane insertion
βG229D + βexon 5 loss	Compound heterozygous	Reduced glycine sensitivity, reduced surface expression
βLine-1 intronic insertion	Autosomal recessive (*Spastic*)	Reduced surface expression
α1 A52S	Autosomal recessive (*Spasmodic*)	Reduced glycine sensitivity
α1 Stop codon	Autosomal recessive (*Oscillator*)	Reduced surface expression
α1 Stop codon	Autosomal recessive (*Myoclonus*)	Reduced surface expression

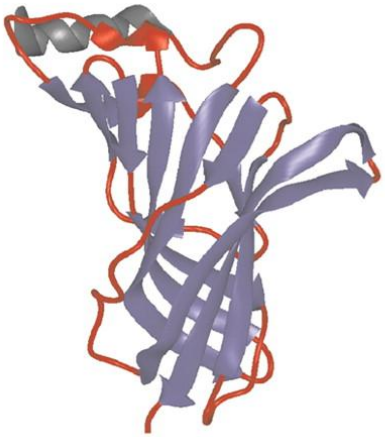
Nicotinicoid receptor structures (Acetylcholine receptor, GABA receptor, Glycine receptor**)**

A

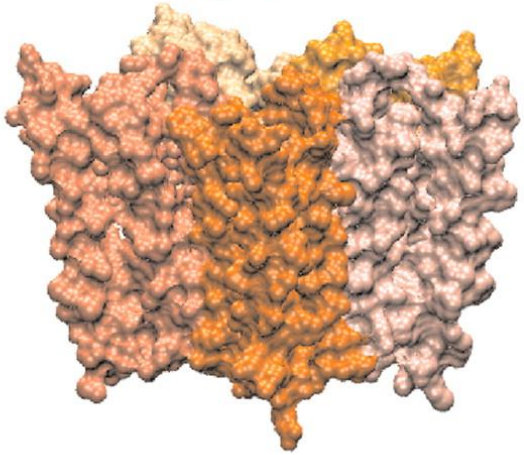


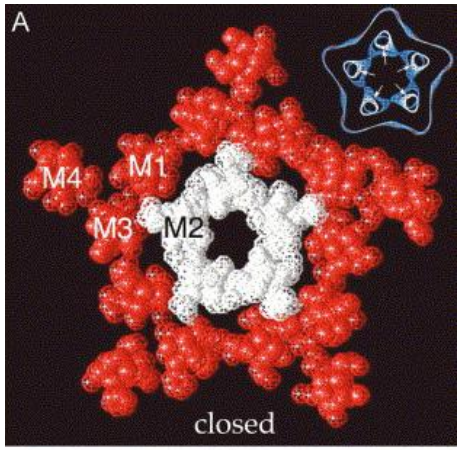
Cascio, M. J. Biol. Chem. 2004;279:19383-19386

