Modelling of cardiac ion channels for proarrhythmia risk assay

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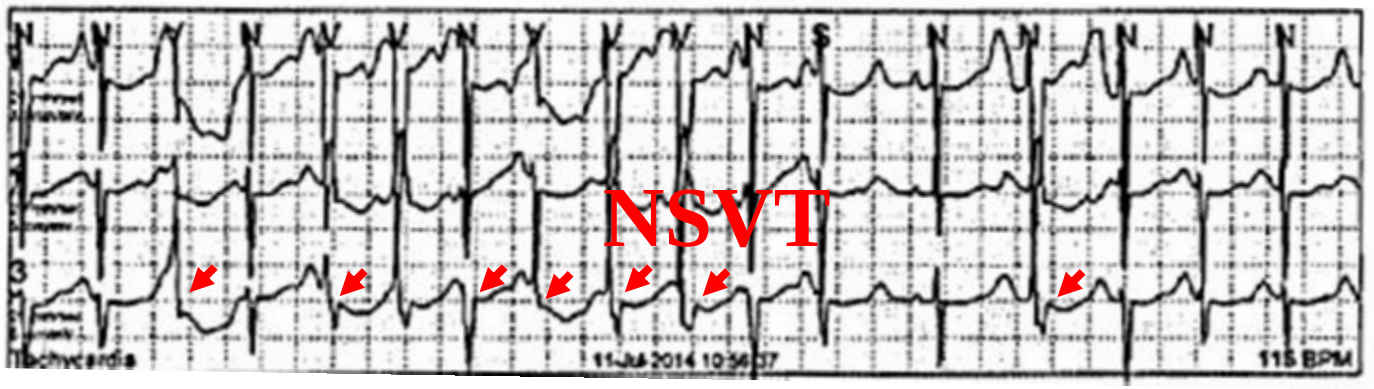
Long QT Syndrome Type 3: Case

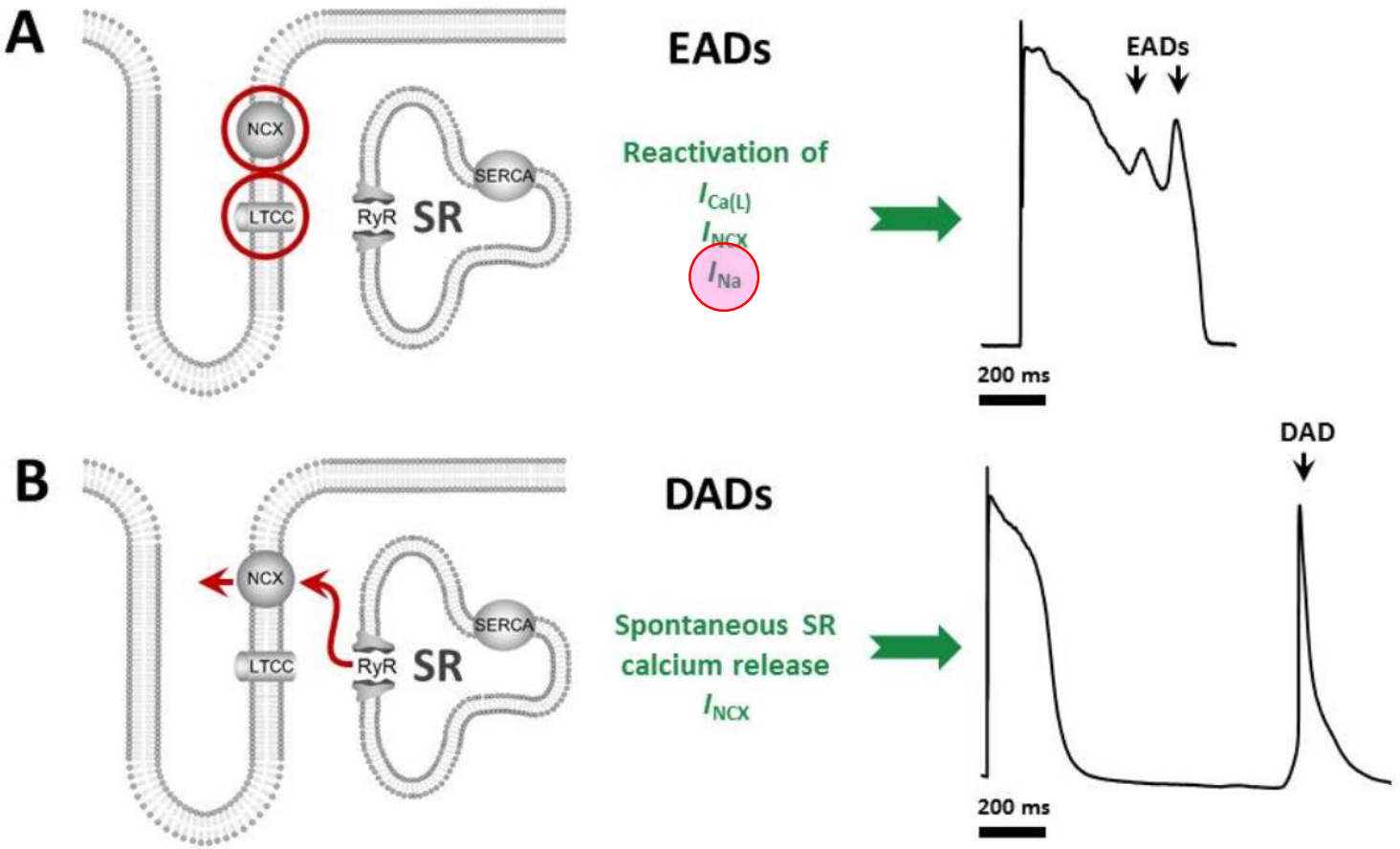
- A male infant was born after 37 weeks' gestation to a 40 years old mother, who had been treated throughout pregnancy with flecainide, 100 mg bid, to treat premature atrial contractions (PACs) and non-sustained atrial tachycardia observed by fetal ECG.
- After birth, a Holter monitoring showed a prolonged QTc interval (~527 ms), ventricular premature complex and non-sustained ventricular tachycardia (NSVT). Thus, Long QT syndrome was suspected.



Long QT Syndrome (LQTS)

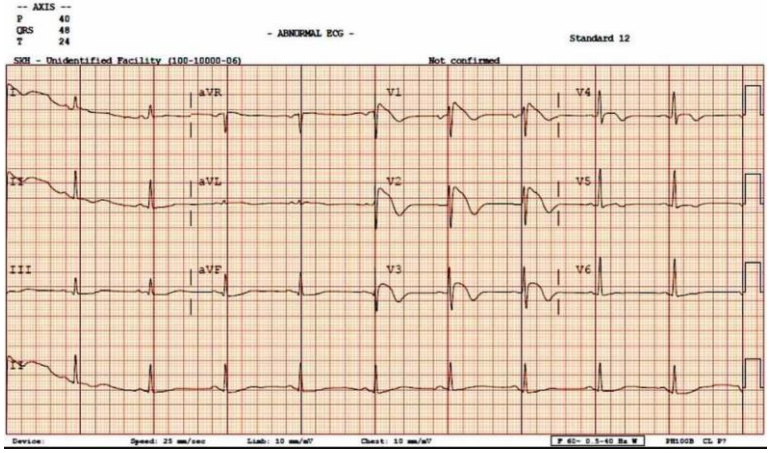
- **LQT1 : loss of function mutation in the *KCNQ*1 (KvLQT1, α subunit of I_{Ks})**
- **LQT2: loss of function mutations in the *KCNH*2 (HERG, α subunit of I_{Kr})**
- **LQT3: gain of function mutations in the *SCN5* ($Na_v1.5$, I_{Na})**
- **LQT4: Ankyrin B**
- **LQT5: loss of function mutations in the *KCNE*1 (minK, β subunit of I_{Ks})**
- **LQT-6: loss of function mutations in the *KCNE*2 (MiRP1, β subunit of I_{Kr})**

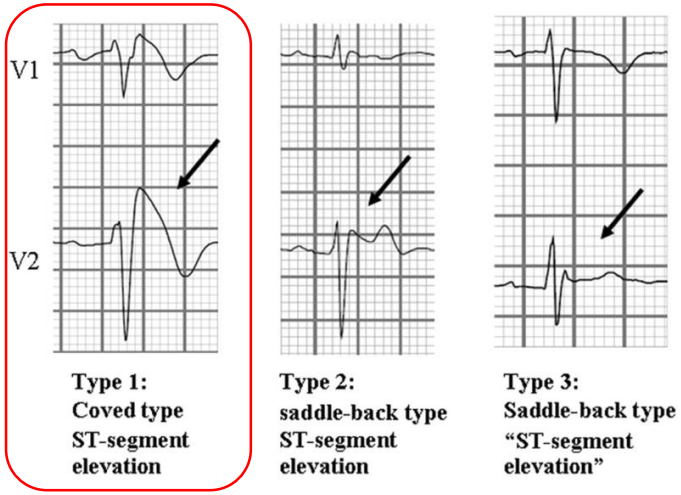
Long QT syndrome begins with early-after depolarizations (EADs)



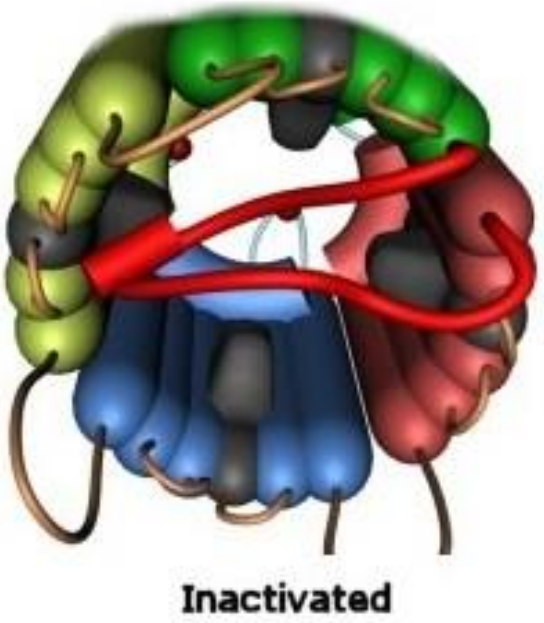
Brugada syndrome

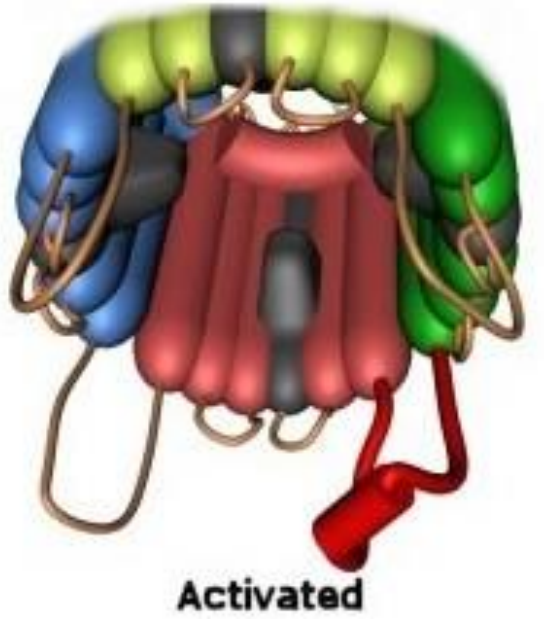
- **A potentially life-threatening heart rhythm disorder**
- **Type 1 Brugada ECG pattern is detected by an ECG test**
- **Many don't have any symptoms. if they have, dizziness, fainting, irregular heartbeats, and sudden death at sleep is common.**
- **20~25% of cases of Brugada syndrome are are associated with mutations in SCN5A.**





Circ Arrhythm Electrophysiol. 2012;5(3):606-616





Na^+-channels

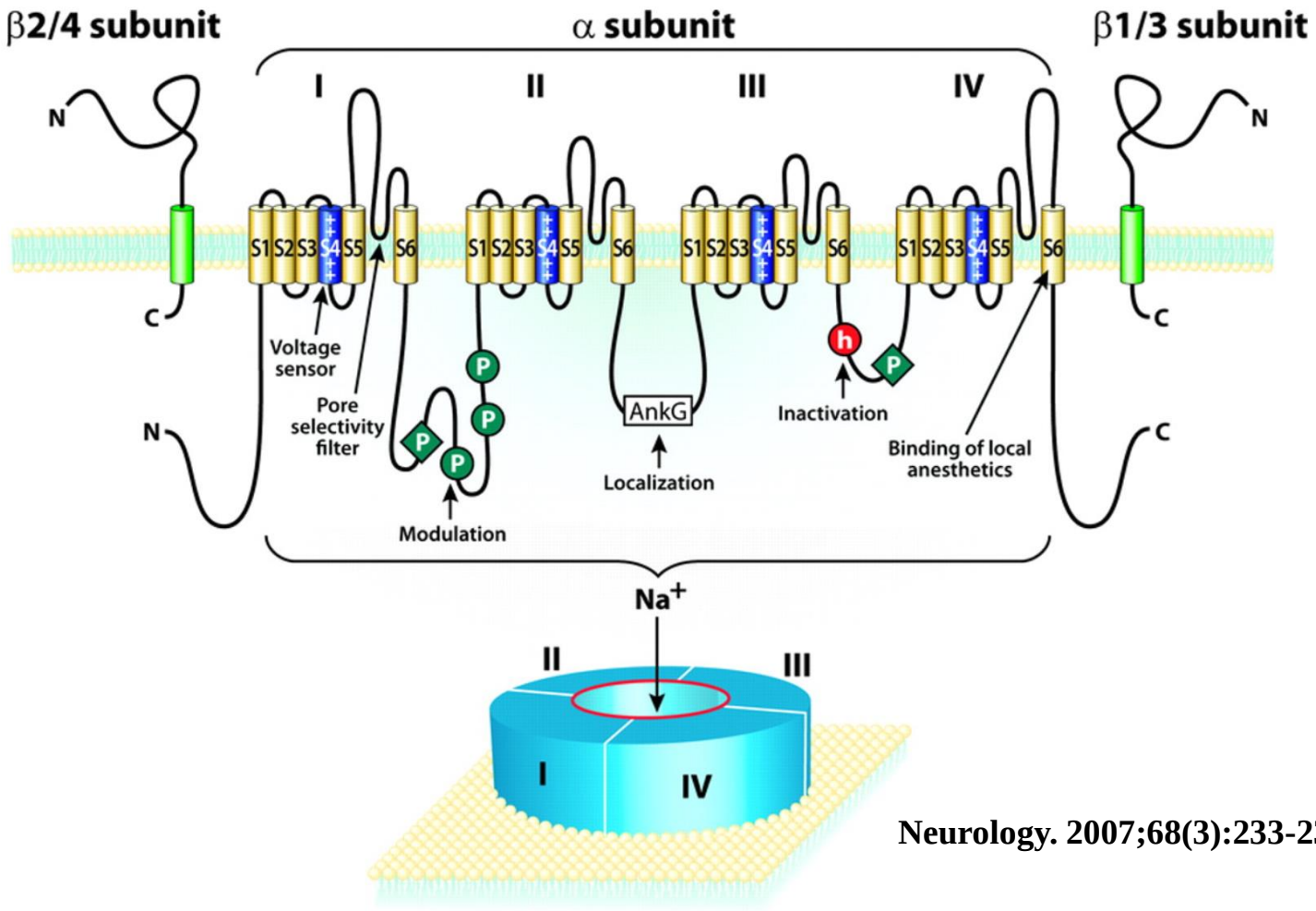
α subunits

- **$Na_V1.1$ – 1.3: CNS, PNS, cardiac(1.3)**
- **$Na_V1.4$: skeletal m.**
- **$Na_V1.5$: cardiac m, Cajal cell**
- **$Na_V1.6$: CNS, DRG, PNS, glia**
- **$Na_V1.7$: DRG, PNS, Schwann cell**
- **Na_V 1.8 - 1.9: DRG**
- **Na_x: heart, uterus, smooth m., glia**

β subunits

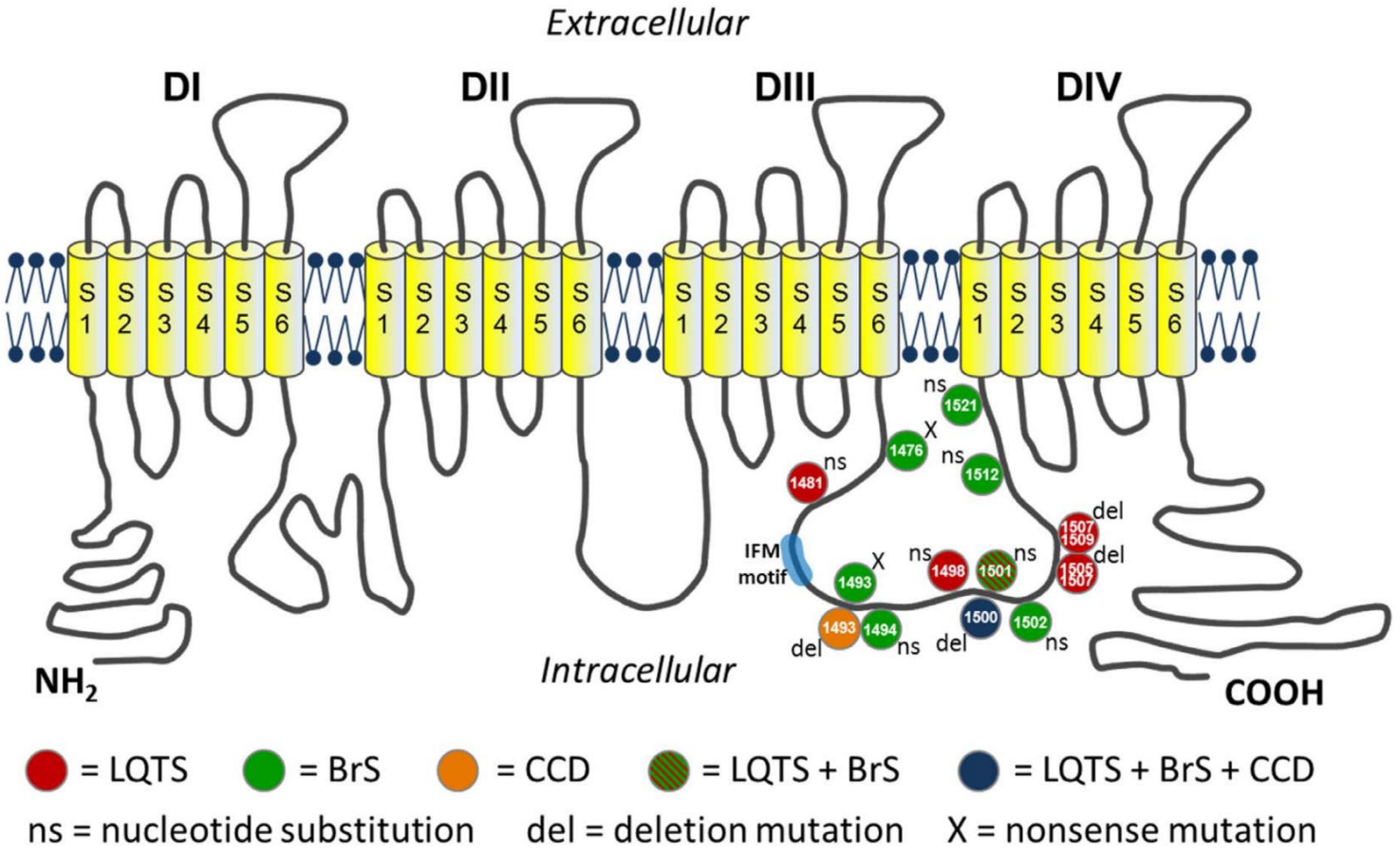
- **type 1 transmembrane glycoproteins with an extracellular N-terminus and a cytoplasmic C-terminus**
- **SCN1B, SCN2B, SCN3B, SCN4B**

Topology of sodium channel



Neurology. 2007;68(3):233-236.

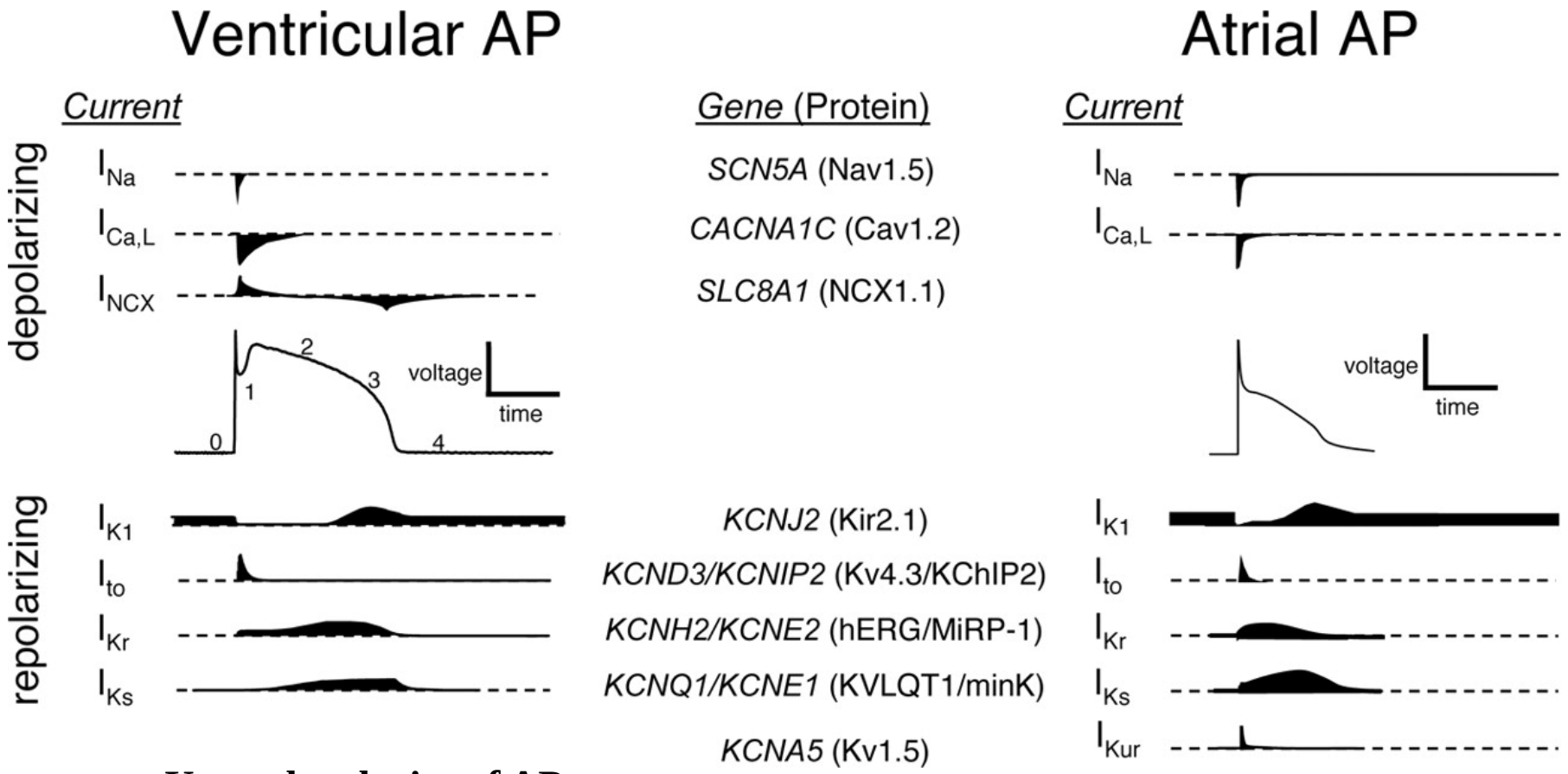
Topological model of the cardiac sodium channel ($Na_v1.5$)



SCN5A is the gene that encodes the cardiac sodium channel (NaV1.5).

PLoS One. 2013;8(6):e67963.