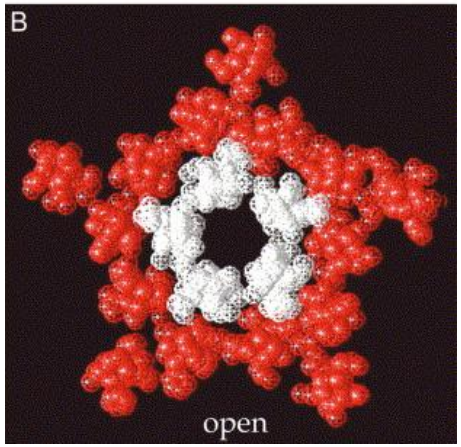
B



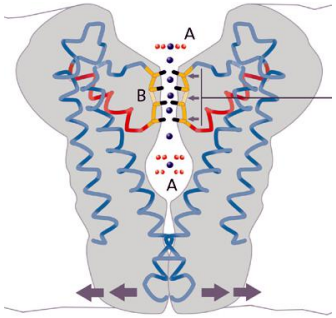
C



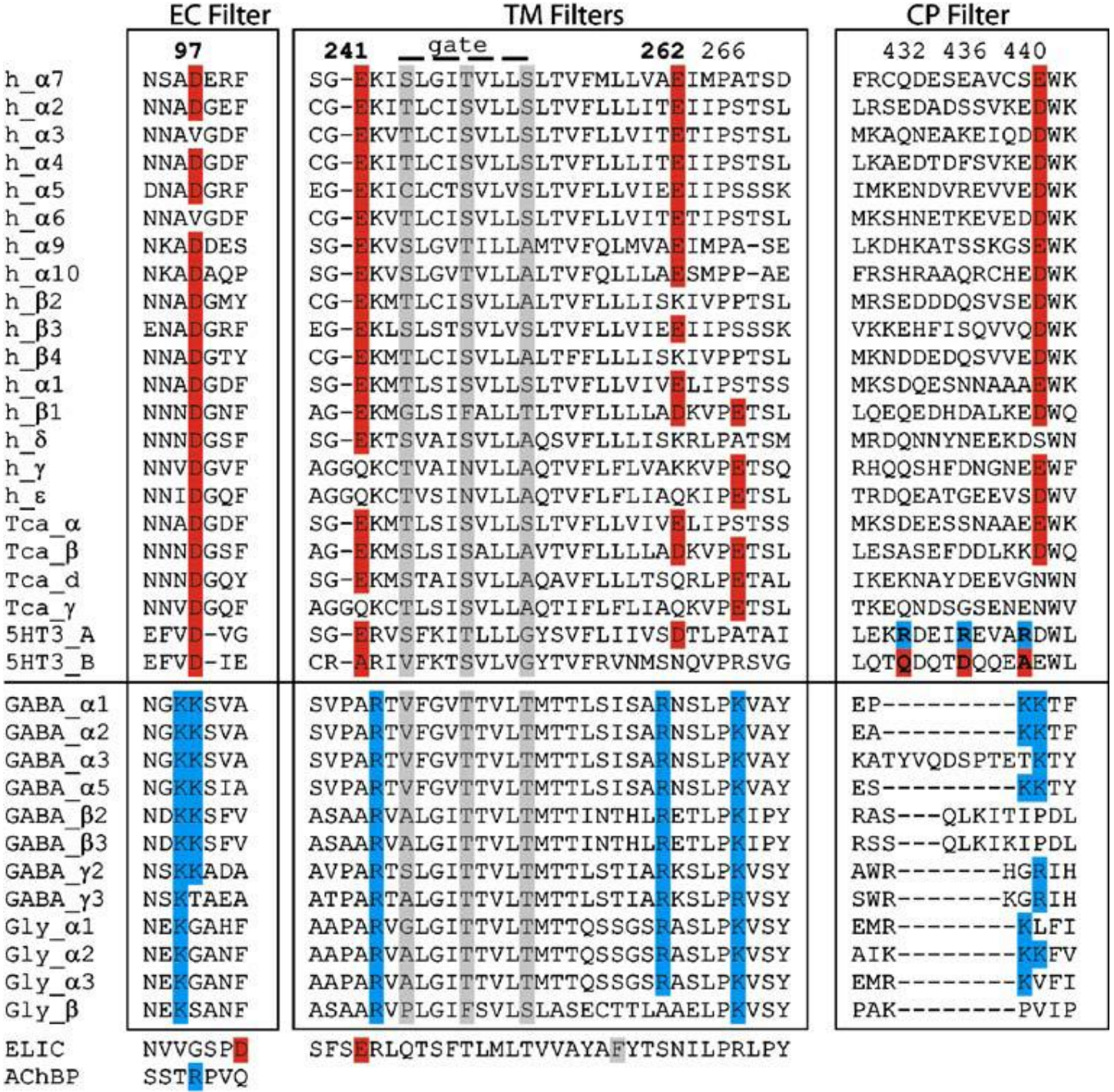




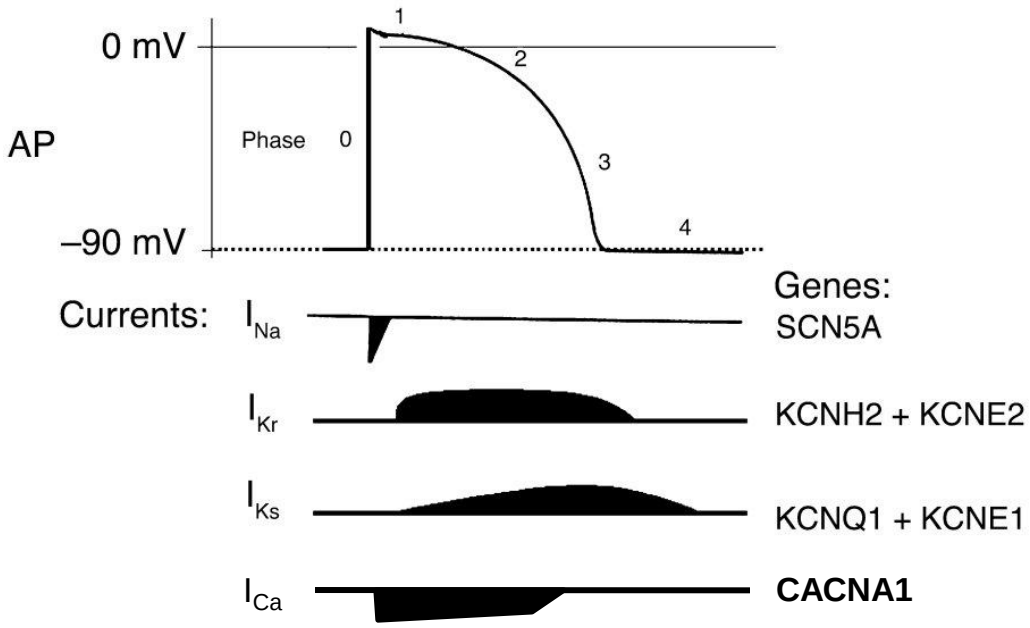
Unwin, N. FEBS Letters 2003; 555:91-95



K^+channel

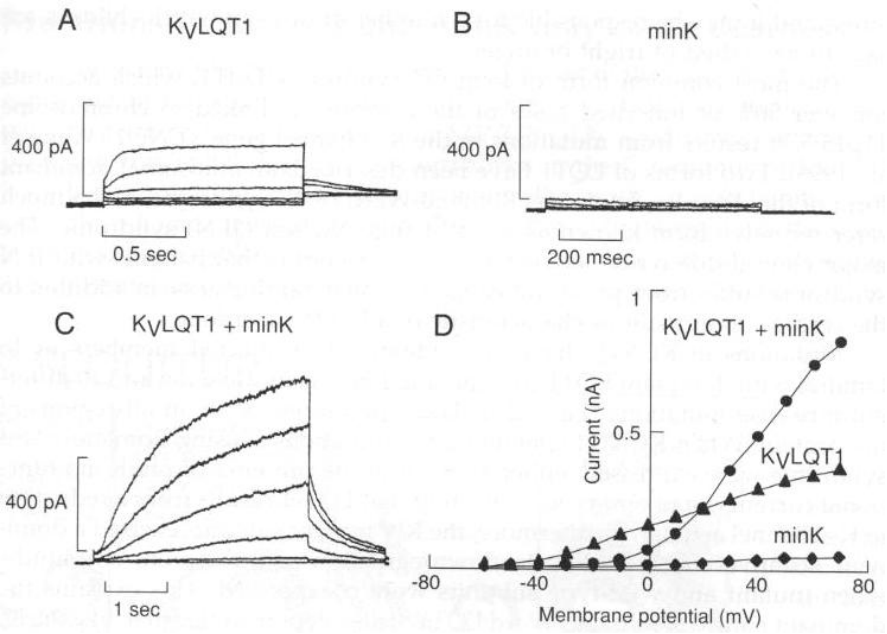

EC Filter
TM Filters
CP Filter
97
241
gate
262
266
432
436
440
h_α7 NSADERF SG-EKISLGITVLLSLTVFMLLVAEIMPATSD FRCQDESEAVCSEWK
h_α2 NNADGEF CG-EKITLCISVLLSLTVFLLLITEIIPSTSL LRSEDADSSVKEDWK
h_α3 NNAVGDF CG-EKVTLCISVLLSLTVFLLVITETIPSTSL MKAQNEAKEIQDDWK
h_α4 NNADGDF CG-EKITLCISVLLSLTVFLLLITEIIPSTSL LKAEDTDFSVKEDWK
h_α5 DNADGRF EG-EKICLCTSVLVSLTVFLLVIEEIIPSSSK IMKENDVREVVEDWK
h_α6 NNAVGDF CG-EKVTLCISVLLSLTVFLLVITETIPSTSL MKSHNETKEVEDDWK
h_α9 NKADDES SG-EKVSLGVTILLAMTVFQLMVAEIMPA-SE LKDHKATSSKGSEWK
h_α10 NKADAQP SG-EKVSLGVTVLLALTVFQLLLAESMPP-AE FRSHRAAQRCHEDWK
h_β2 NNADGMY CG-EKMTLCISVLLALTVFLLLISKIVPPTSL MRSEDDDQSVSEDWK
h_β3 ENADGRF EG-EKLSLSTSVLVSLTVFLLVIEEIIPSSSK VKKEHFISQVVQDWK
h_β4 NNADGTY CG-EKMTLCISVLLALTFFLLLISKIVPPTSL MKNDDEDQSVVEDWK
h_α1 NNADGDF SG-EKMTLSISVLLSLTVFLLVIVELIPSTSS MKSDQESNNAAAEWK
h_β1 NNNDGNF AG-EKMGLSIFALLTLTVFLLLLADKVPETSL LQEQEDHDALKEDWQ
h_δ NNNDGSF SG-EKTSVAISVLLAQSVFLLLISKRLPATSM MRDQNNYNEEKDSWN
h_γ NNVDGVF AGGQKCTVAINVLLAQTVFLFLVAKKVPETSQ RHQQSHFDNGNEEWF
h_ε NNIDGQF AGGQKCTVSINVLLAQTVFLFLIAQKIPETSL TRDQEATGEEVSDWV
Tca_α NNADGDF SG-EKMTLSISVLLSLTVFLLVIVELIPSTSS MKSDEESSNAAEEWK
Tca_β NNNDGSF AG-EKMSLSISALLAVTVFLLLLADKVPETSL LESASEFDDLKKDWQ
Tca_d NNNDGQY SG-EKMSTAISVLLAQAVFLLLTSQRLPETAL IKEKNAYDEEVGNWN
Tca_γ NNVDGQF AGGQKCTLSISVLLAQTIFLFLIAQKVPETSL TKEQNDSGSENENWV
5HT3_A EFVD-VG SG-ERVSFKITLLLGYSVFLIIVSDTLPATAI LEKRDEIREVARDWL
5HT3_B EFVD-IE CR-ARIVFKTSVLVGYTVFRVNMSNQVPRSVG LQTQDQTDQQEAEWL
GABA_α1 NGKKSVA SVPARTVFGVTTVLTMTTLSISARNSLPKVAY EP---------KKTF
GABA_α2 NGKKSVA SVPARTVFGVTTVLTMTTLSISARNSLPKVAY EA---------KKTF