Contribution of $Na_v1.5$ to cardiac action potential



- **Upstrok velocity of AP**
- **Peak of AP**
- **Conduction velocity in heart tissue**

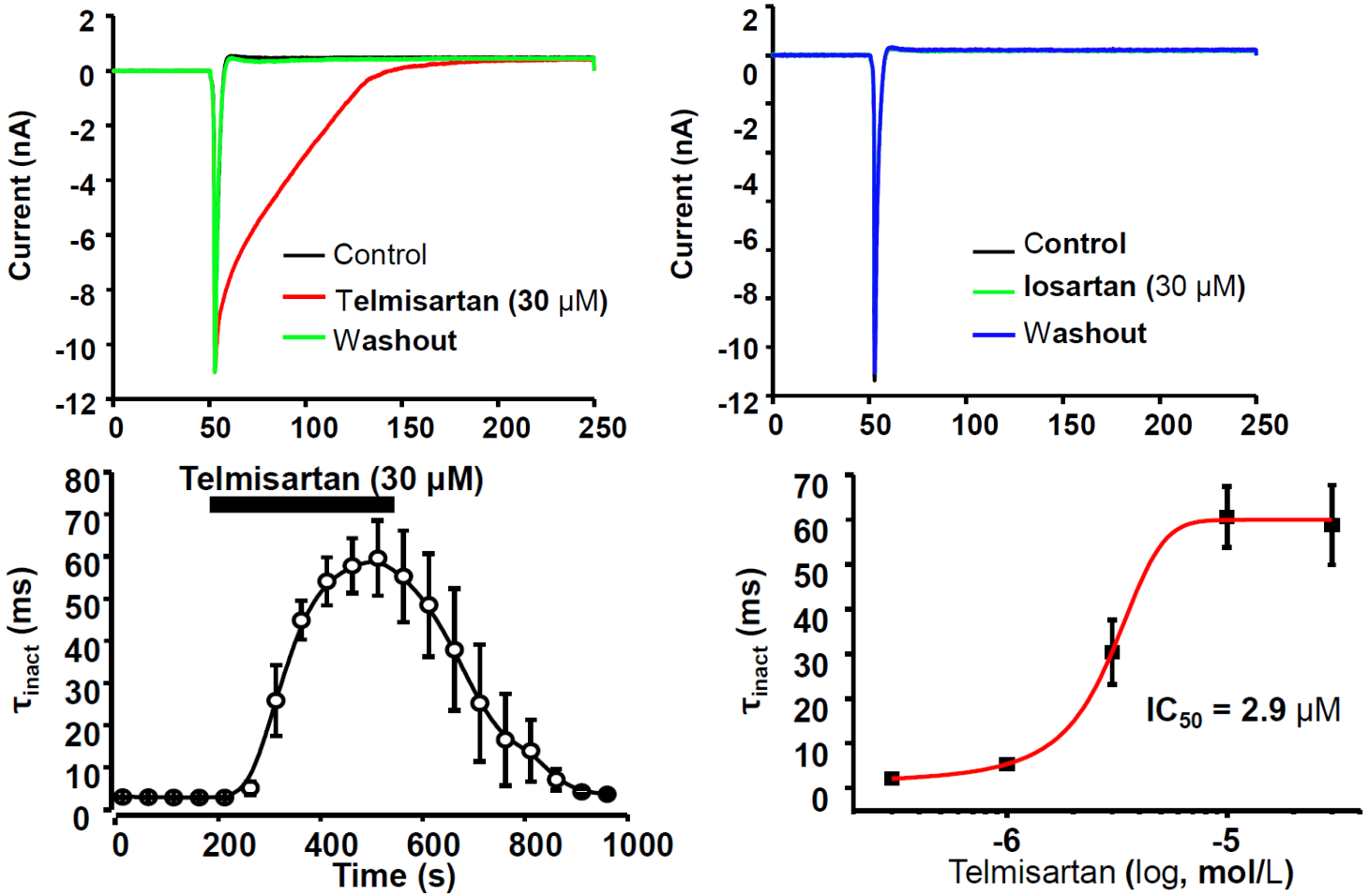
Alterations of I_{Na} by drugs

Pflugers Arch - Eur J Physiol
DOI 10.1007/s00424-012-1170-3

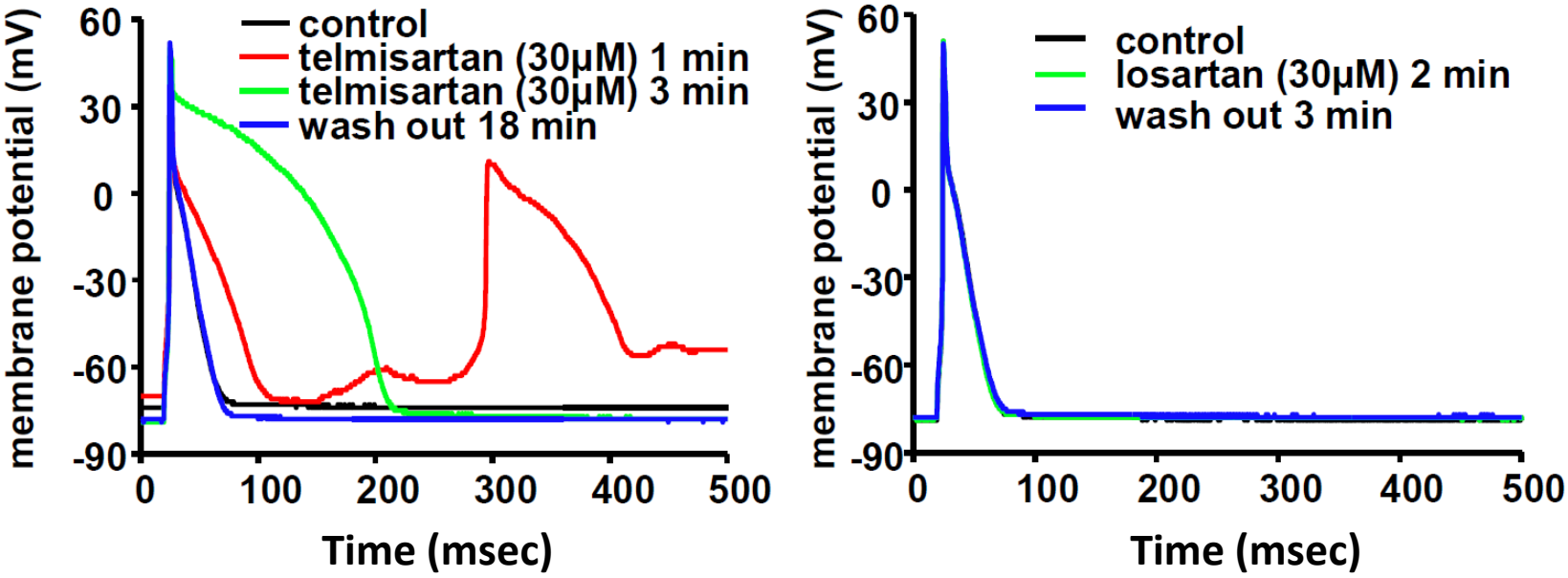
ION CHANNELS, RECEPTORS AND TRANSPORTERS

The angiotensin receptor blocker and PPAR-γ agonist, telmisartan, delays inactivation of voltage-gated sodium channel in rat heart: novel mechanism of drug action

Telmisartan slows inactivation of I_{Na}.

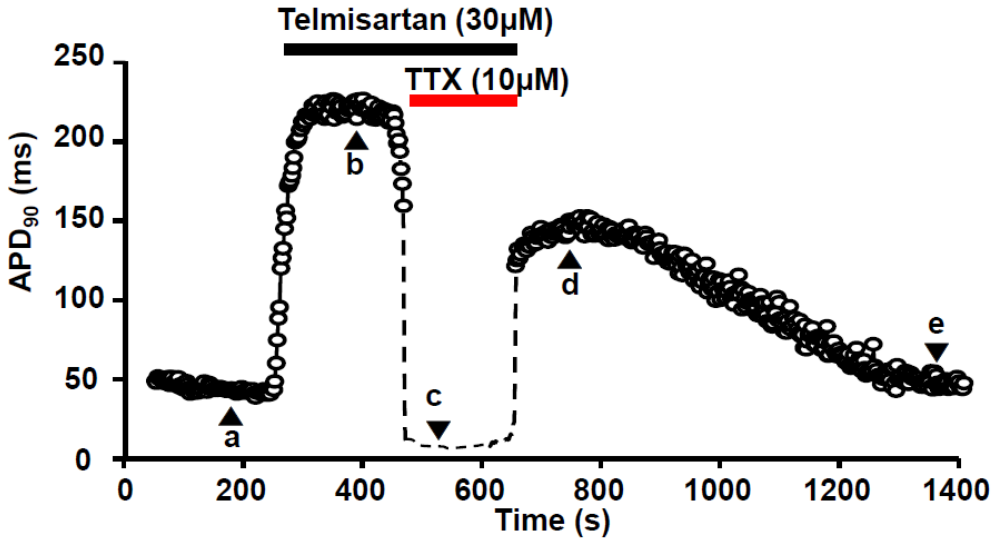


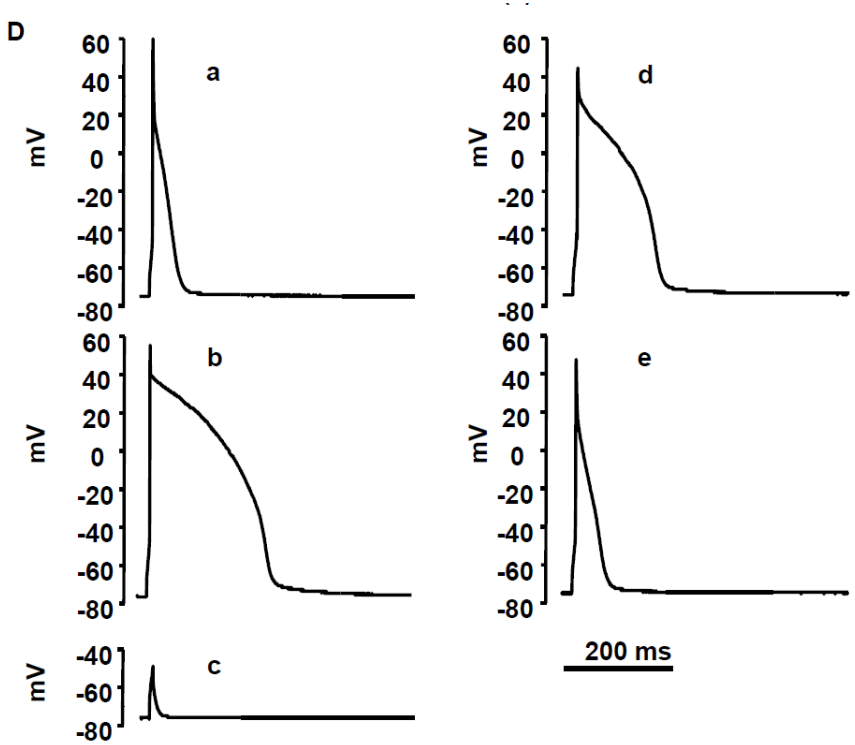
Slowing of I_{Na} inactivation elongates the action potential (AP).



Pflugers Arch. 2012;464(6):631-43.

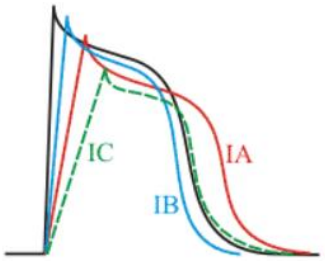
The effect of telmisartan is TTX-sensitive.





Pflugers Arch. 2012;464(6):631-43.

Pharmacology of $Na_V1.5$



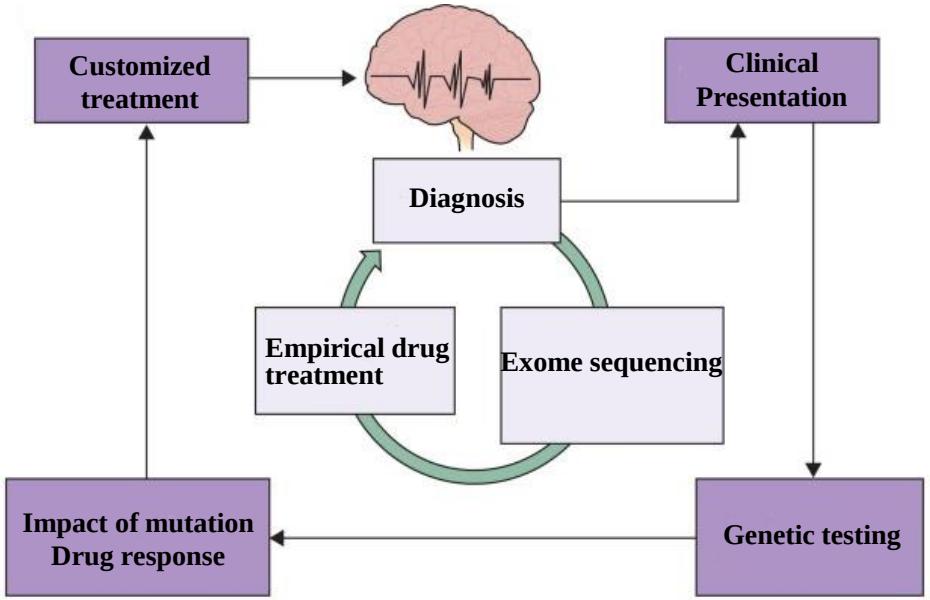
Ventricular Action Potential

- Class IA: e.g., quinidine
  - Moderate Na^+-channel blockade
  - ↑ ERP
- Class IB: e.g., lidocaine
  - Weak Na^+-channel blockade
  - ↓ ERP
- Class IC: e.g., flecainide
  - Strong Na^+-channel blockade
  - → ERP

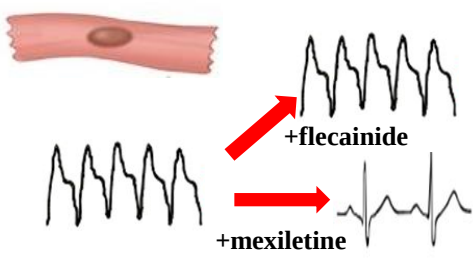
Class IA: atrial fibrillation, flutter; supraventricular & ventricular tachyarrhythmias		
quinidine*	anticholinergic (moderate)	cinchonism (blurred vision, tinnitus, headache, psychosis); cramping and nausea; enhances digitalis toxicity
procainamide	anticholinergic (weak); relatively short half-life	lupus-like syndrome in 25-30% of patients
disopryamide	anticholinergic (strong)	negative inotropic effect
Class IB: ventricular tachyarrhythmias (VT)		
lidocaine*	IV only; VT and PVCs	good efficacy in ischemic myocardium
tocainide	orally active lidocaine analog	can cause pulmonary fibrosis
mexiletine	orally active lidocaine analog	good efficacy in ischemic myocardium
Class IC: life-threatening supraventricular tachyarrhythmias (SVT) and ventricular tachyarrhythmias (VT)		
flecainide*	SVT	can induce life-threatening VT
propafenone	SVT & VT;	β-blocking and Ca^{++}-channel blocking activity can worsen heart failure
moricizine	VT; IB activity	

Richard E. Klabunde, PhD.
https://www.cvpharmacology.com/antiarrhy/sodium-blockers

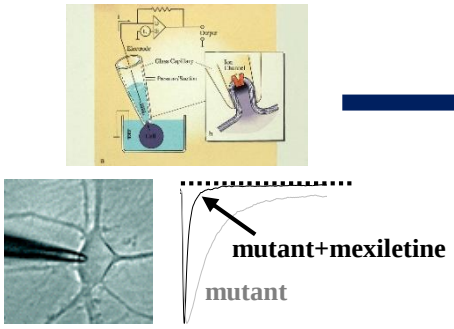
A strategy of customized treatment for genetic disorder



① Patient-specific iPSCs-CMs

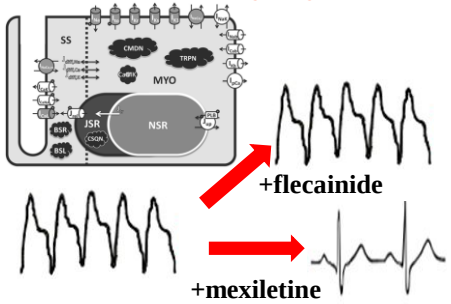


② i) 293T cells expressing mutant channels:
Mutation & Drug response test



ii) Prediction of drug efficacy

Virtual human cardiomyocytes

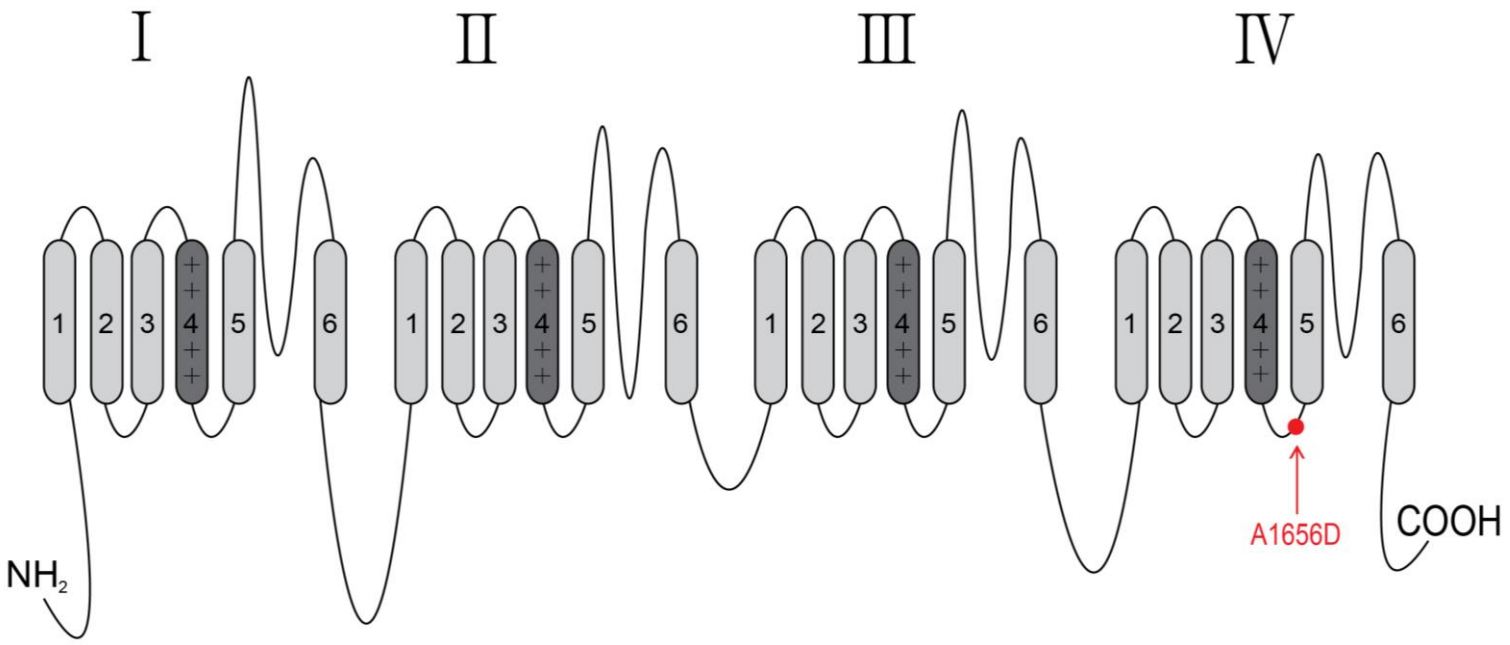


Genetic Testing

- **Genetic testing**
- **Sanger sequencing**
- ***SCN5A* c.4967C>A, p.A1656D; novel, de novo, variant of unknown significance**
- **no mutations in major ion channels and transporters such as KCNQ1, KCNE1, KCNH2 or KCNE2 or other channel-related LQTS genes were identified.**