GABA_α3 NGKKSVA SVPARTVFGVTTVLTMTTLSISARNSLPKVAY KATYVQDSPTETKTY
GABA_α5 NGKKSIA SVPARTVFGVTTVLTMTTLSISARNSLPKVAY ES---------KKTY
GABA_β2 NDKKSFV ASAARVALGITTVLTMTTINTHLRETLPKIPY RAS---QLKITIPDL
GABA_β3 NDKKSFV ASAARVALGITTVLTMTTINTHLRETLPKIPY RSS---QLKIKIPDL
GABA_γ2 NSKKADA AVPARTSLGITTVLTMTTLSTIARKSLPKVSY AWR-------HGRIH
GABA_γ3 NSKTAEA ATPARTALGITTVLTMTTLSTIARKSLPRVSY SWR-------KGRIH
Gly_α1 NEKGAHF AAPARVGLGITTVLTMTTQSSGSRASLPKVSY EMR--------KLFI
Gly_α2 NEKGANF AAPARVALGITTVLTMTTQSSGSRASLPKVSY AIK--------KKFV
Gly_α3 NEKGANF AAPARVALGITTVLTMTTQSSGSRASLPKVSY EMR--------KVFI
Gly_β NEKSANF ASAARVPLGIFSVLSLASECTTLAAELPKVSY PAK--------PVIP
ELIC NVVGSPD SFSERLQTSFTLMLTVVAYAFYTSNILPRLPY
AChBP SSTRPVQ

Cardiac action potential



I_{Kr}: rapidly activating K^+current
I_{Ks}: slowly activating K^+current

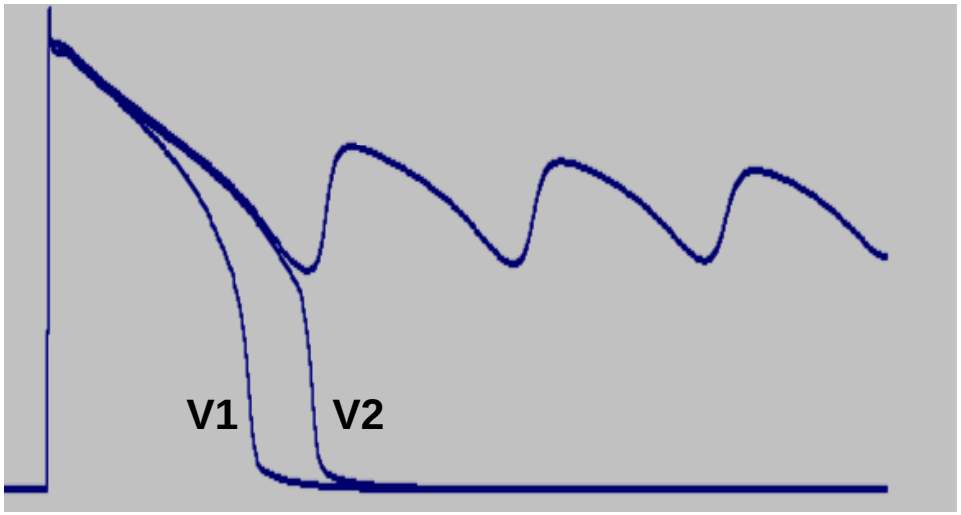
Arthur J. Moss, Robert S. Kass (2005) JCI 115:2018



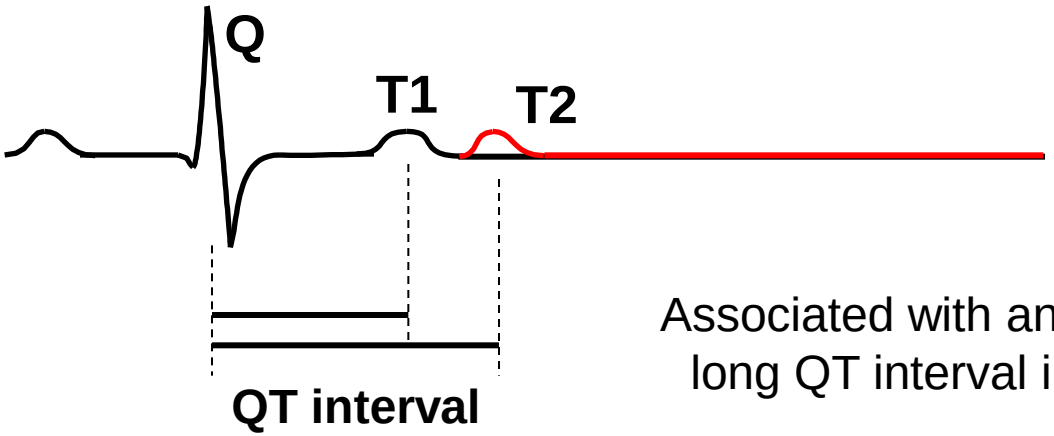
LQT syndrome

Can lead to fatal cardiac arrhythmia, usually precipitated by physical or emotional stress

Ventricular action potential



ECG



Associated with an abnormally long QT interval in the ECG

LQT syndrome

Prolonged depolarization due to enhanced inward Na^+ current?

Slower repolarization due to reduced outward K^+ current?

Disease	Gene (historical name)	Protein
LQT1	*KCNQ1* (KVLQT1)	$I_{Ks}K^+$ channel α subunit
LQT2	*KCNH2* (HERG)	$I_{Kr}K^+$ channel α subunit
LQT3	SCN5A	$I_{Na}Na^+$ channel α subunit
LQT4	ANKB	Ankyrin-B
LQT5	*KCNE1* (minK)	$I_{Ks}K^+$ channel β subunit
LQT6	*KCNE2* (MiRP1)	$I_{Kr}K^+$ channel β subunit
LQT7	KCNJ2	$I_{Kr2.1}K^+$ channel α subunit
LQT8	*CACNA1*	Cav1.2 Calcium channel α subunit

(a selection of) CHANNELOPATHIES

K^+ Channel	Episodic ataxia type 1 Hyperinsulinemic hypoglycemia of infancy Oncogenic potential Benign familial neonatal convulsions Hereditary hearing loss Type II diabetes Antenatal variant of Bartter syndrome Andersen's syndrome Total colour blindness Periodic Paralysis **Long QT syndrome** (type 1,2,5) Myokymia
Na^+ Channel	Liddle's syndrome **Hyperkaelemic Periodic Paralysis** **Paramyotonia congenita** **Congenital indifference to pain** Generalized epilepsy with febrile seizures types 1 & 2 **Long QT syndrome** 3 Pseudohypoaldosteronism Potassium-aggravated myotonia **Paroxysmal extreme pain disorder** Severe myoclonic epilepsy of infancy Brugada syndrome Isolated cardiac conduction disease
Ca^{2+} Channel	Episodic ataxia type 2 Familial hemiplegic migraine Spinocerebellar ataxia type 6 Hypokaelemic periodic paralysis type I Malignant hyperthermia Generalized epilepsy Central core disease Congenital night blindness Expressed in advanced prostate cancer Stationary night blindness
Cl^- Channel	**Myotonia congenita** Dent's disease (proteinuria and hypercalciuria) Osteopetrosis Bartter syndrome Cystic Fibrosis
Glycine receptor	**Hyperekplexia (stiff baby syndrome)** **Startle disease**