Novel A1656D mutation on the SCN5A gene

A



B

```
mouse   1620 FSPTLFRVIRLARIGRILRLIRGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYSIFGM 1679
rat     1619 FSPTLFRVIRLARIGRILRLIRGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYSIFGM 1678
guinea  1615 FSPTLFRVIRLARIGRILRLIRGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYSIFGM 1674
human   1617 FSPTLFRVIRLARIGRILRLIRGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYSIFGM 1676
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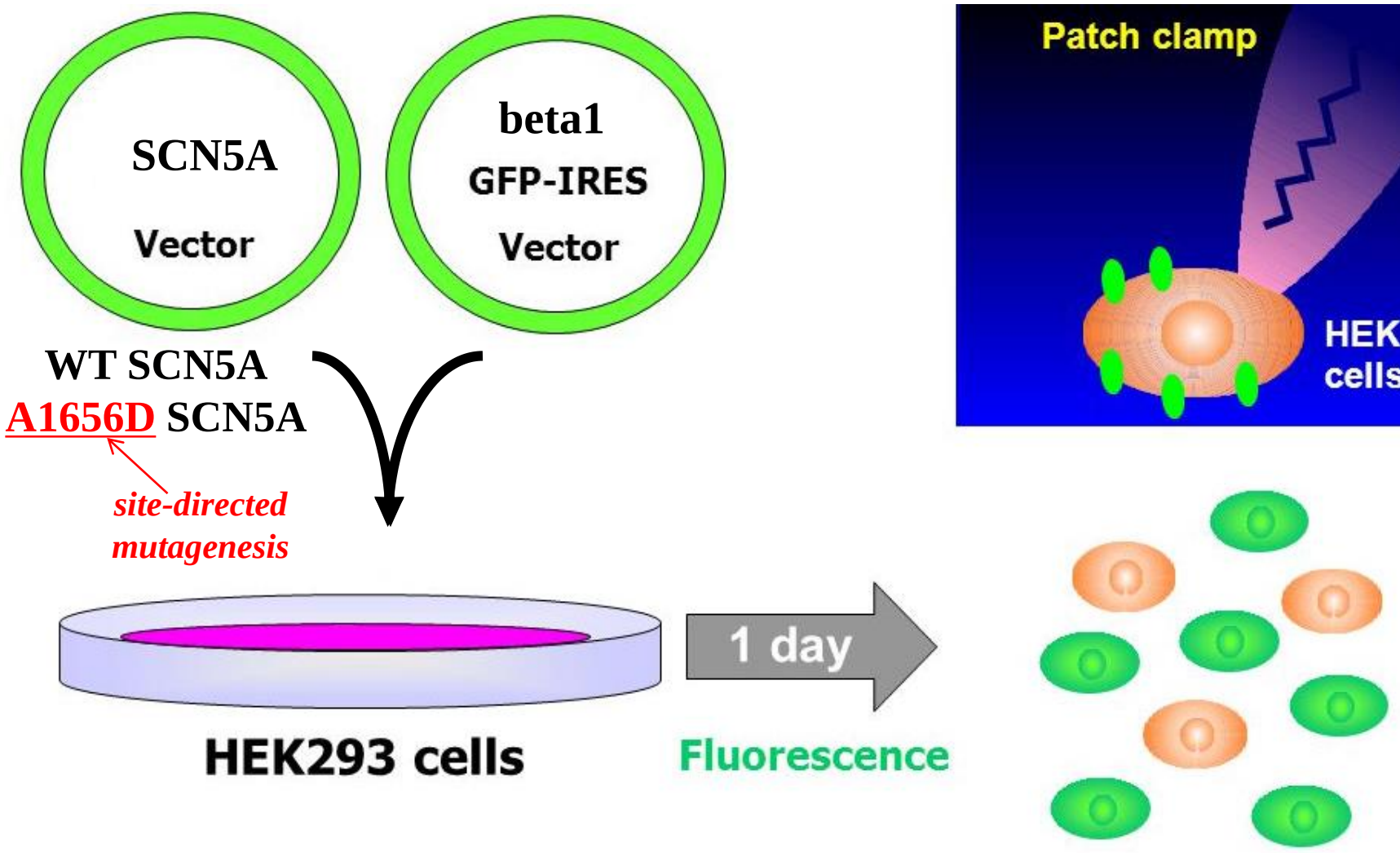
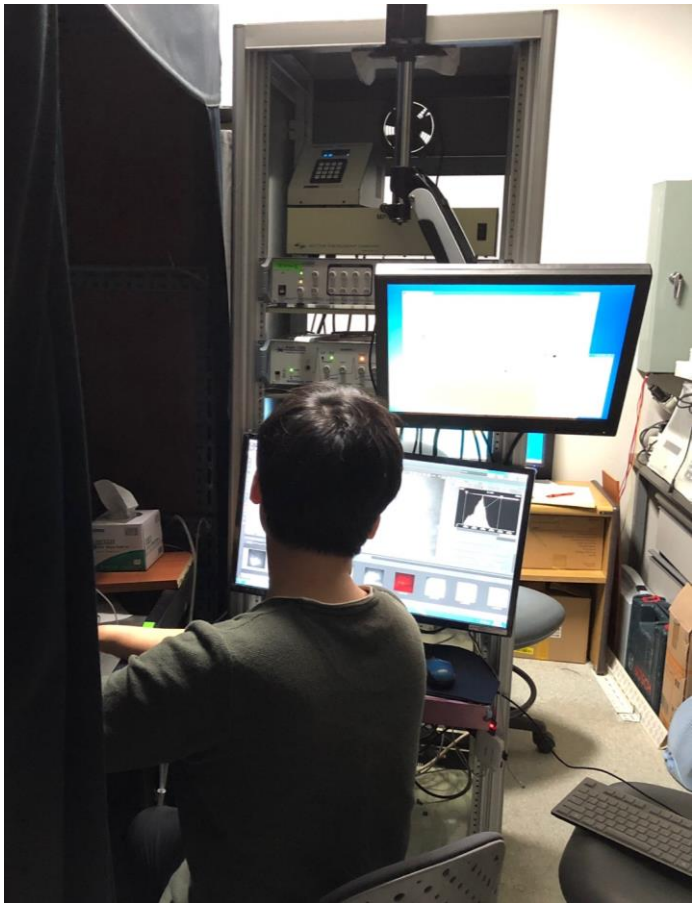
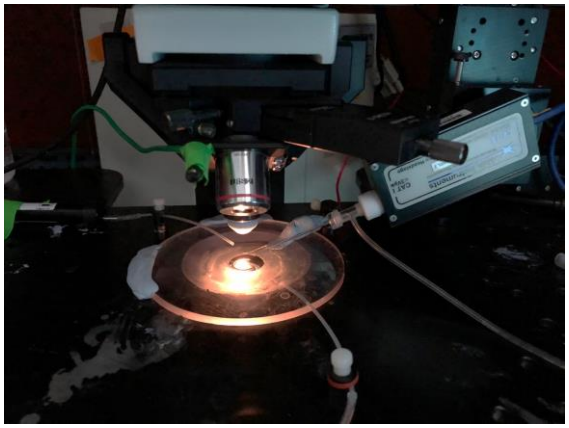
Assessing Functional Consequences of SCN5A Variants

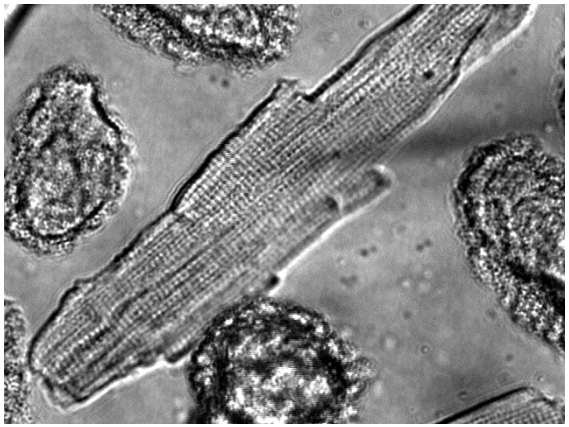


Assessing Functional Consequences of SCN5A Variants

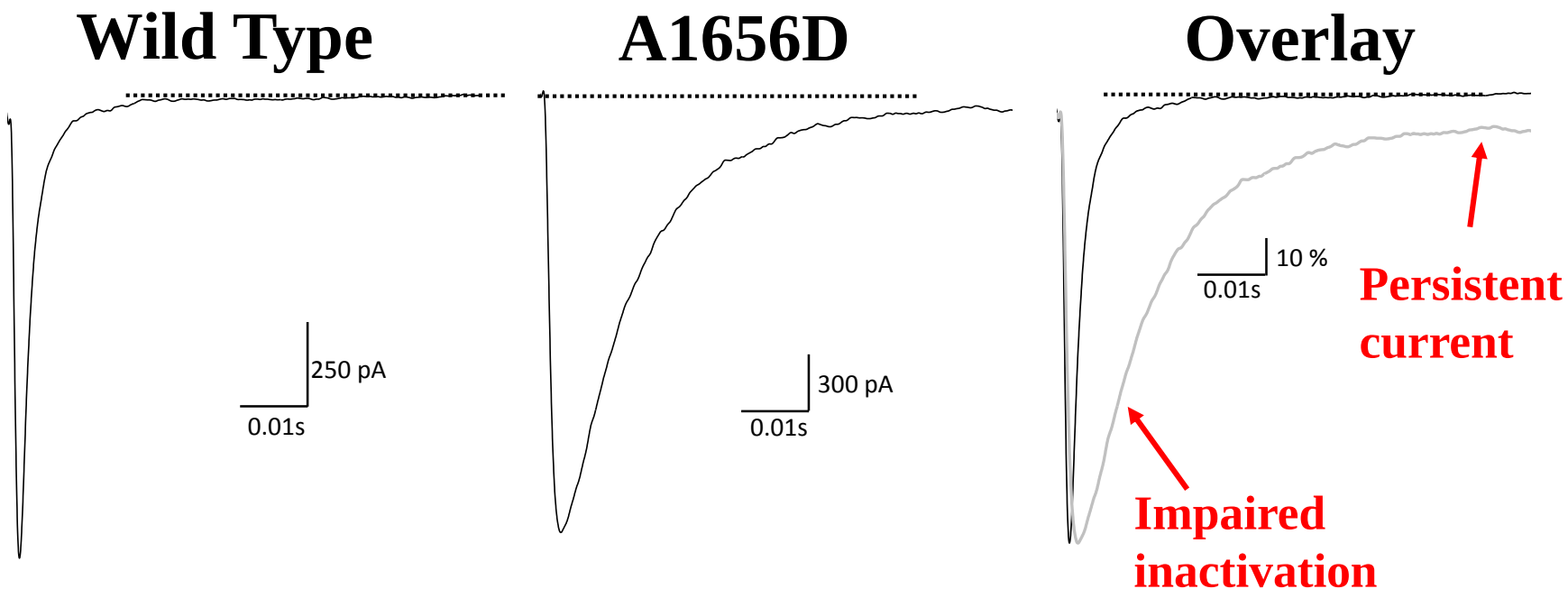
Whole-cell patch clamp



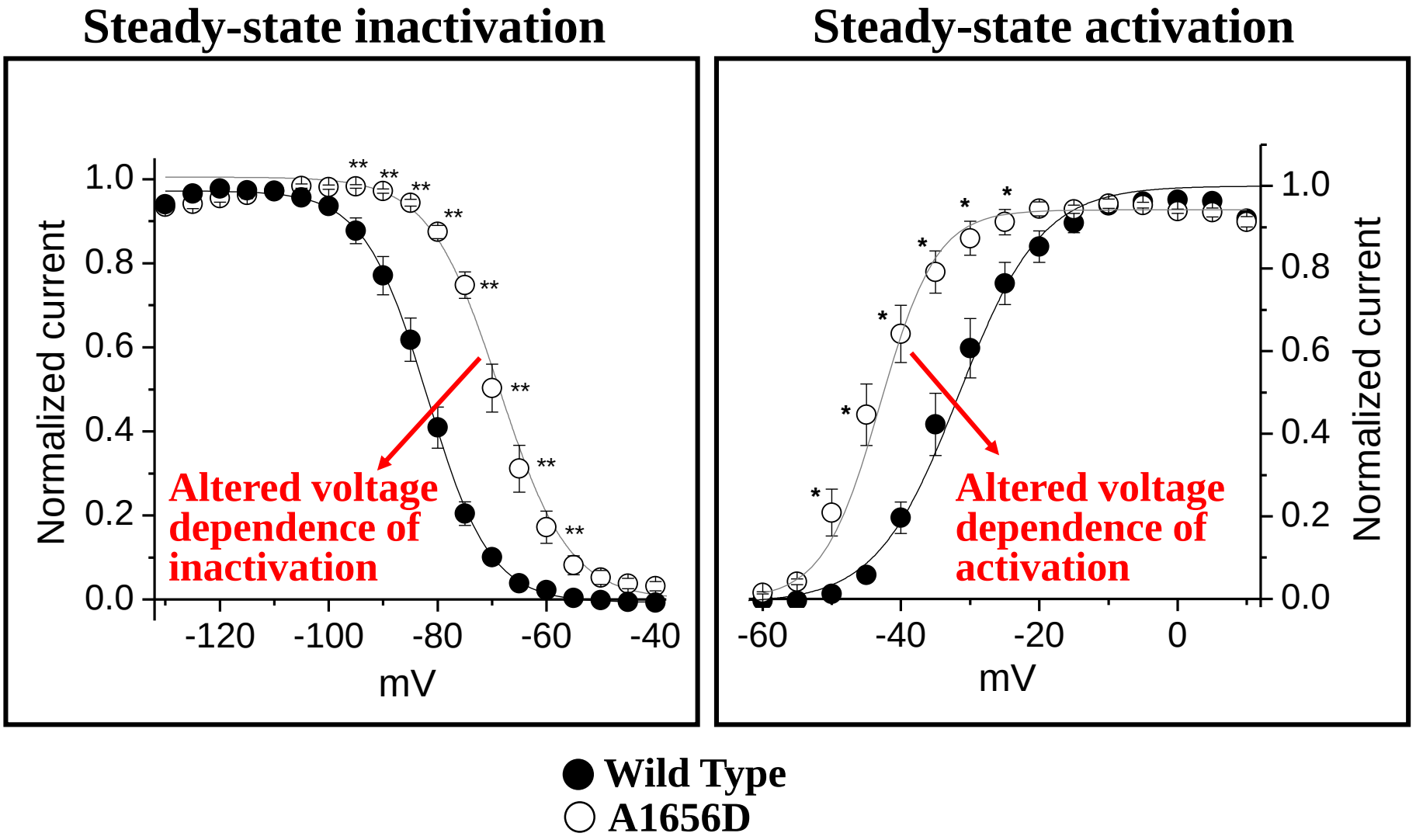




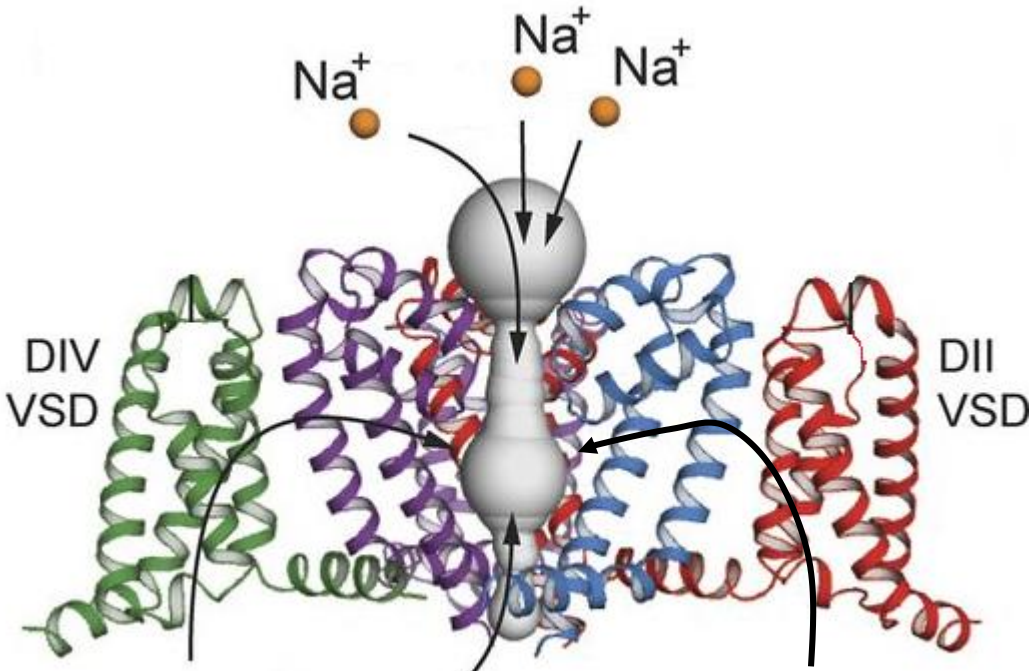
Gain-of-function effects of A1656D SCN5A mutation on channel gating



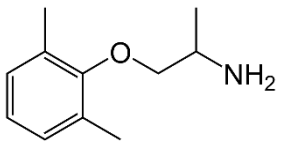
Underlying molecular mechanisms of A1656D SCN5A mutation in arrhythmia



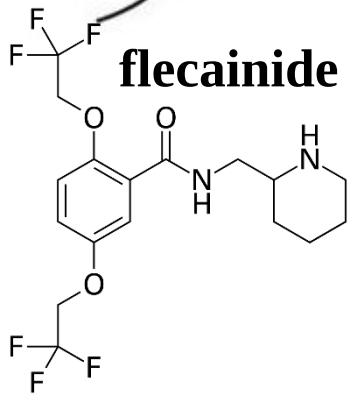
Na^+ channel blockers for long QT mutant Na^+ channels



mexiletine



flecainide



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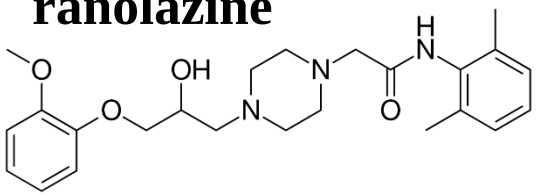
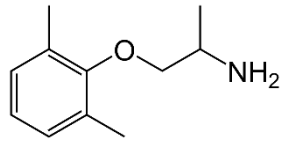


Distinct Pharmacology of A1656D Mutant Channels



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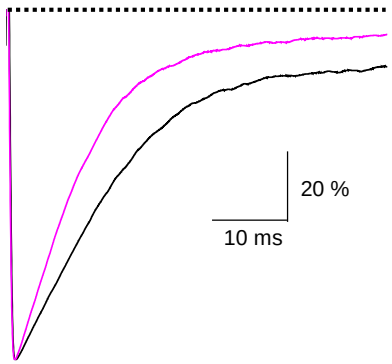

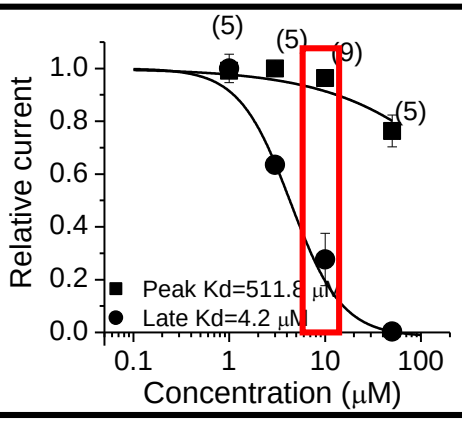

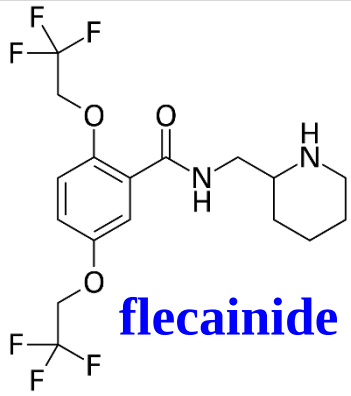

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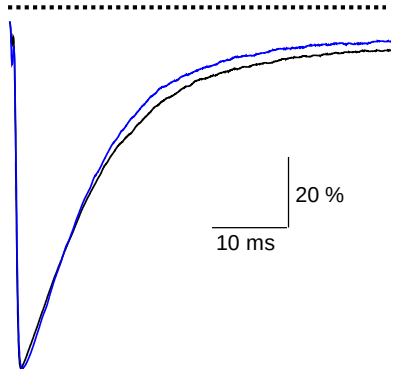
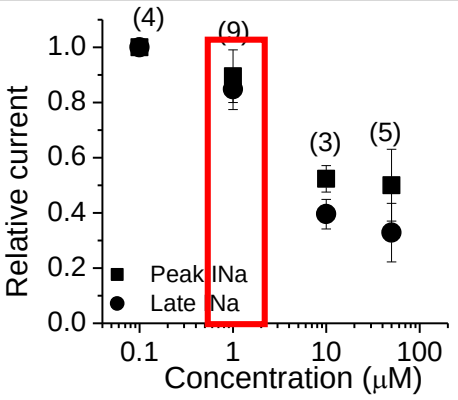
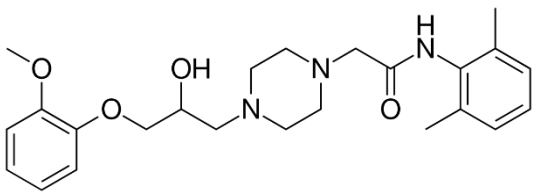
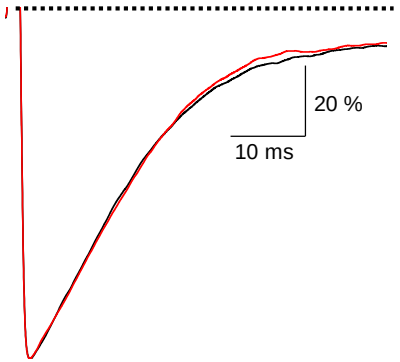
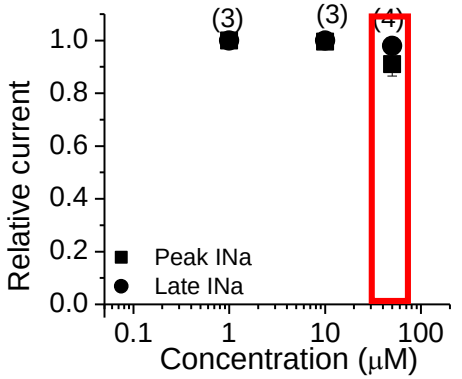




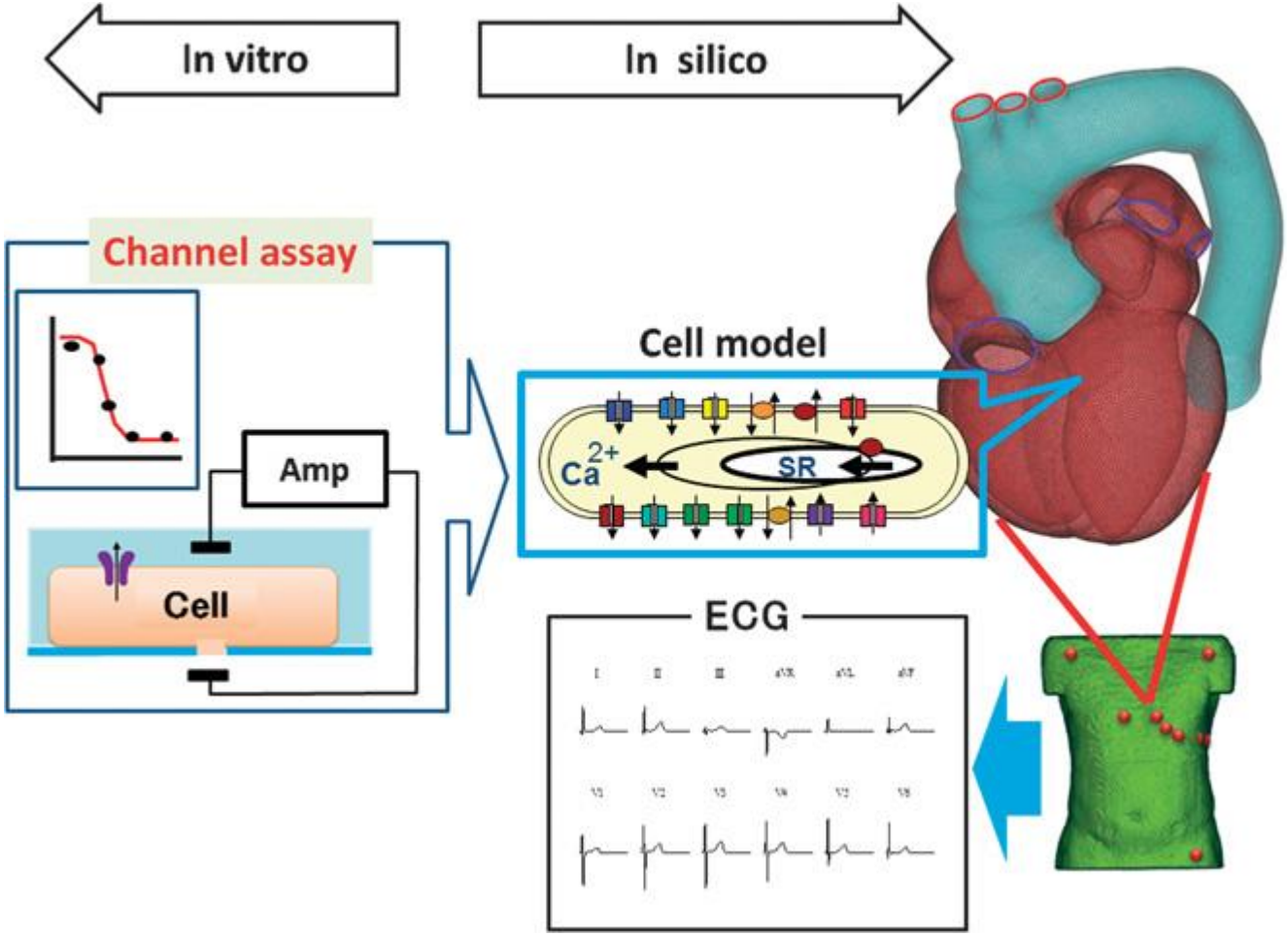


ranolazine



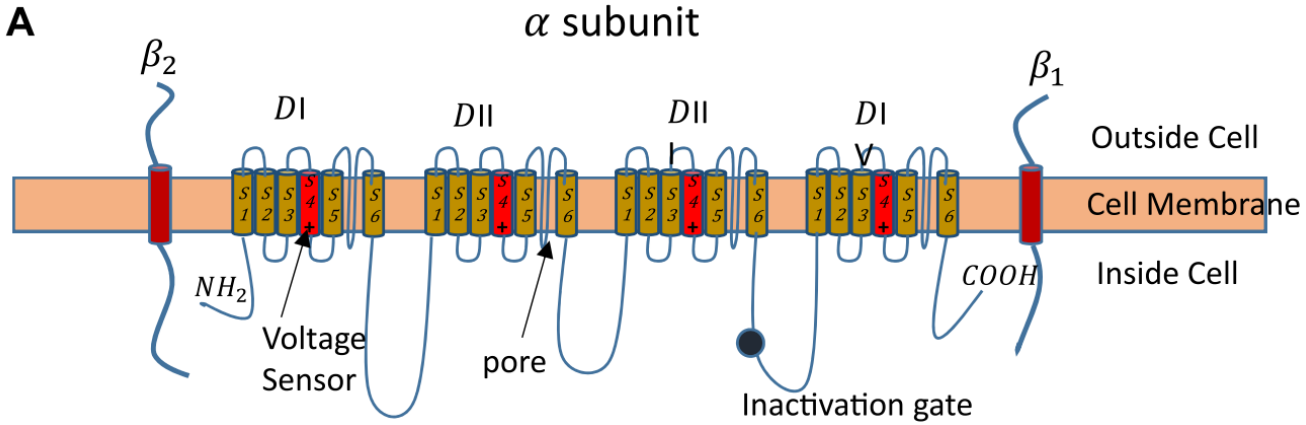


In silico Modeling for Prediction of Drug Effects

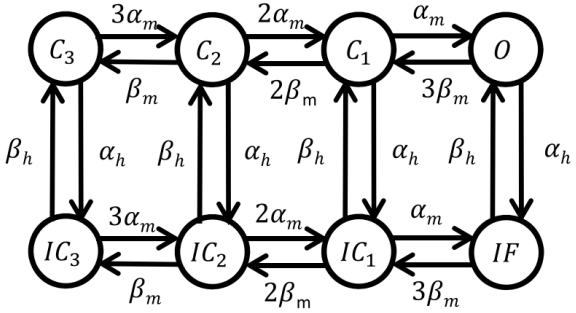


Science Advances **1, no. 4, e1400142**

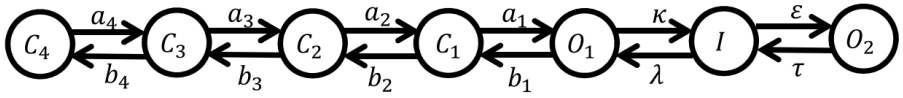
Model of Na^+ channel



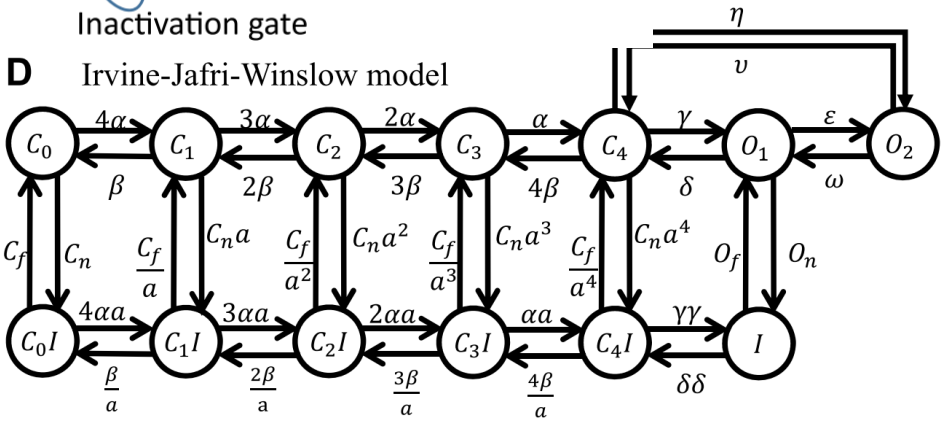
B Hodgkin-Huxley model



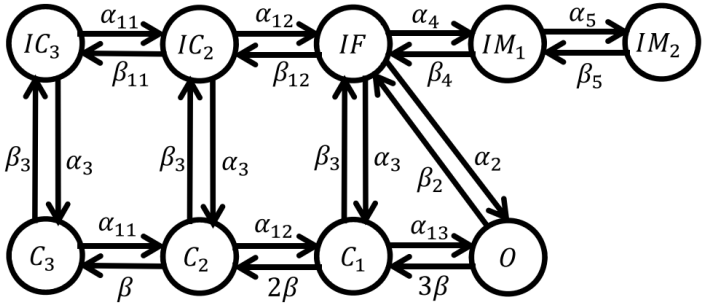
C Armstrong-Bezanilla model



D Irvine-Jafri-Winslow model



E Clancy-Rudy model

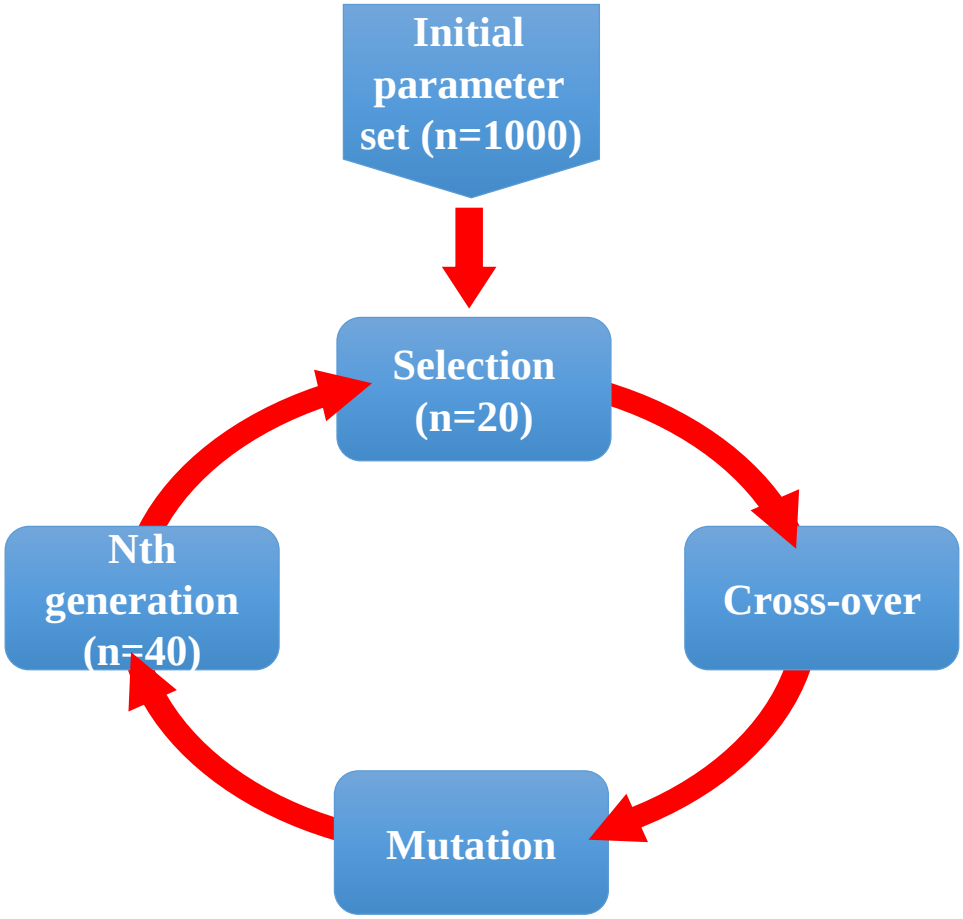


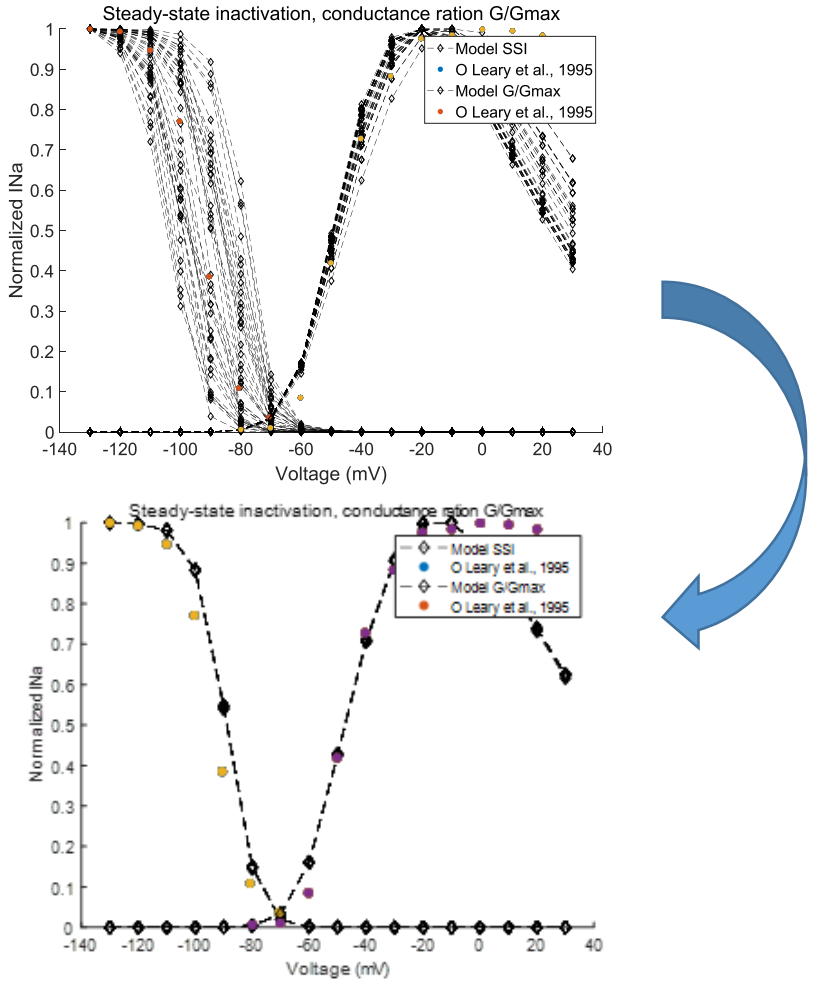
$$I_{Na} = g_{Na} \cdot O \cdot (V - E_{Na})$$

J Membr Biol. 2019 Feb;252(1):77-103

Model fitting
- genetic algorithm -

Estimate best fits for experimental data using machine learning genetic algorithm





Model fitting
- fitting using R -

RStudio

File Edit Code View Plots Session Build Debug Profile Tools Help

Fit_5StateModel_v3.R

```
{
  dc1dt = p['kco']*y['c2']-p['kco']*y['c1']
  dc2dt = p['kco']*y['c1']+p['koc']*y['c3']-(p['kco']+p['koc'])*y['c2']
  dc3dt = p['kco']*y['c2']+p['koc']*y['o']-(p['kco']+p['koc'])*y['c3']
  dodt = p['kco']*y['c3']+p['kio']*y['i']-(p['koi']+p['koc'])*y['o']
  didt = p['koi']*y['o']-p['kio']*y['i']
  return(list(c(dc1dt,dc2dt,dc3dt,dodt,didt)))
}

# Prediction for numerical integration ------------------------------------------------------
Fpred <- function(ival,theta)
{
  y0 = deSolve::lsoda(y=c(c1=ival[1],c2=ival[2],c3=ival[3],o=ival[4],i=ival[5]),
                      times = seldata$time,
                      func=StateDE,
                      parms = c(kco=theta[1],
                                koc=theta[2],
                                koi=theta[3],
                                kio=theta[4]
                                ))
  return(y0[,'o'])
}

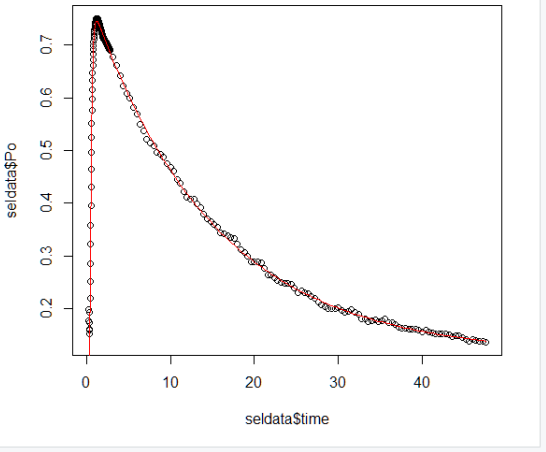
# Setup condition ---------------------------------------------------------
Erev = 60                   # ^^ reversal potential for the channel
Vh = -120                   # ^^ holding potential
firstV = -60                # ^^ first test potential
StepV = 5                   # ^^ step pulse increment
episodes <- 2:ncol(rawD)    # ^^ 1st column is time, from 2nd colume: membrane current

fitmatrix <- matrix(nrow = length(episodes), ncol = 10)      # ^^ should match formula below, if number of st0 element is 6, then ncol should be 6+
testv <- matrix(nrow=length(episodes), ncol=2)               # for test, first column is voltage, second column is what you want to see

initvar = c(6.99,2.39,0.21,0.0088)
#initvar = c(0.099,0.086,0.76,0.03)
ivals <- c(0.98,0,0,0.02,0)
gm <- 0.06      # maximum conductance

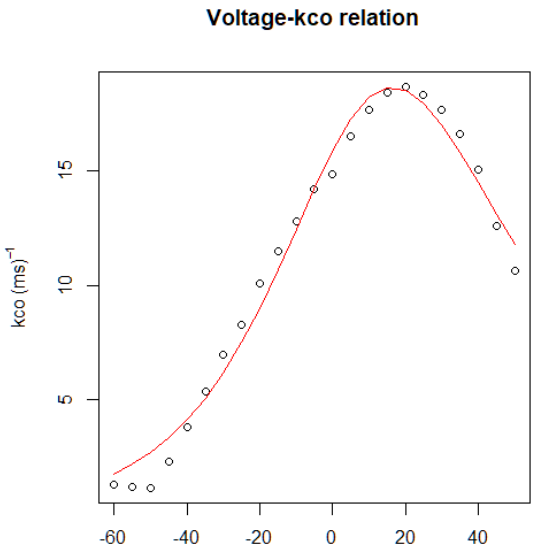
# Fit current -------------------------------------------------------------------------
nof <- 1        # for fitmatrix
for (i in episodes)
{
  Vm = firstV + (i-2)*StepV      # set first voltage and size of steps
  seldata = data.frame(
    time=rawD[c(15:nrow(rawD)),1],
    Po=c(rawD[c(15:nrow(rawD)),i]/(Vm-Erev)/gm)
    )
  resmodel <- nls(Po ~ Fpred(ivals,theta),
                  data=seldata,
                  start=list(theta=initvar),
```

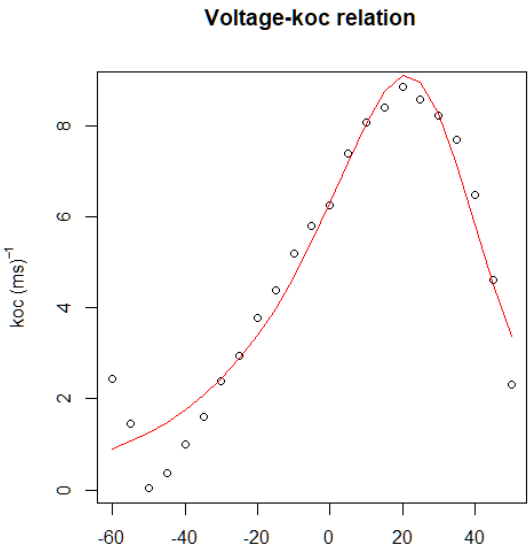
```
> # Setup condition ---------------------------------------------------------
> Erev = 60                     # ^^ reversal potential for the channel
> Vh = -120                     # ^^ holding potential
> firstV = -60                  # ^^ first test potential
> StepV = 5                     # ^^ step pulse increment
> episodes <- 2:ncol(rawD)      # ^^ 1st column is time, from 2nd colume: membrane current
>
> fitmatrix <- matrix(nrow = length(episodes), ncol = 10)      # ^^ should match formula below, if number of st0 element is 6, then ncol should be 6+1.
> testv <- matrix(nrow=length(episodes), ncol=2)               # for test, first column is voltage, second column is what you want to see
>
```



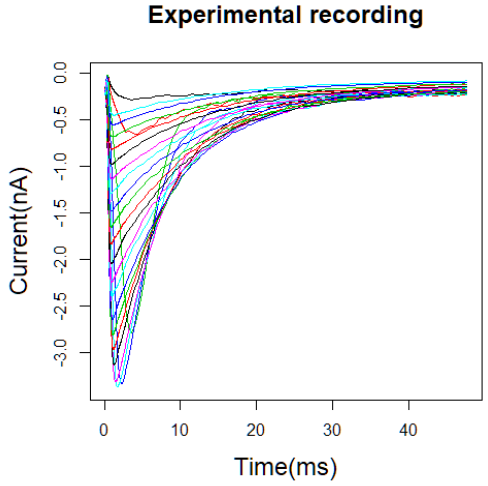
(1) Get rate constant set for model of Na^+ channel at each voltage

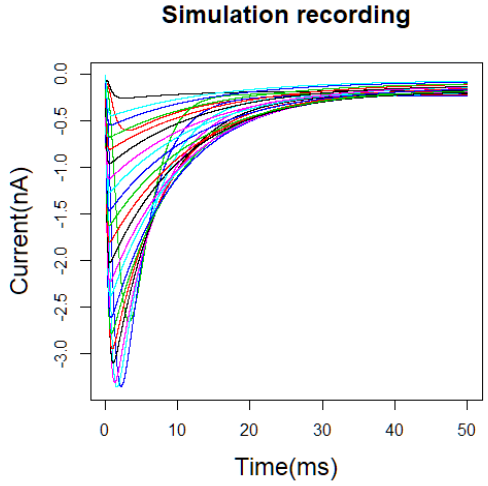
Model fitting





(2) fit relation between voltage and rate constant set





(3) Simulate voltage-gated Na^+ currents

Model fitting
- IV curve fitting -

RStudio

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Fit_5StateModel_v3.R | test_module_v5(par).R*

```
  minf <- sapply(ivdata$x, function(x) {
    1/(1+exp(-(x - parm[1])/parm[2]))
  })
  hinf <- sapply(ivdata$x, function(x) {
    1/(1+exp((x - parm[3])/parm[4]))
  })

  yval <- parSapply(cl,1:c(length(ivdata$x)), function(x) {
    oval <- deSolve::lsoda(y=c(m=0,h=1),
                           times=df[sp,1],
                           func=StateDE,
                           parms=c(af=minf[x]/mtau[x],ab=(1-minf[x])/mtau[x],bf=hinf[x]/htau[x],bb=(1-hinf[x])/htau[x]))
    min(1e-9*parm[5]*oval[,'m']^3*oval[,'h']*(ivdata[x,'x']-65))
  })
  return (yval)
}

# 6. fit
fitmodel <- nls(y ~ PredMin(pa),
                data=ivdata,
                start=list(pa=c(-33,5.6,-81,5.6,0.1)),
                algorithm='port',
                lower=c(-100,1,-100,2,0.00001),
                upper=c(-20,20,0,20,100),
                control=nls.control(maxiter=2000,warnOnly=TRUE))

# 7. visualize
plot(ivdata,type='b',lty=1, xlab='voltage(mV)',ylab='peak(A)')
test <- coef(fitmodel)
result <- as.numeric(test)
clusterExport(cl,"result")
points(ivdata$x,PredMin(result),type='b',lty=1,col=2)

stopCluster(cl)

# 8. check whole cell current
minf <- sapply(ivdata$x, function(x) {
  1/(1+exp(-(x - result[1])/result[2]))
})
hinf <- sapply(ivdata$x, function(x) {
  1/(1+exp((x - result[3])/result[4]))
})
yval <- sapply(1:c(length(ivdata$x)), function(x) {
  oval <- deSolve::lsoda(y=c(m=0,h=1),
                         times=df[sp,1],
                         func=StateDE,
                         parms=c(af=minf[x]/mtau[x],ab=(1-minf[x])/mtau[x],bf=hinf[x]/htau[x],bb=(1-hinf[x])/htau[x]))
  1e-9*result[5]*oval[,'m']^3*oval[,'h']*(ivdata[x,'x']-65)
})
matplot(yval,type='l',lty=1,xlim=c(0,300))
```

History | Connections

To Console | To Source

```
stopCluster(cl)
```

Console | Terminal | Jobs

```
> # 6. fit
> fitmodel <- nls(y ~ PredMin(pa),
+                 data=ivdata,
+                 start=list(pa=c(-33,5.6,-81,5.6,0.1)),
+                 algorithm='port',
+                 lower=c(-100,1,-100,2,0.00001),
+                 upper=c(-20,20,0,20,100),
+                 control=nls.control(maxiter=2000,warnOnly=TRUE))
>
> # 7. visualize
> plot(ivdata,type='b',lty=1, xlab='voltage(mV)',ylab='peak(A)')
> test <- coef(fitmodel)
> result <- as.numeric(test)
> clusterExport(cl,"result")
> points(ivdata$x,PredMin(result),type='b',lty=1,col=2)
>
> stopCluster(cl)
>
```

Environment | Files | Plots | Packages | Help | Viewer

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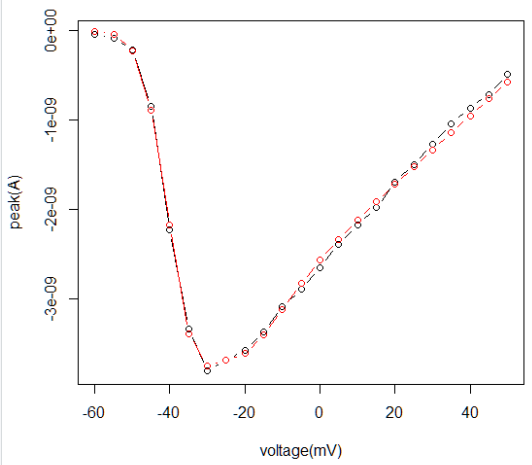
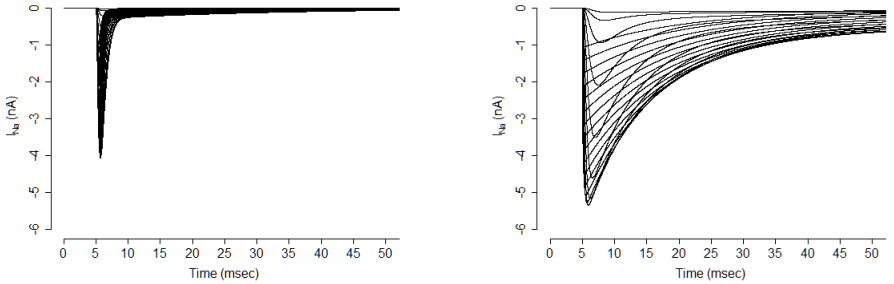
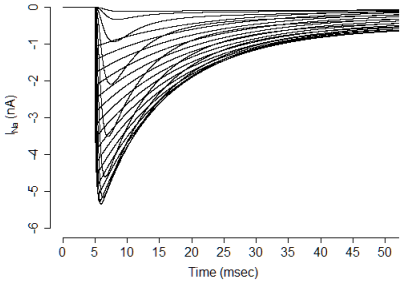


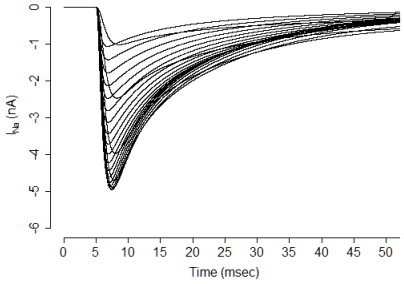
Simulated current traces of WT and A1656D

WT ⇒ **A1656D**

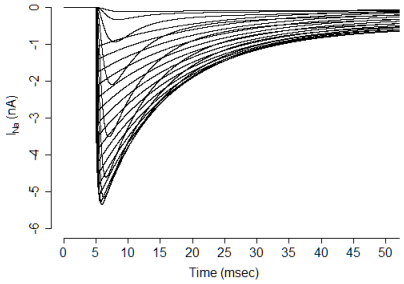


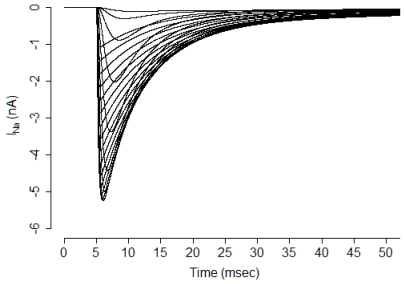
A1656D ⇒ **A1656D + Flecainide**



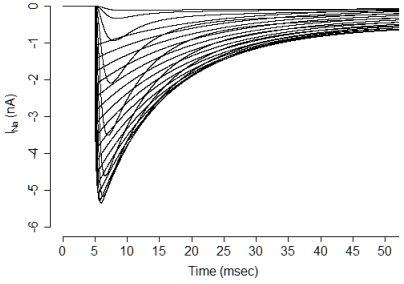


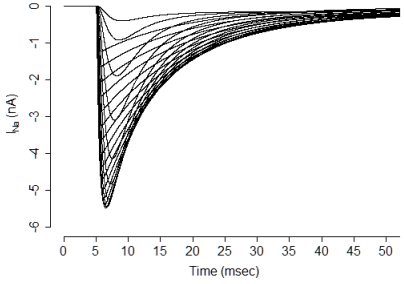
A1656D ⇒ **A1656D + Mexiletine**



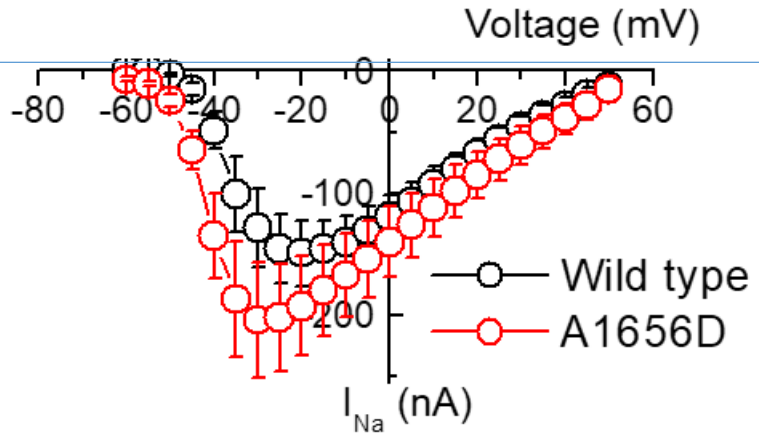


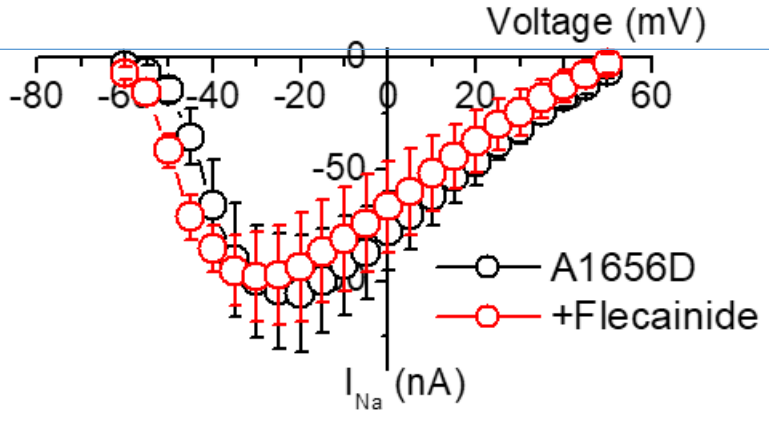
A1656D ⇒ **A1656D + Ranolazine**

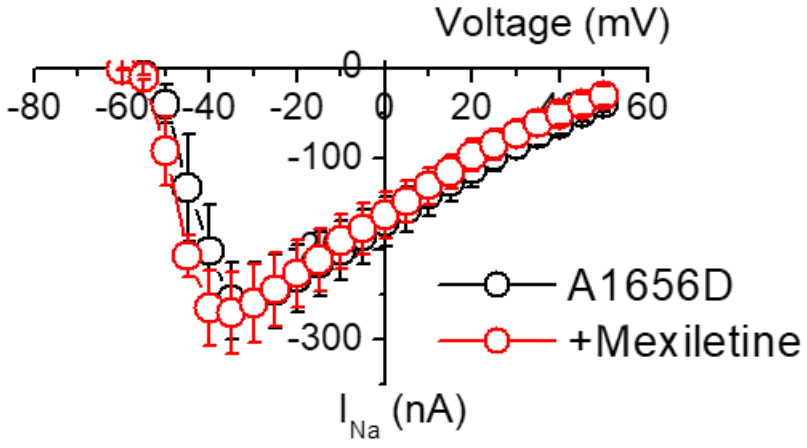


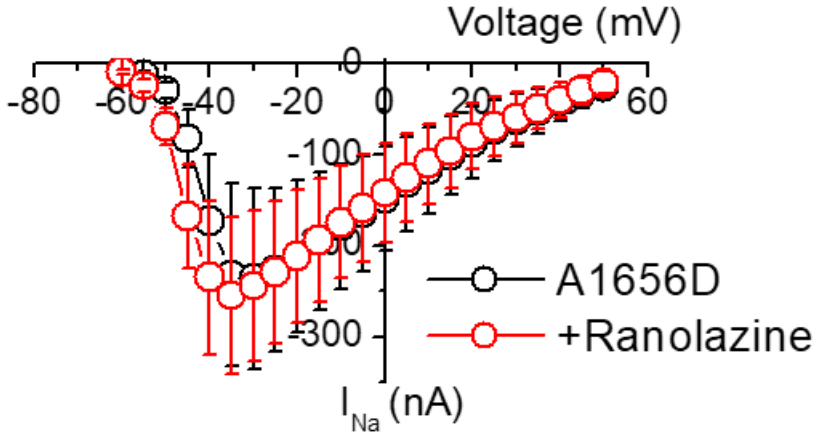


Current-voltage relations and their drug responses

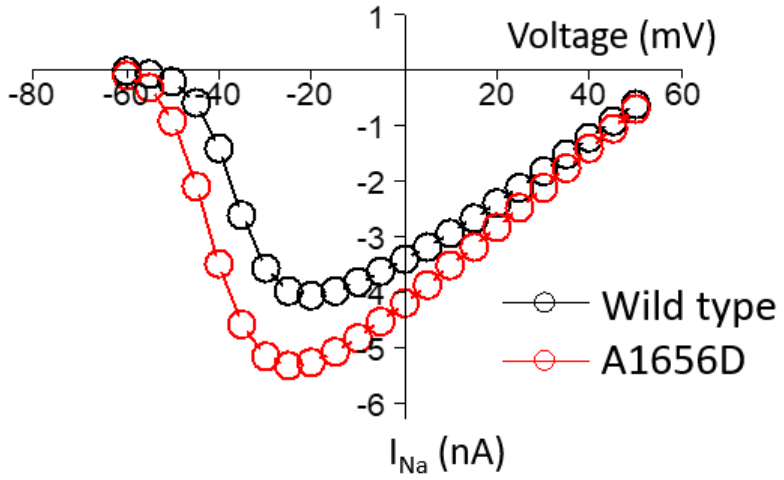


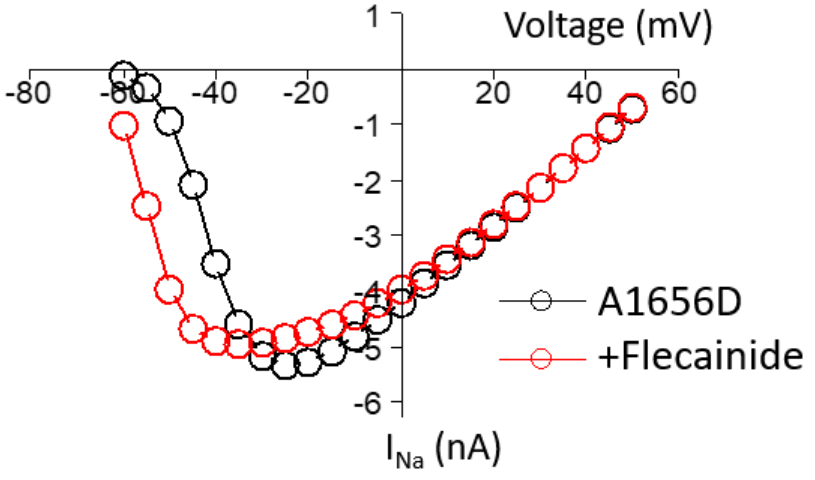


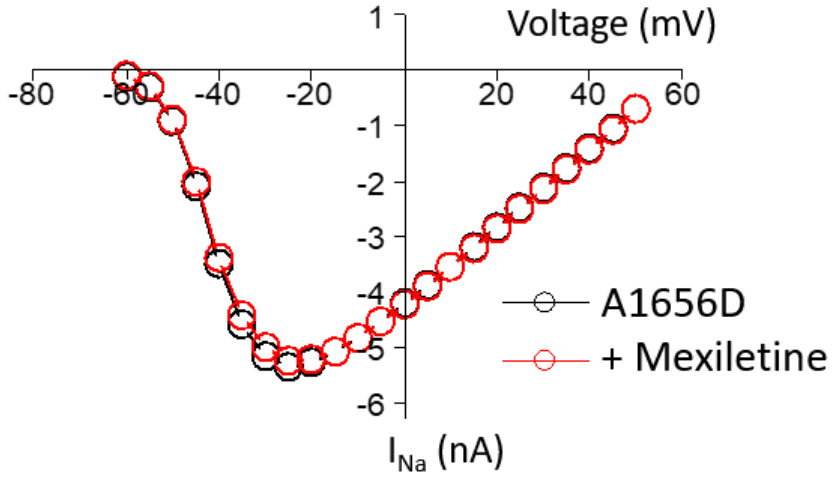


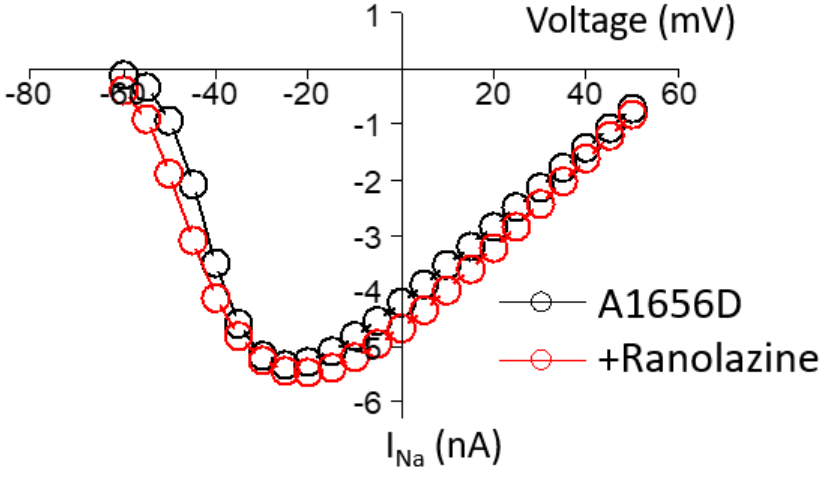


Simulated current-voltage relations and their drug responses



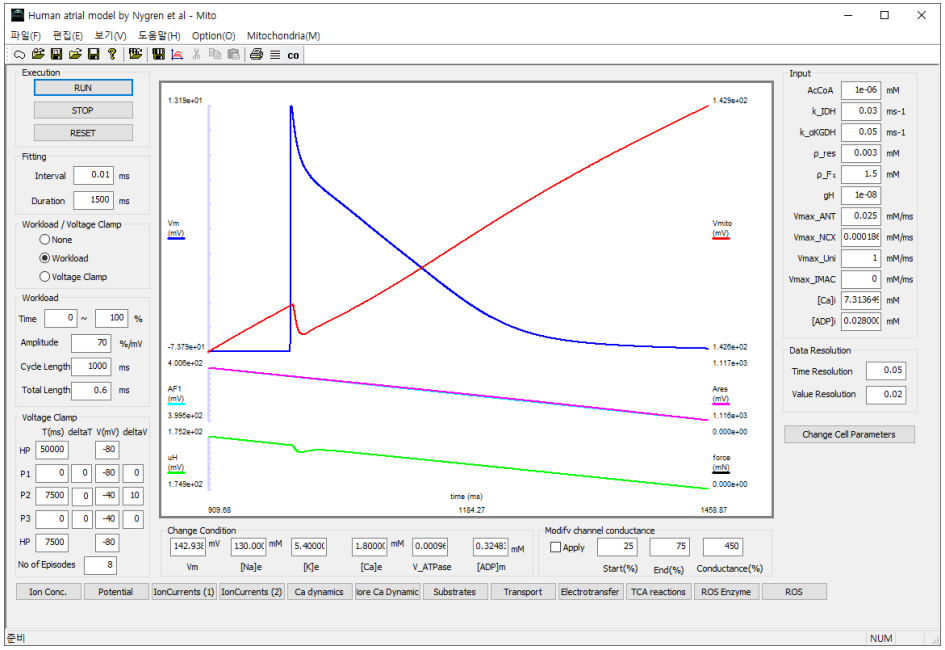


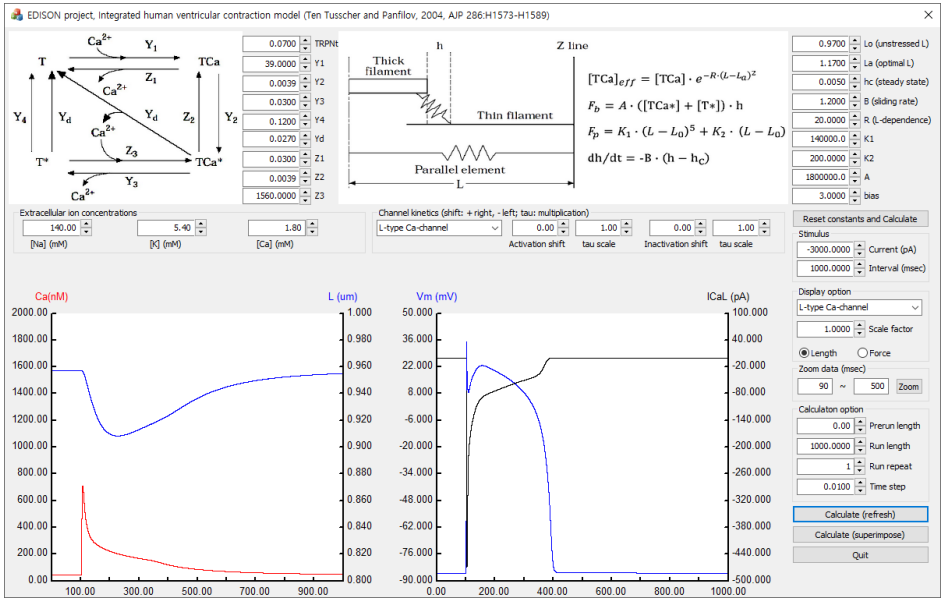




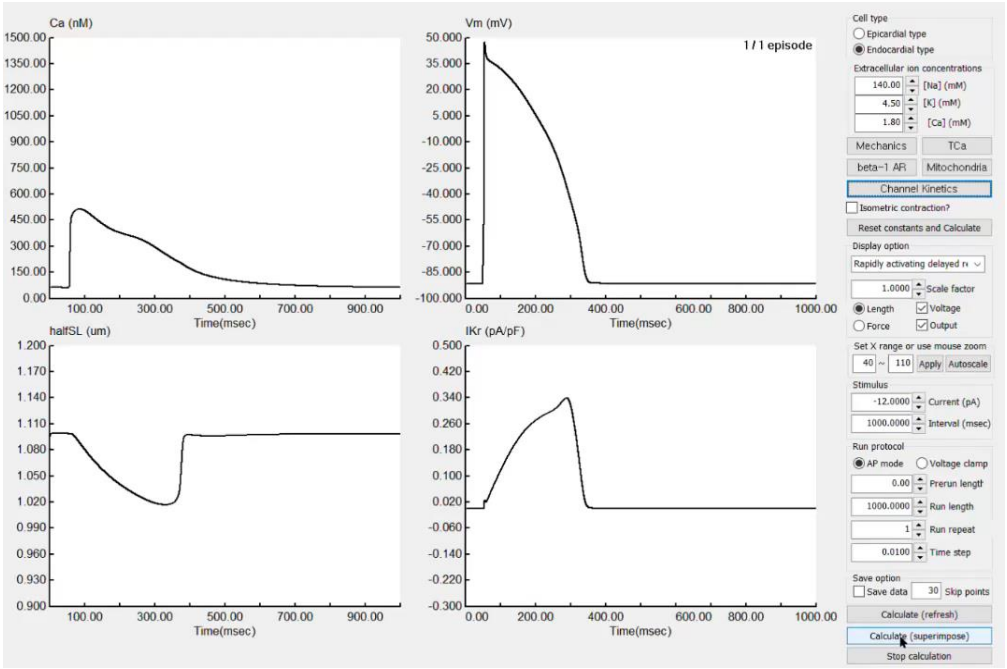
Integration into human atrial and ventricular myocyte models

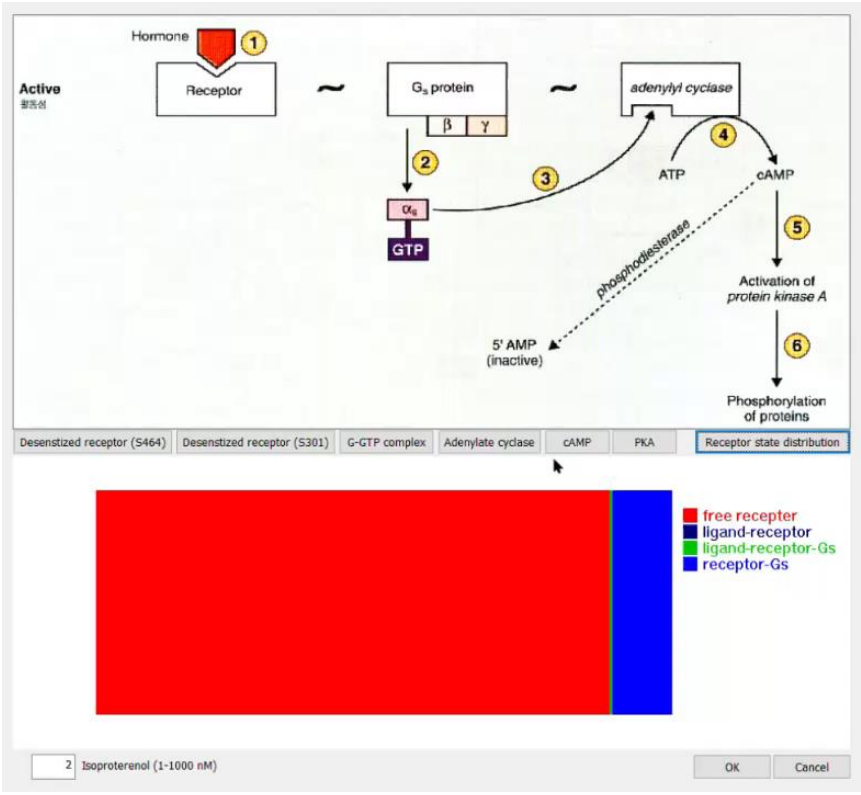
Nygren et al. (1998), Circulation Research





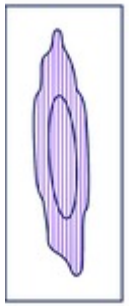
Ten Tusscher and Panfilov (2004), AJP

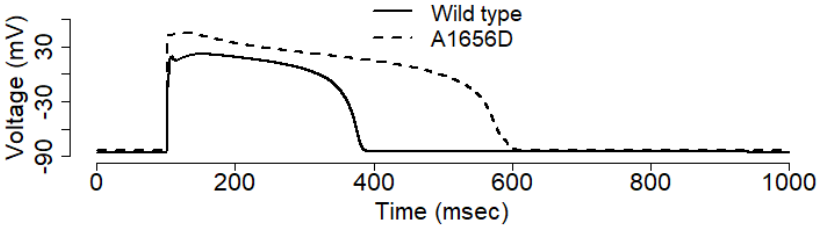

Ca (nM)
Vm (mV)
1 / 1 episode
halfSL (um)
IKr (pA/pF)
Time(msec)
Cell type
Epicardial type
Endocardial type
Extracellular ion concentrations
140.00 [Na] (mM)
4.50 [K] (mM)
1.80 [Ca] (mM)
Mechanics
TCa
beta-1 AR
Mitochondria
Channel Kinetics
Isometric contraction?
Reset constants and Calculate
Display option
Rapidly activating delayed r
1.0000 Scale factor
Length
Voltage
Force
Output
Set X range or use mouse zoom
40 ~ 110 Apply Autoscale
Stimulus
-12.0000 Current (pA)
1000.0000 Interval (msec)
Run protocol
AP mode
Voltage clamp
0.00 Prerun length
1000.0000 Run length
1 Run repeat
0.0100 Time step
Save option
Save data
30 Skip points
Calculate (refresh)
Calculate (superimpose)
Stop calculation

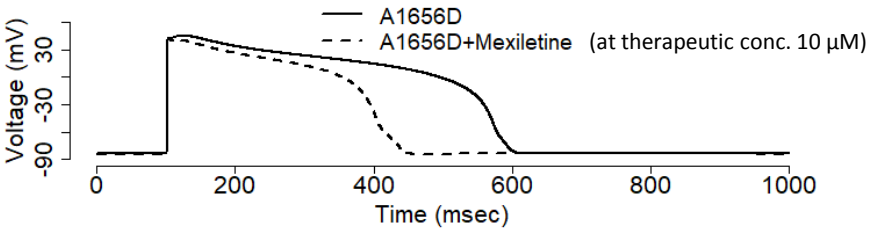

Hormone
Active
Receptor
G_s protein
β γ
adenylyl cyclase
ATP
cAMP
α_s
GTP
phosphodiesterase
5' AMP
(inactive)
Activation of
protein kinase A
Phosphorylation
of proteins
Desenstized receptor (S464)
Desenstized receptor (S301)
G-GTP complex
Adenylate cyclase
cAMP
PKA
Receptor state distribution
free recepter
ligand-receptor
ligand-receptor-Gs
receptor-Gs
2 Isoproterenol (1-1000 nM)
OK
Cancel

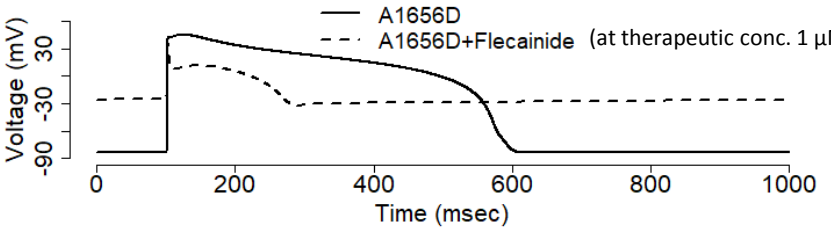
Predicted effect of drugs on human ventricular myocytes

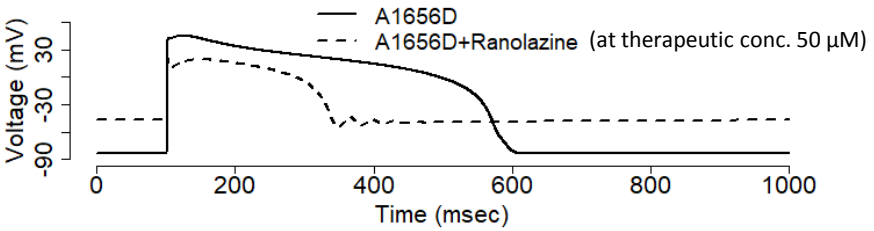
Human ventricular myocytes





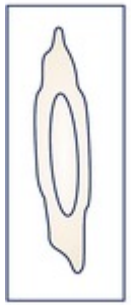


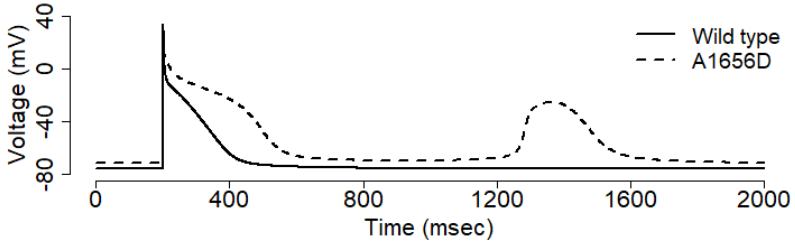


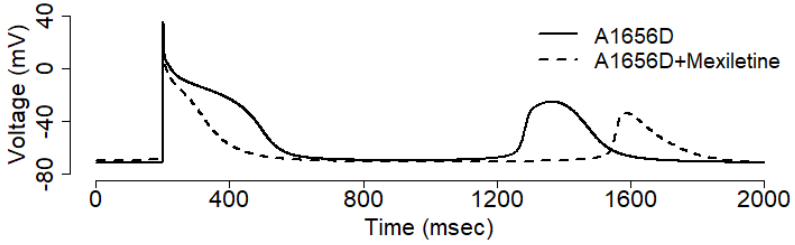


Predicted effect of drugs on human atrial myocytes

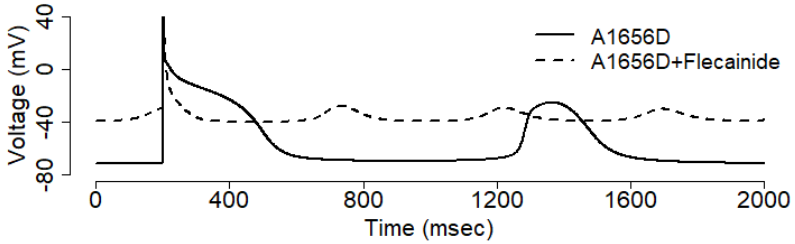
Human atrial myocytes



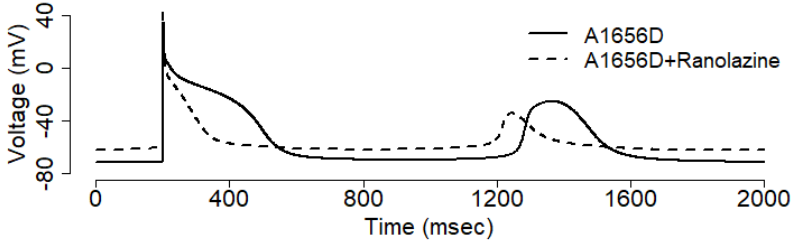




(at therapeutic conc. 10 µM)



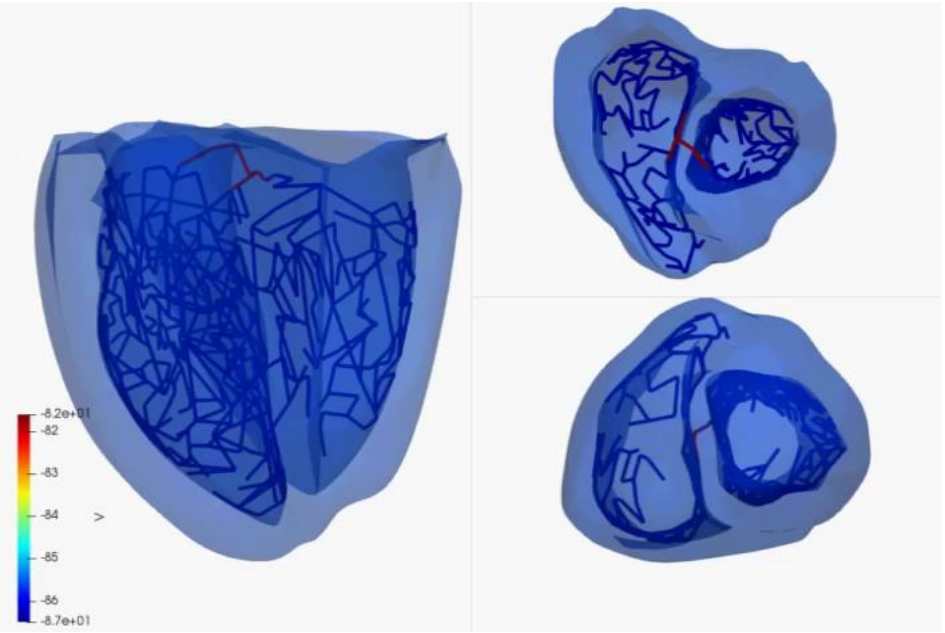
(at therapeutic conc. 1 µM)

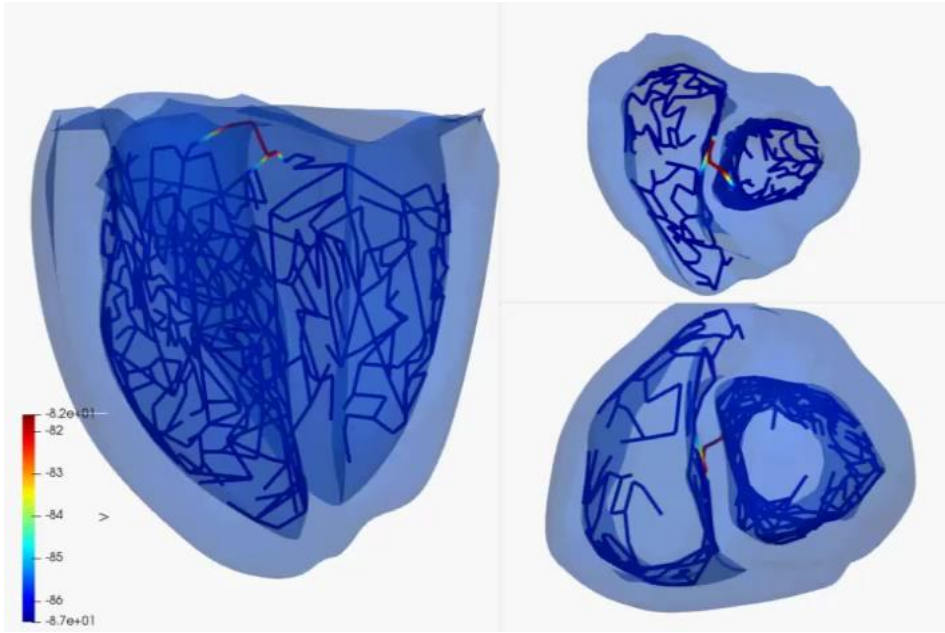


(at therapeutic conc. 50 µM)

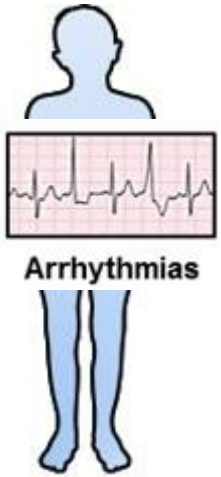
WT

A1656D

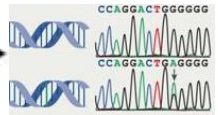




Is prediction correct?



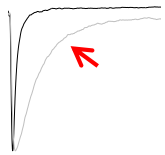
DNA sequence



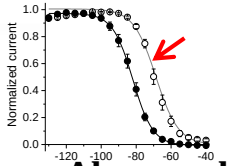
SCN5A
A1656D

Patch Clamp

A1656D SCN5A defects



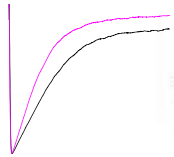
Persistent current



Abnormal inactivation

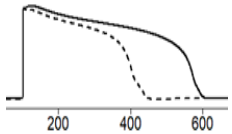
Treat with candidate drugs

+ Mexiletine



Drug response

In Silico Simulation



Defects improve

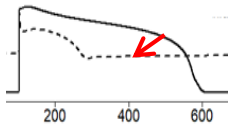
Compare *in vitro* results to clinical outcomes

+ Mexiletine

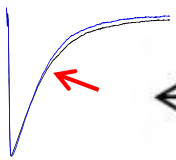
?

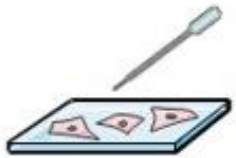
+ Flecainide

?



Defects persist



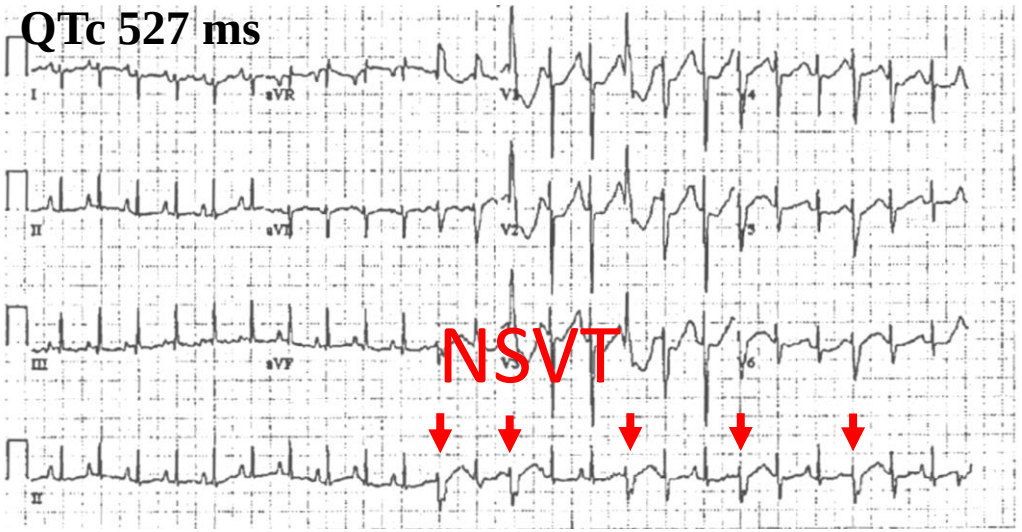


+ Flecainide

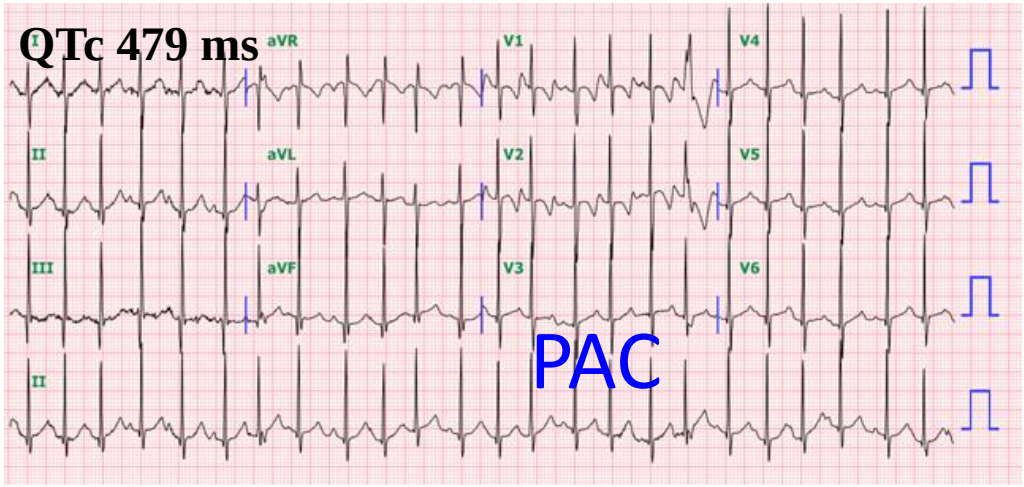
Mexiletine preferentially resolves ventricular arrhythmias in a proband

Flecainide

b-blockers

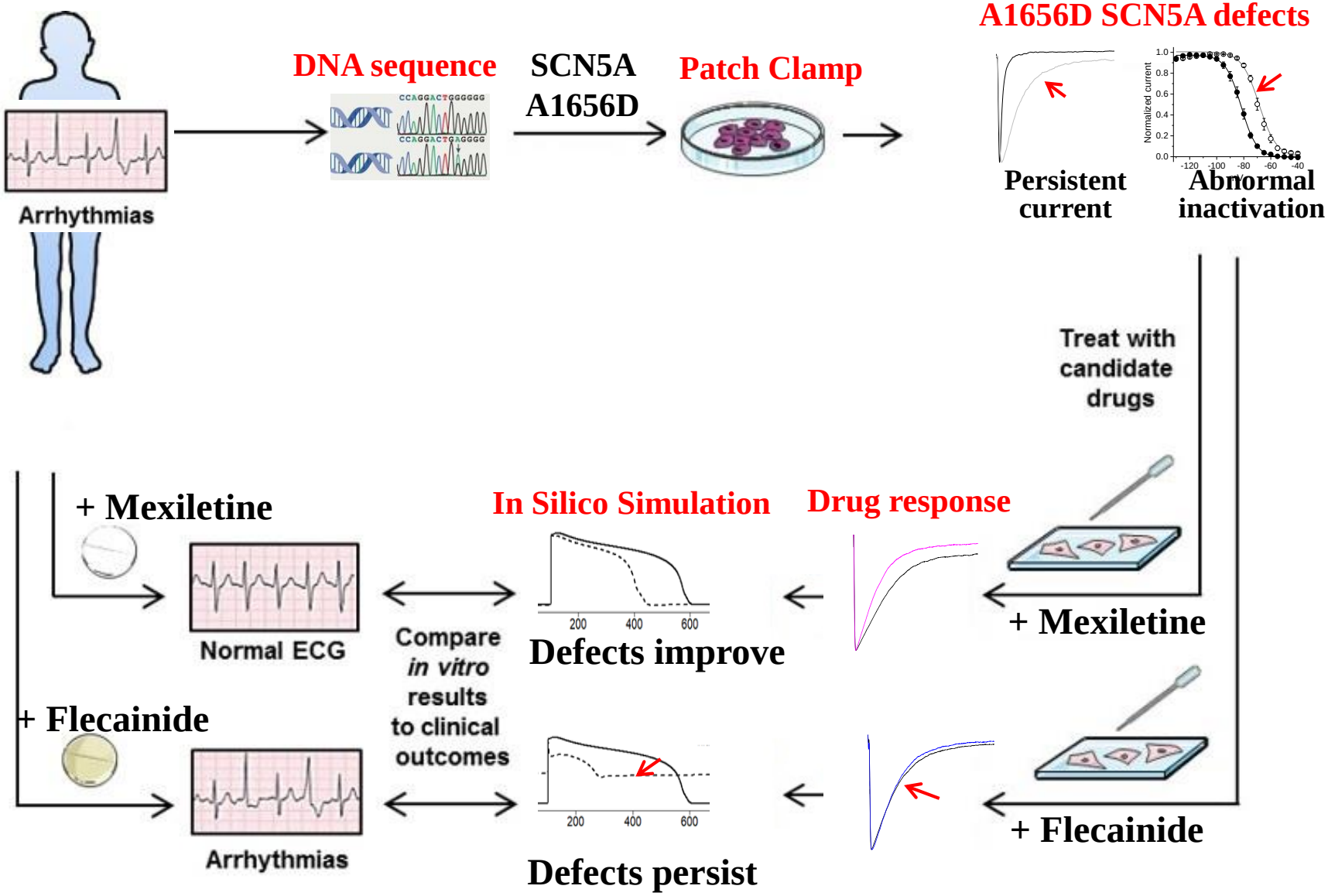


Mexiletine



Revision in JMCC

Is prediction correct? Yes



Part 2: CiPA

A brief introduction to CiPA (Comprehensive *In Vitro* Proarrhythmia Assay)

TCP Transl Clin Pharmacol
2019;27(1):12-18
https://doi.org/10.12793/tcp.2019.27.1.12

Introduction to *in silico* model for proarrhythmic risk assessment under the CiPA initiative

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In 2005, the International Council for Harmonization (ICH) established cardiotoxicity assessment guidelines to identify the risk of Torsade de Pointes (TdP). It is focused on the blockade of the human ether-à-go-go-related gene (hERG) channel known to cause QT/QTc prolongation and the QT/QTc prolongation shown on the electrocardiogram. However, these biomarkers are not the direct risks of TdP with low specificity as the action potential is influenced by multiple channels along with the hERG channel. Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative emerged to address limitations of the current model. The objective of CiPA is to develop a standardized *in silico* model of a human ventricular cell to quantitively evaluate the cardiac response for the cardiac toxicity risk and to come up with a metric for the TdP risk assessment. *In silico* working group under CiPA developed a standardized and reliable *in silico* model and a metric that can quantitatively evaluate cellular cardiac electrophysiologic activity. The implementation mainly consists of hERG fitting, Hill fitting, and action potential simulation. In this review, we explained how the *in silico* model of CiPA works, and briefly summarized current overall CiPA studies. We hope this review helps clinical pharmacologists to understand the underlying estimation process of CiPA *in silico* modeling.

frontiers in Physiology
ORIGINAL RESEARCH
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Three-Dimensional Heart Model-Based Screening of Proarrhythmic Potential by *in silico* Simulation of Action Potential and Electrocardiograms

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Drugs Withdrawn from Market Due to QTc Prolongation or Torsade de Pointes

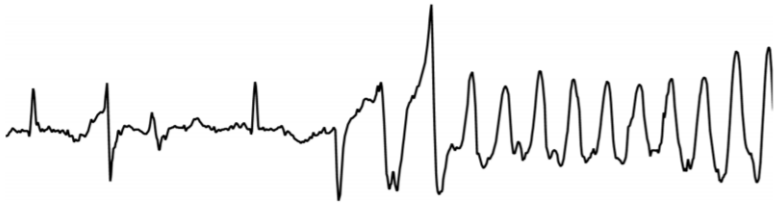
Drug	Therapeutic Class	Year of Withdrawal
Prenylamine	Antianginal	1988 (EU, not marketed in US)
Terodiline	Antianginal/urinary incontinence	1991 (EU, not marketed in US)
Terfenadine	Antihistamine	1998
Sertindole	Antipsychotic	1998 (not marketed in US, EU reintroduction in 2002)
Astemizole	Antihistamine	1999
Sparfloxacin	Antibiotic	2001
Cisapride	Gastric prokinetic	2000
Droperidol	Tranquilizer/analgesic	2001
Levacetylmethadol	Methadone substitution	2003
Thioridazine	Antipsychotic	2005 (ex-US)
Propoxyphene	Opioid analgesic	2010

Adapted from Table 1 in **Stockbridge et al. Drug Safety (2013) 36:167-82**
EU, European Union; **US**, United States

https://www.fda.gov/media/104642/download

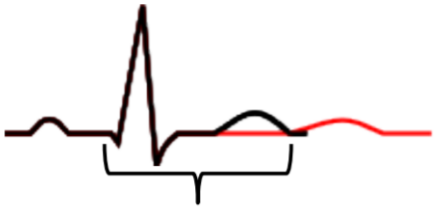
Torsade de Pointes and QT prolongation

Torsade de pointes …



↓

Is associated with QT prolongation …



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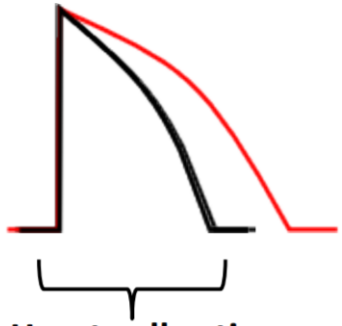
Is associated with action potential prolongation …



Is associated with hERG channel block →

Not all QT prolonging drugs cause torsade de pointes!!!

https://c-path.org/wp-content/uploads/2019/09/BmPWksp-3_6-CiPA_Mode-ZLi.pdf

Regulatory (ICH) guidelines

- **ICH S7B: The nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals**
- **ICH E14: The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs – randomized, placebo- and positive-controlled study in healthy volunteers to evaluate QT/QTc interval at supratherapeutic dose levels**

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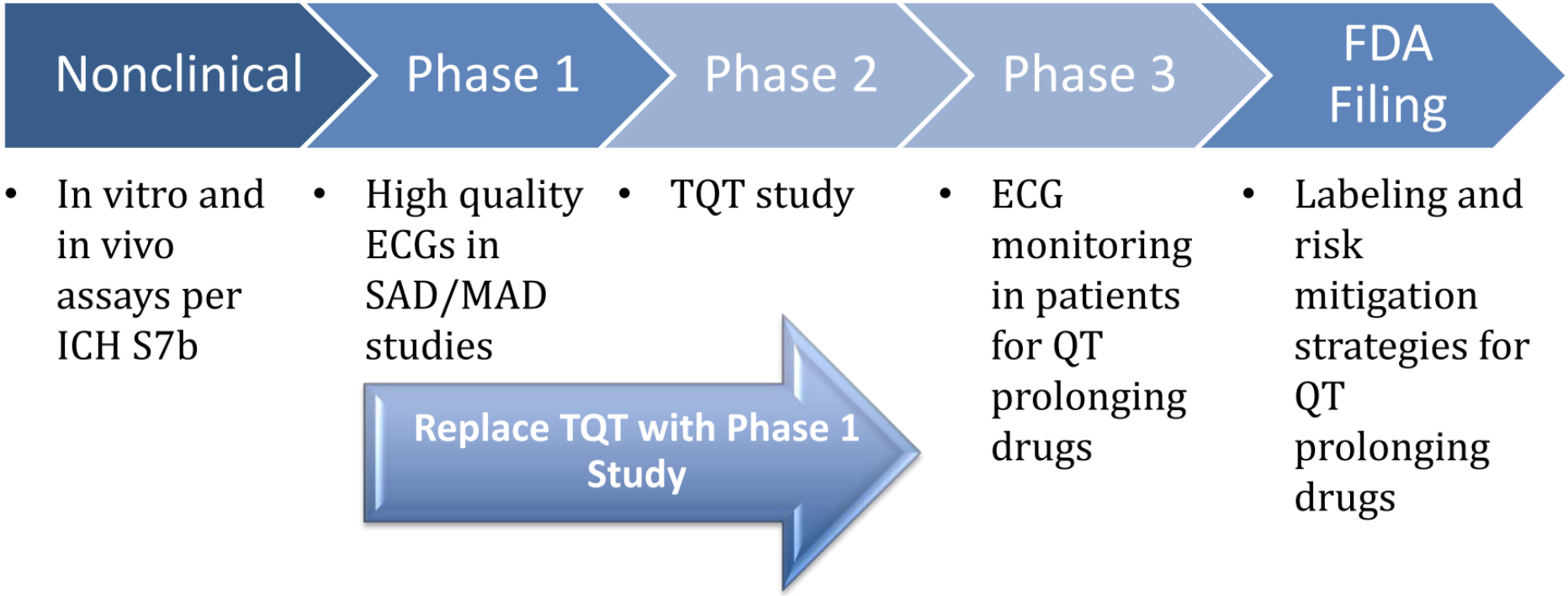
QTc Evaluation in Drug Development



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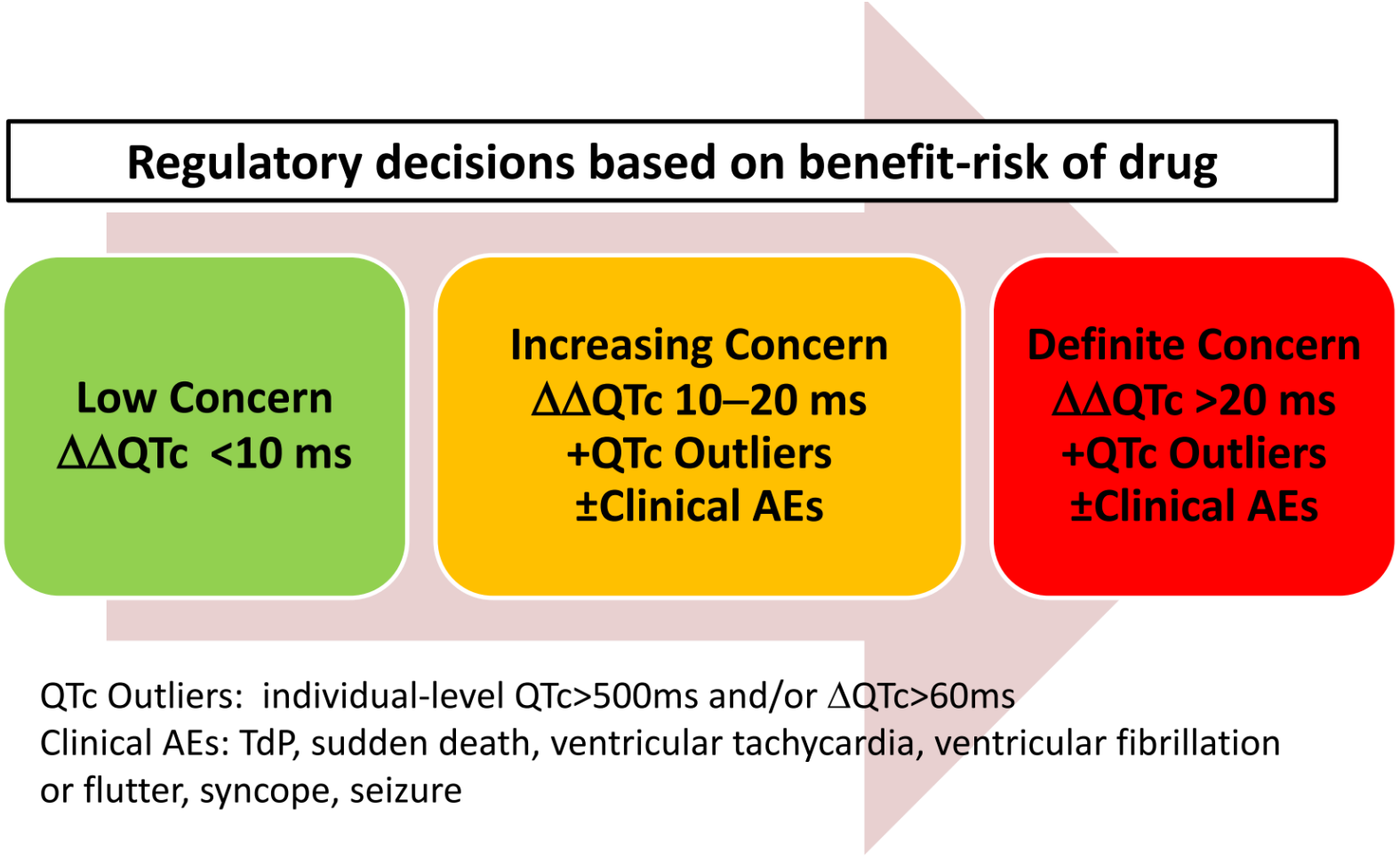
QTc Prolongation and Concern for Torsade de Pointes Risk

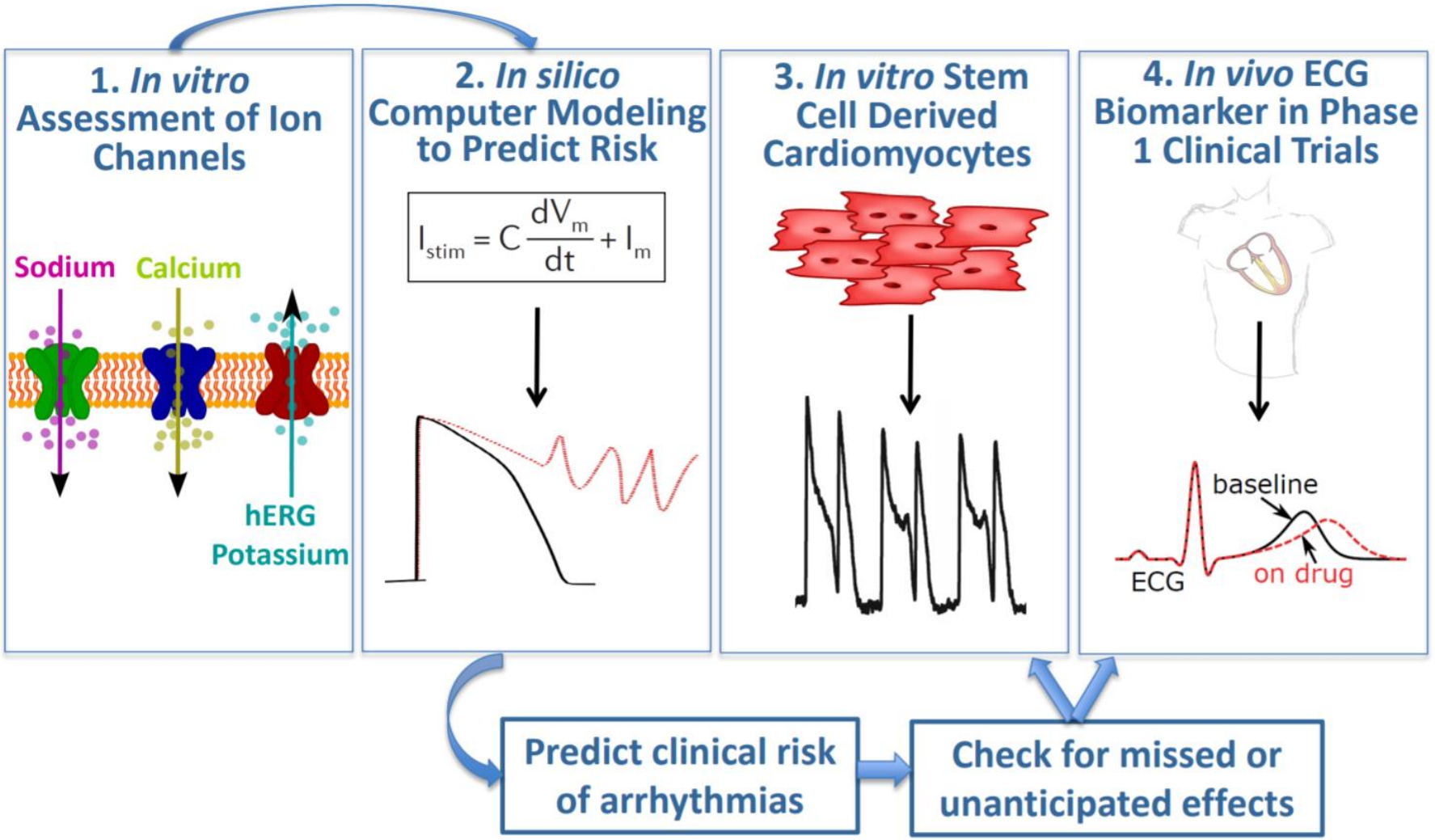


QTc Outliers: individual-level QTc>500ms and/or ΔQTc>60ms
Clinical AEs: TdP, sudden death, ventricular tachycardia, ventricular fibrillation or flutter, syncope, seizure

ΔΔQTc, change from baseline QTc placebo corrected; **AE**, adverse event; **TdP**, torsade de pointes

https://www.fda.gov/media/104642/download

Comprehensive in vitro Proarrhythmia Assay (CiPA)



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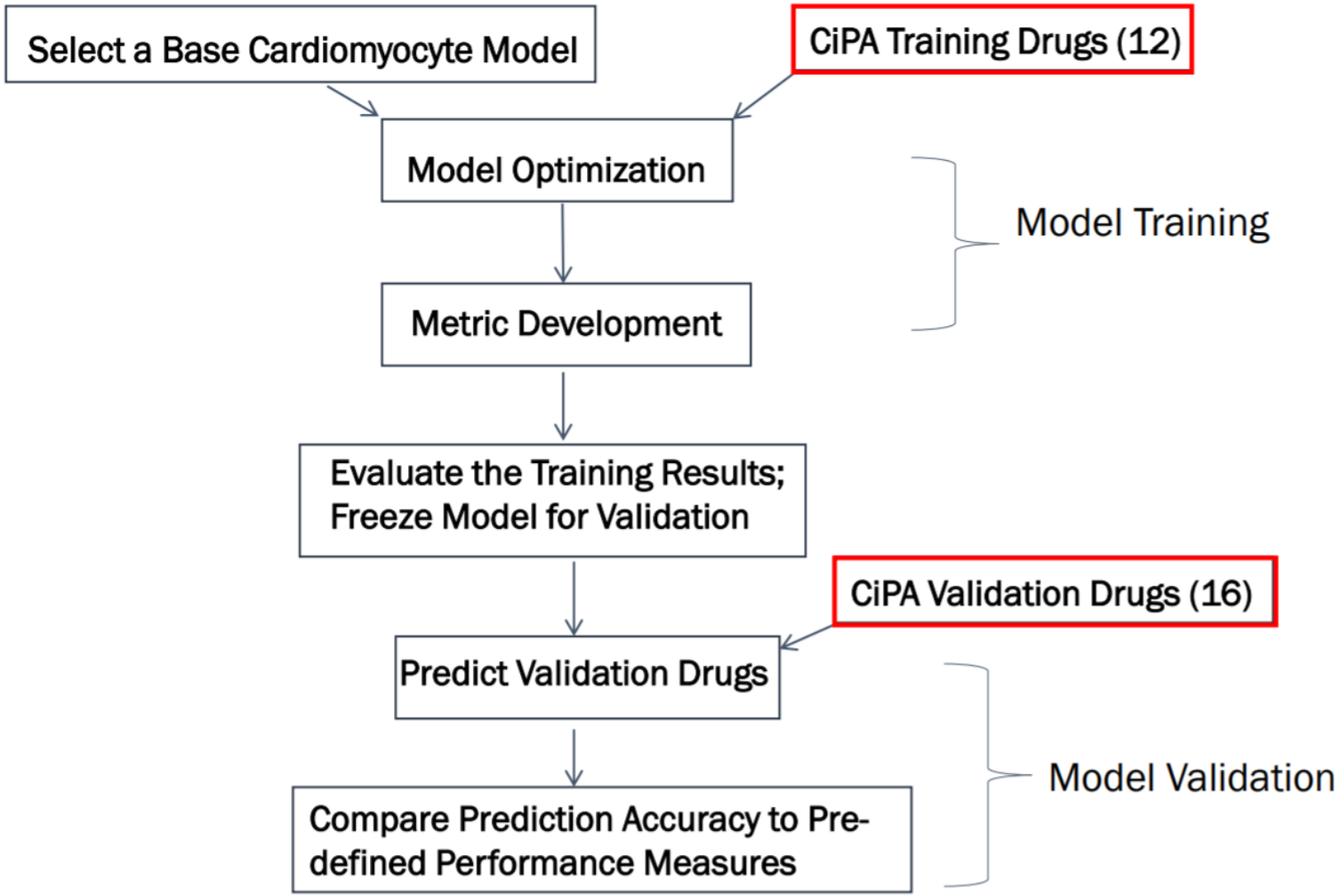
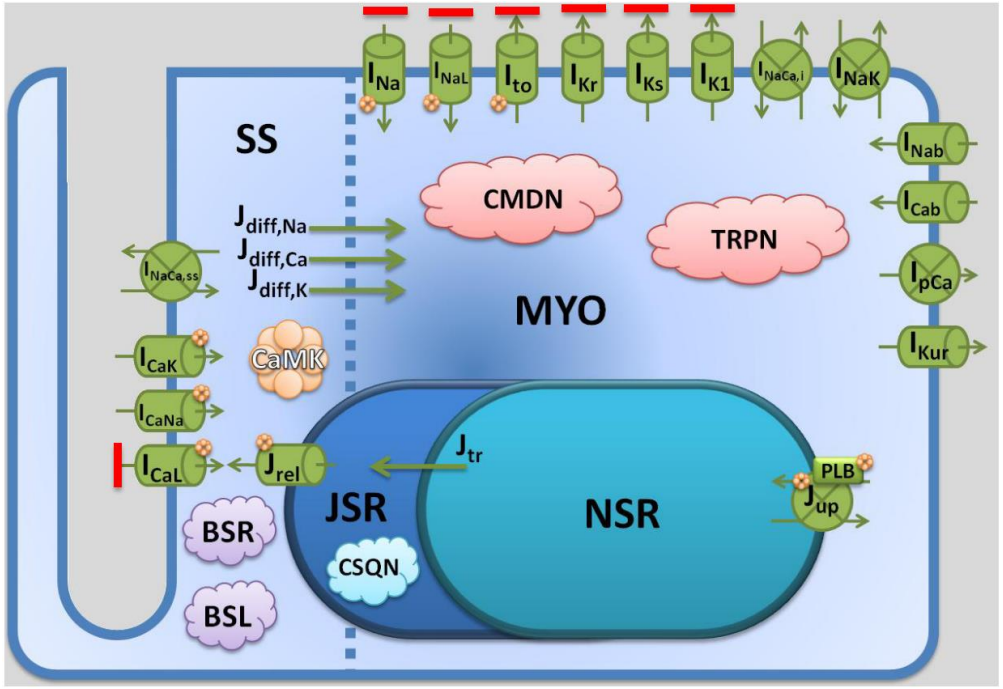
Model Development and Validation Strategy



https://c-path.org/wp-content/uploads/2019/09/BmPWksp-3_6-CiPA_Mode-ZLi.pdf

Model Development and Validation Strategy

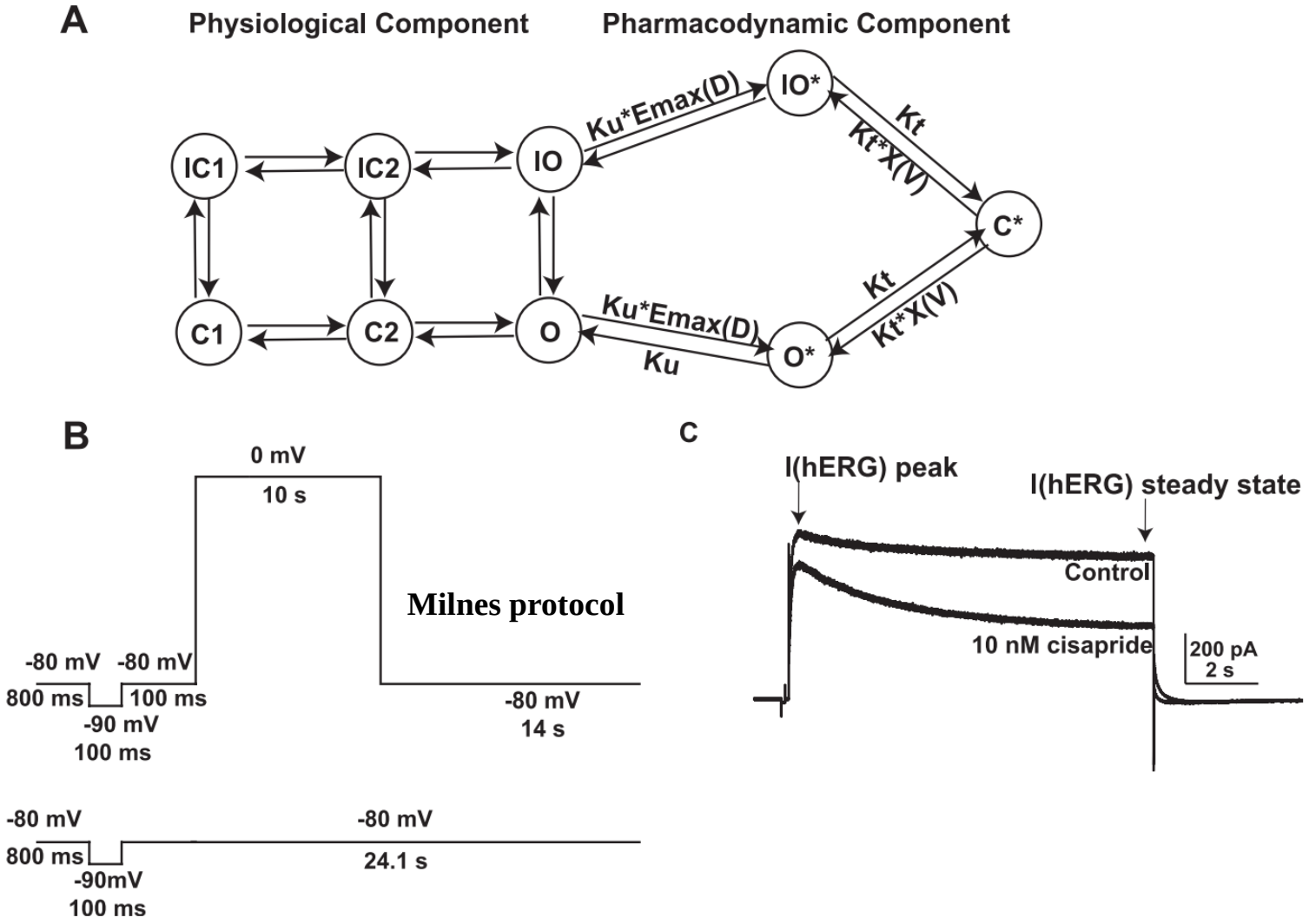


Base cardiomyocyte model: O'Hara T, Virag L, Varro A, & Rudy Y (2011) PLoS Comput Biol 7(5):e1002061.

1. Modeling dynamic drug-hERG interactions rather than using simple IC50s
2. Optimizing model parameters so that the model can better recapitulate experimental data
3. Developing a statistical framework to translate experimental variability into prediction uncertainty

https://c-path.org/wp-content/uploads/2019/09/BmPWksp-3_6-CiPA_Mode-ZLi.pdf

Modeling dynamic drug-hERG interactions



Circ Arrhythm Electrophysiol. 2017;10:e004628. DOI: 10.1161/CIRCEP.116.004628.)

Trapping behaviors of drugs are revealed by Milnes protocol

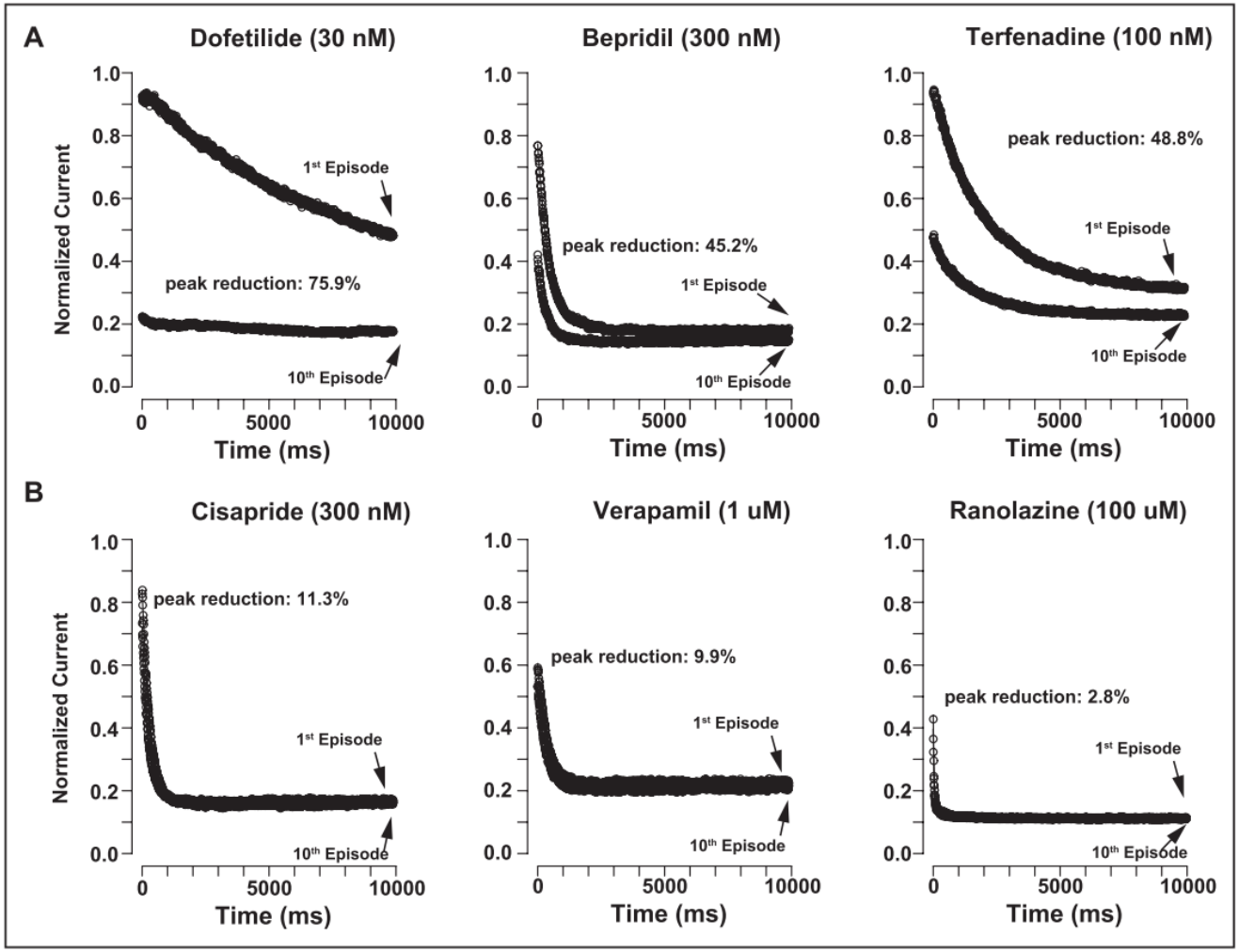
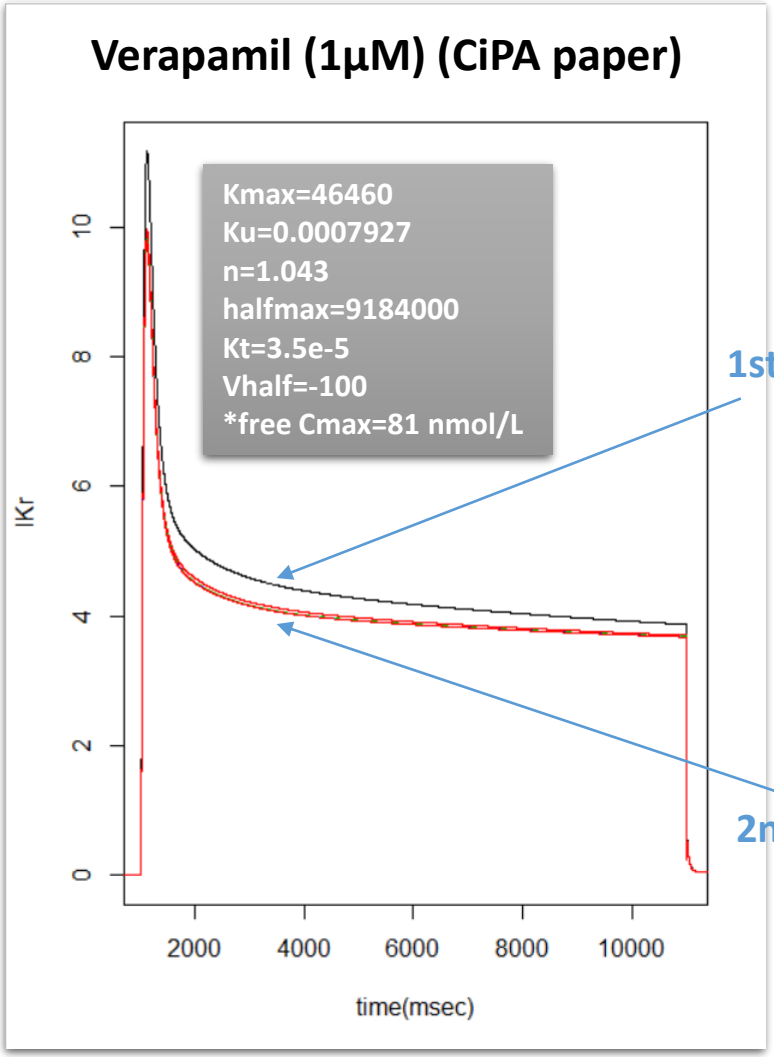


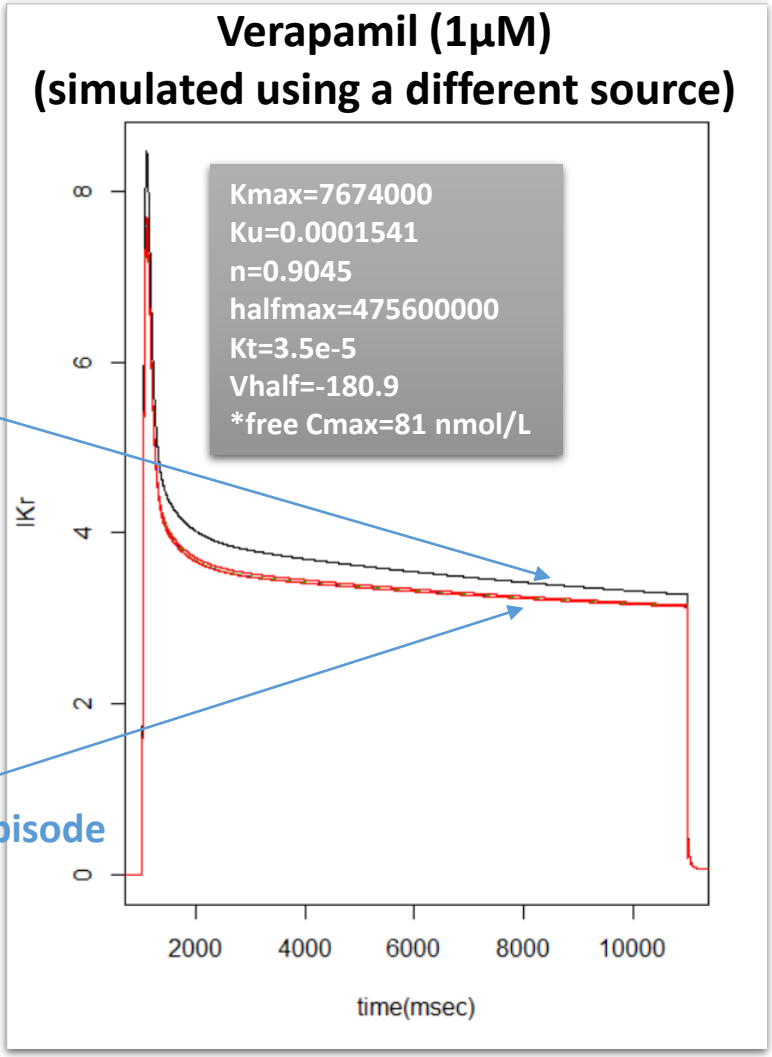
Figure 2. Different patterns for trapped and nontrapped drugs assessed by Milnes protocol. Shown are the mean normalized currents for the first and tenth episodes during the sustained depolarization at 0 mV after equilibration in 3 trapped drugs (**A**, dofetilide, bepridil, and terfenadine) and 3 nontrapped drugs (**B**, cisapride, verapamil, and ranolazine). Time zero corresponds to depolarization from –80 to 0 mV. Note for trapped drugs (**A**), there is a significant decrease in current peaks, whereas for nontrapped drugs (**B**), the first and tenth episodes look almost identical.

Circ Arrhythm Electrophysiol. 2017;10:e004628. DOI: 10.1161/CIRCEP.116.004628.)

Reconstruction of trapping behaviors of drugs by hERG-drug binding model

free Cmax = 81 nmol/L



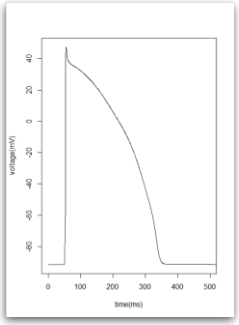


1st Episode

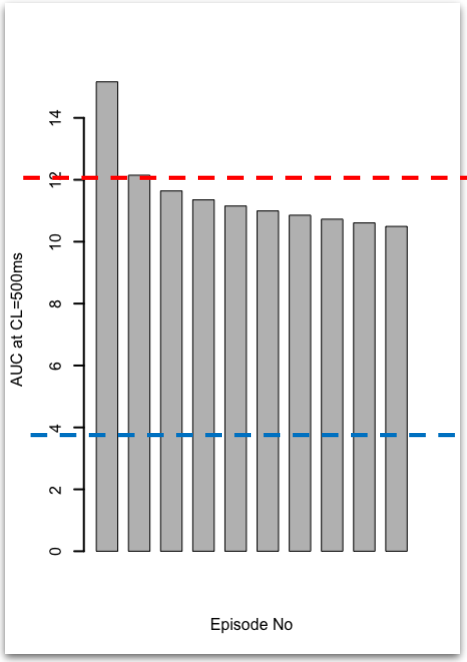
2nd ~ 10 th Episode

Evaluation of reverse-use dependency (RUD): Verapamil

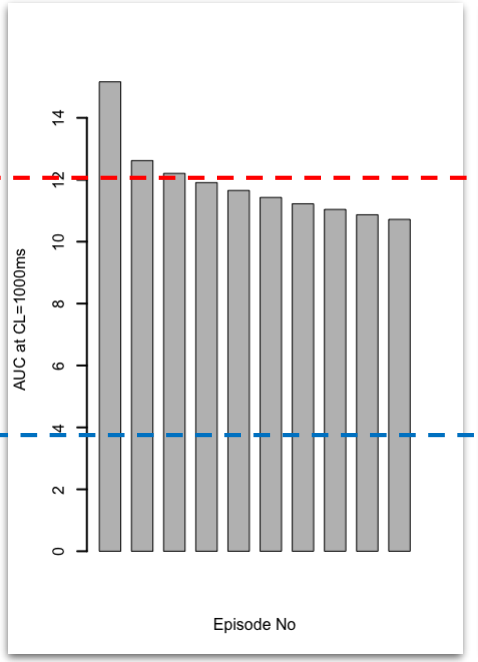
AP clamp (25x Cmax verapamil)
- AUC (area under curve)



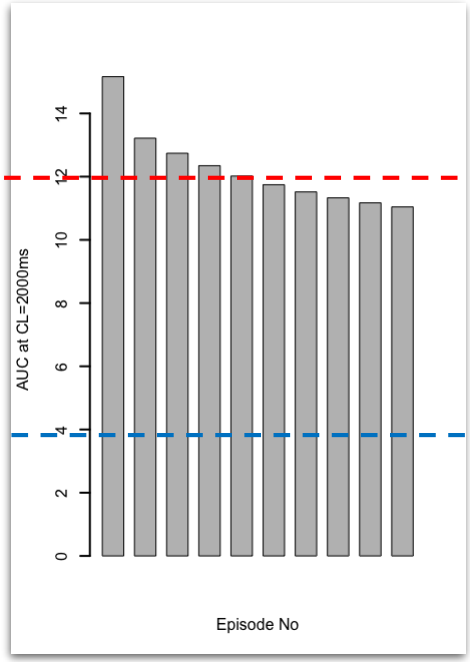
CL = 500 ms (2Hz)



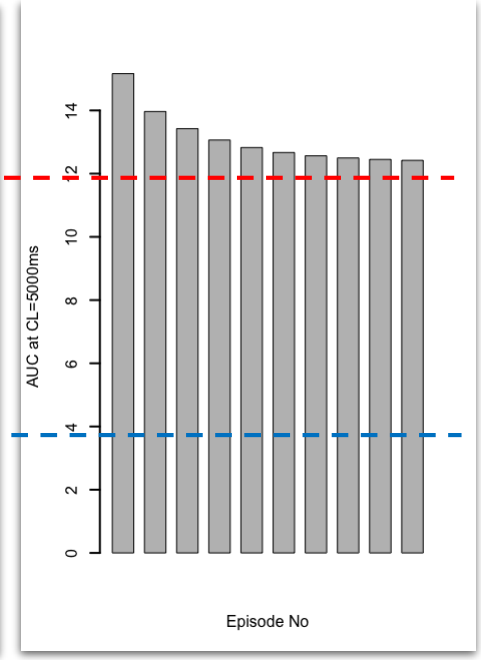
CL = 1000 ms (1Hz)



CL = 2000 ms (0.5Hz)



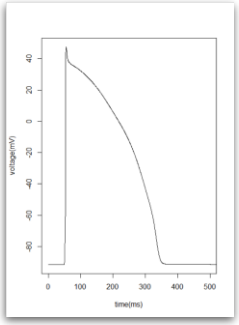
CL = 5000 ms (0.2Hz)



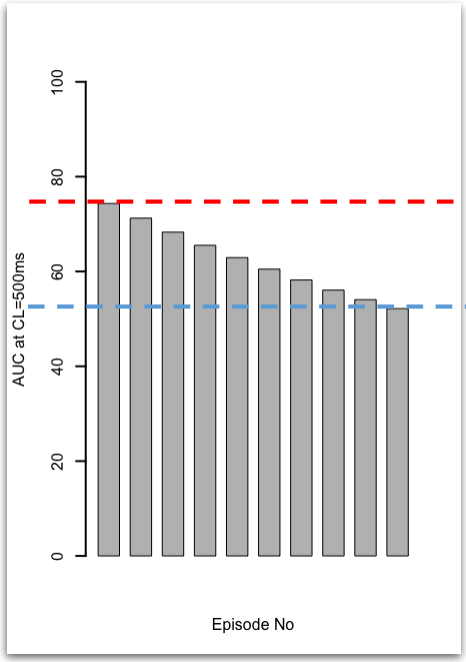
RUD: degree of APD prolongation
***more pronounced at slower heart rates**

Evaluation of reverse-use dependency (RUD): Dofetilide

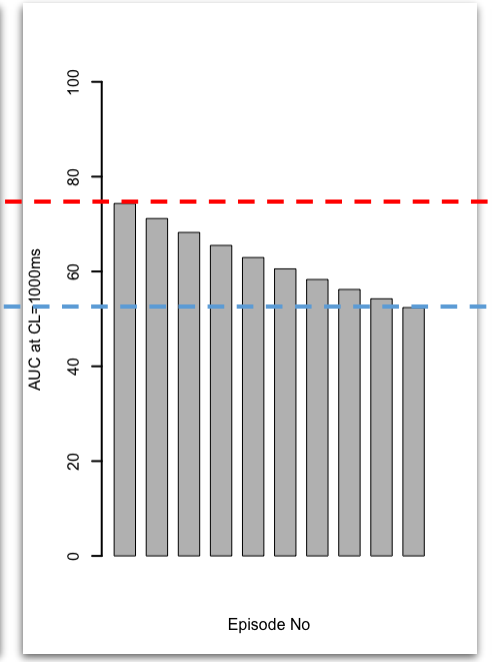
AP clamp (25x Cmax dofetilide)
- AUC (area under curve)



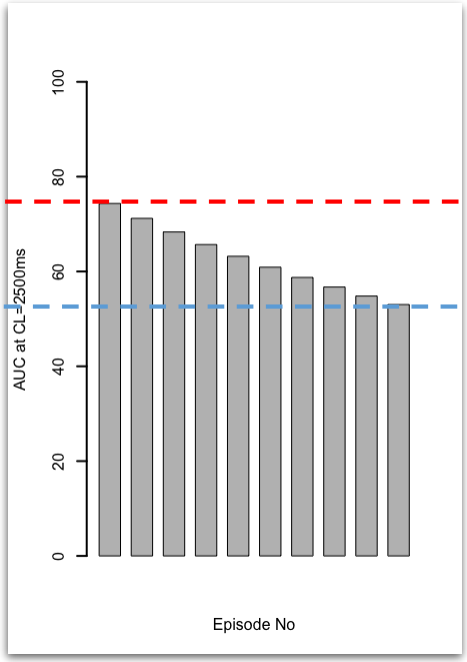
CL = 500 ms (2Hz)



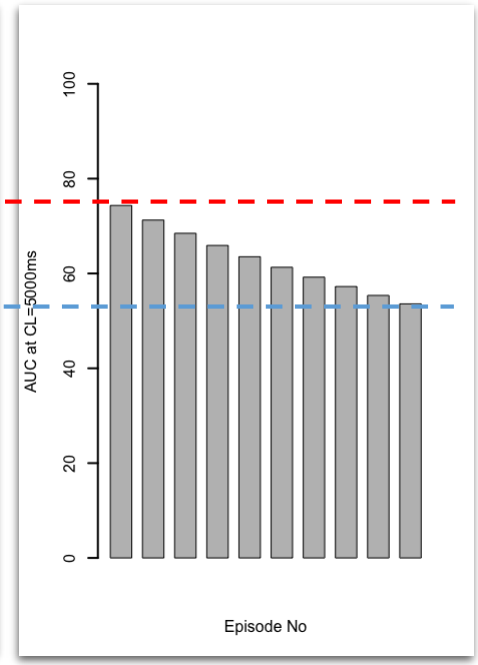
CL = 1000 ms (1Hz)



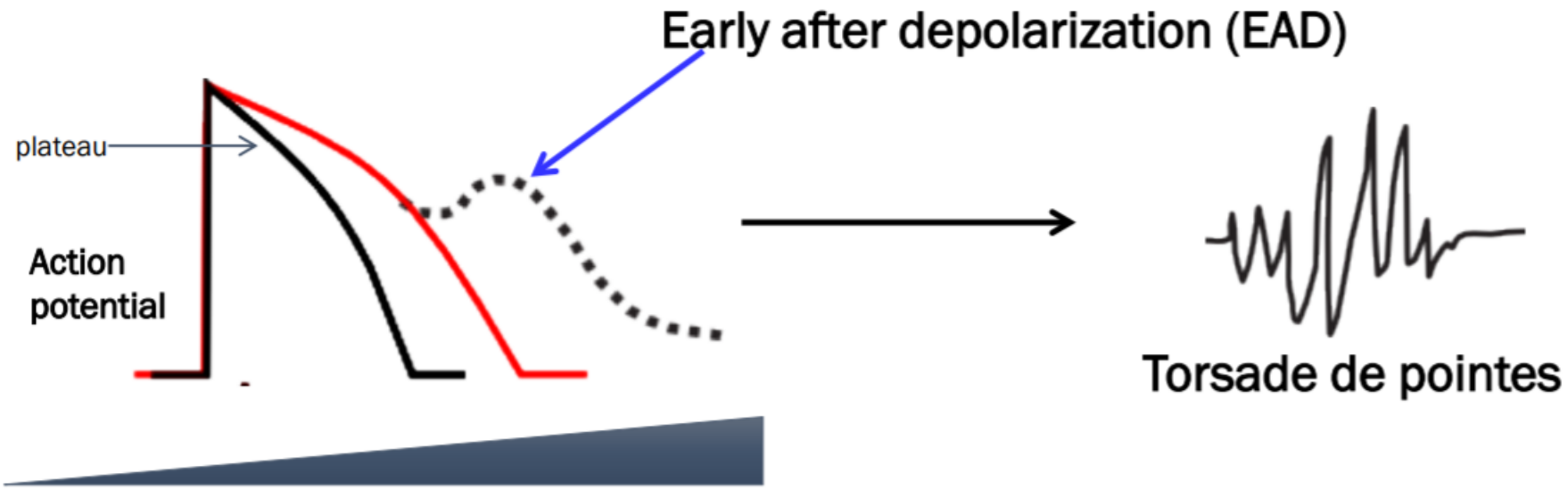
CL = 2000 ms (0.5Hz)



CL = 5000 ms (0.2Hz)



Key Mechanism of TdP: Imbalance of Inward and Outward Currents



Increased ratio between inward and outward currents

Inward	Outward
I_{CaL} (L-type Ca-current)	I_{Kr} (hERG + MiRP1) (Rapidly activating delayed recitifier K-current)
I_{NaL} (Late Na-current)	I_{Ks} (Slowly activating delayed rectifier K-current)
	I_{K1} (Inward retifier K-current)
	I_{to} (Transient outward K-current)

The net current between inward and outward currents reflect their balance.

$$I_{net} = I_{CaL}+I_{NaL}+I_{Kr}+I_{Ks}+I_{K1}+I_{to}$$

qNet: Amount of electronic charge carried by Inet

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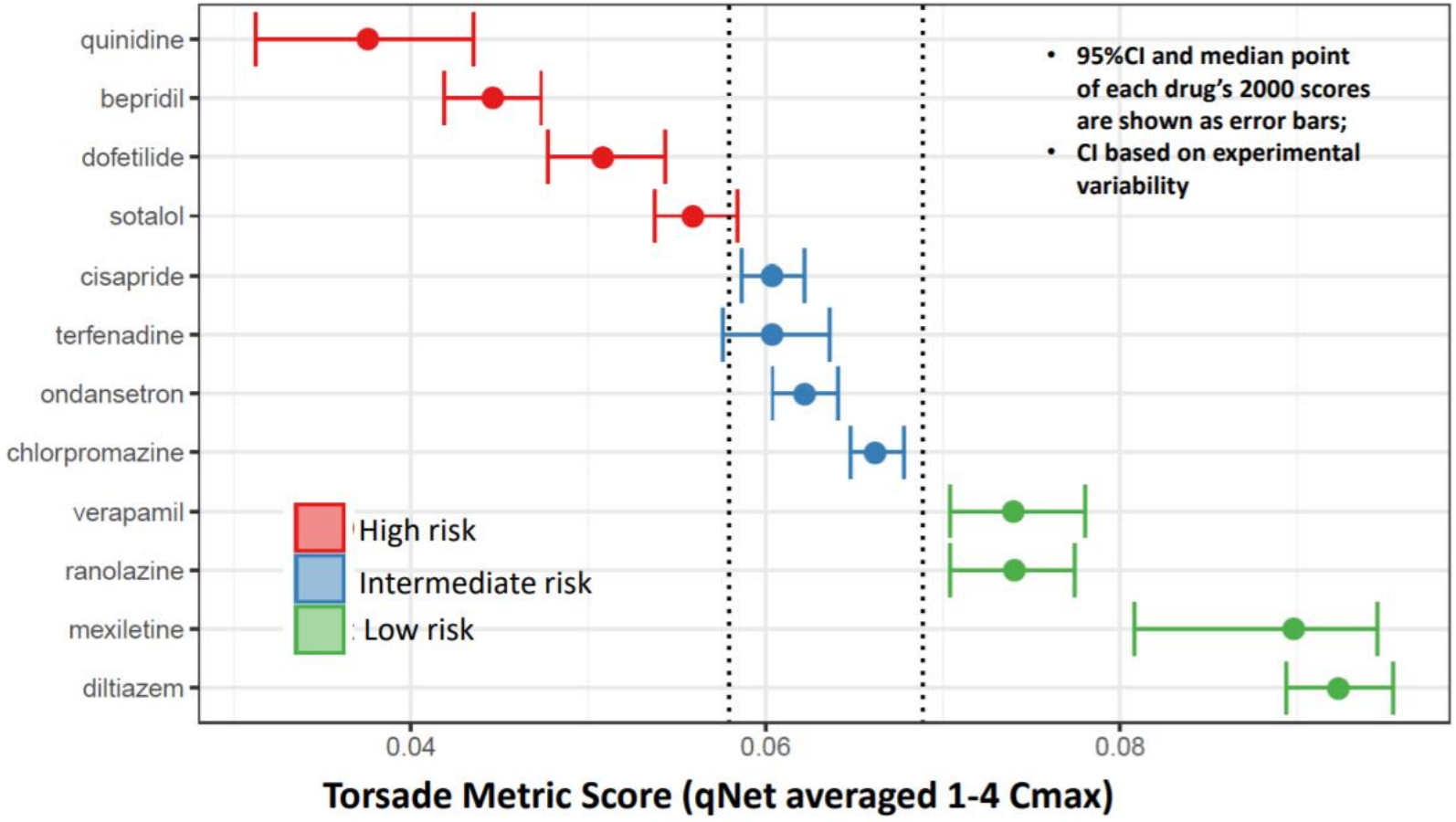
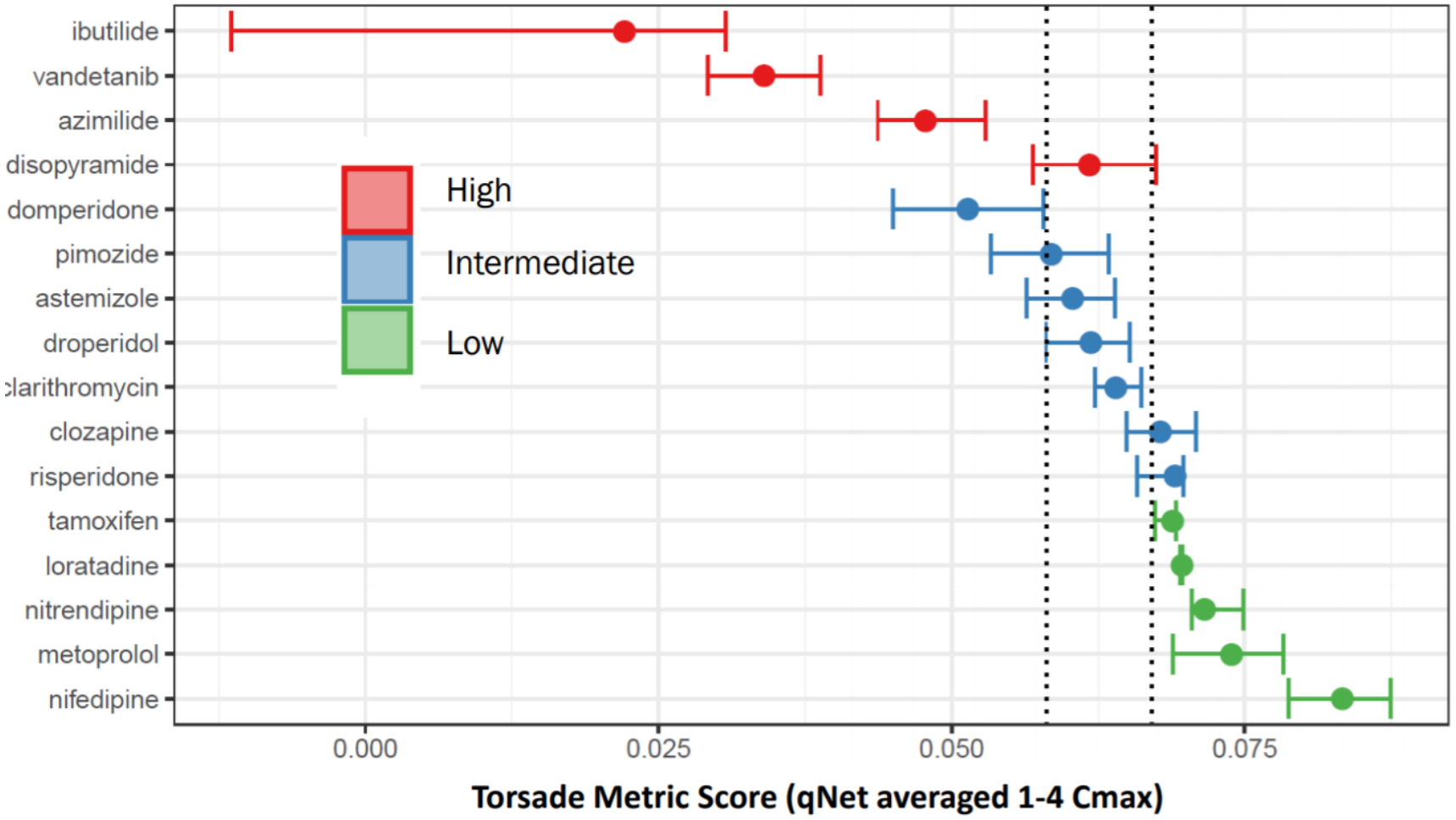
Torsade Metric Score for Manual Training Data



hERG (potassium channel) data: manual patch clamp
Non-hERG (sodium and calcium channel) data: manual patch clamp

https://c-path.org/wp-content/uploads/2019/09/BmPWksp-3_6-CiPA_Mode-ZLi.pdf

Prediction of the 16 Validation Drugs (Hybrid Data)



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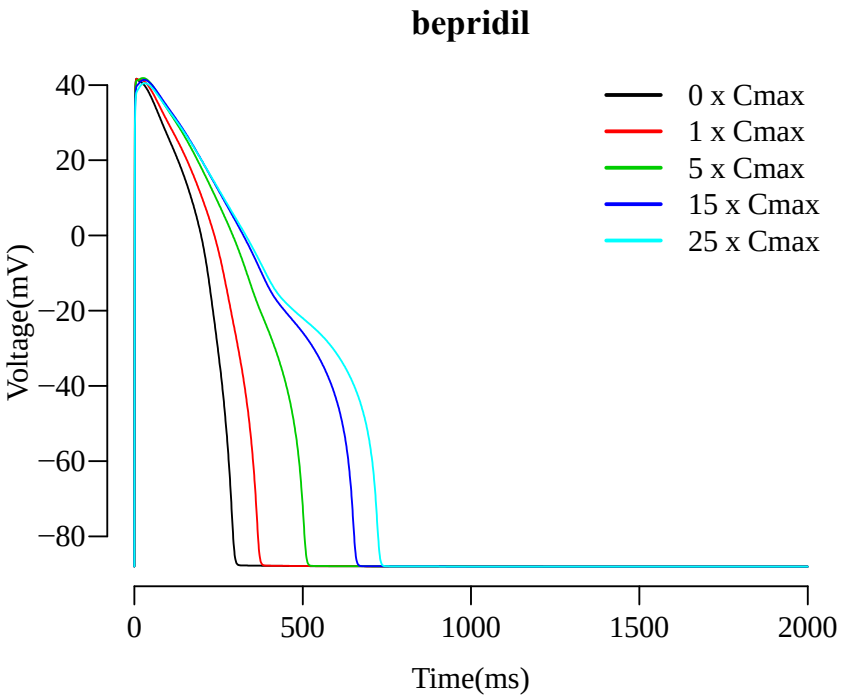
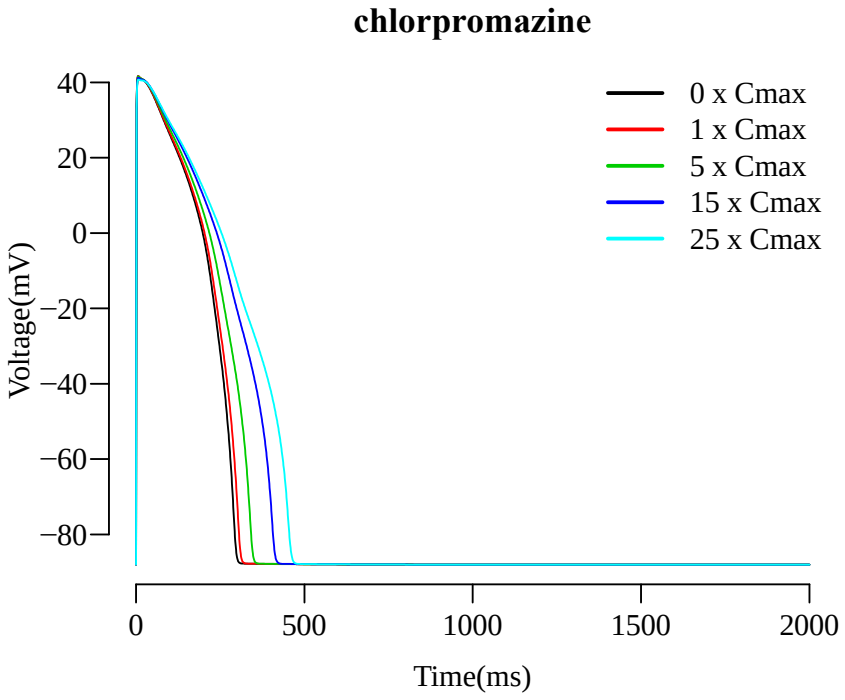
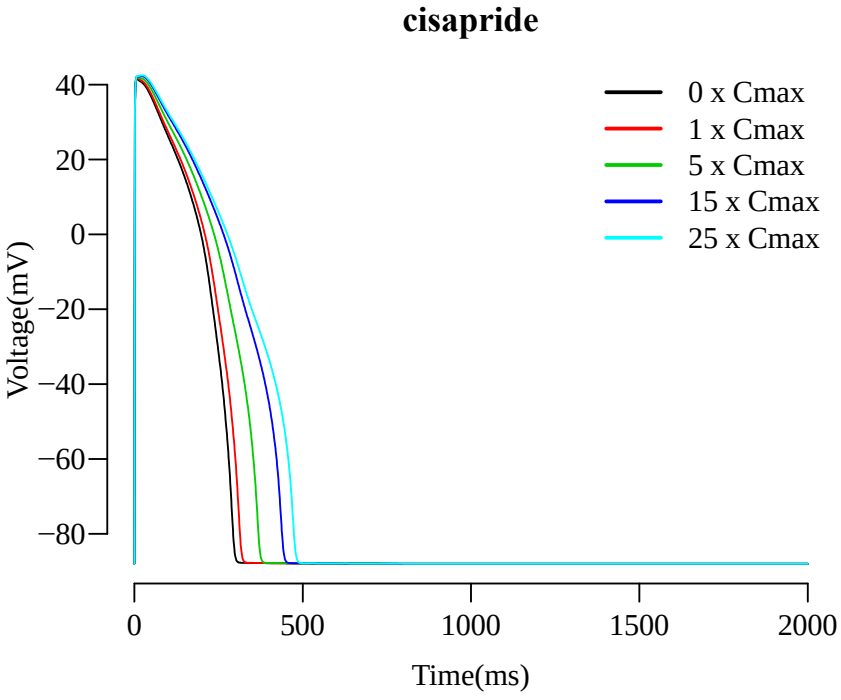
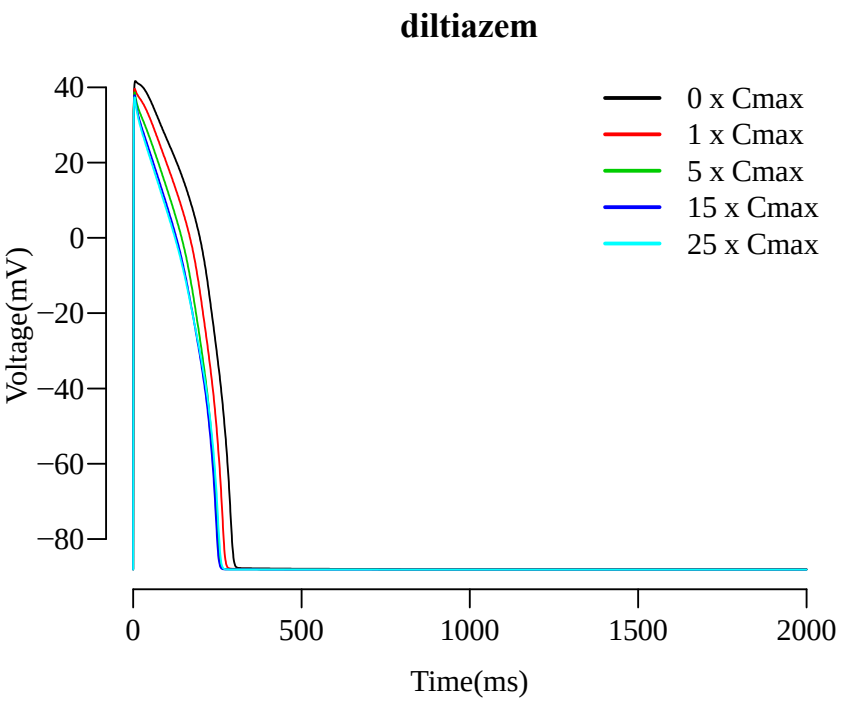
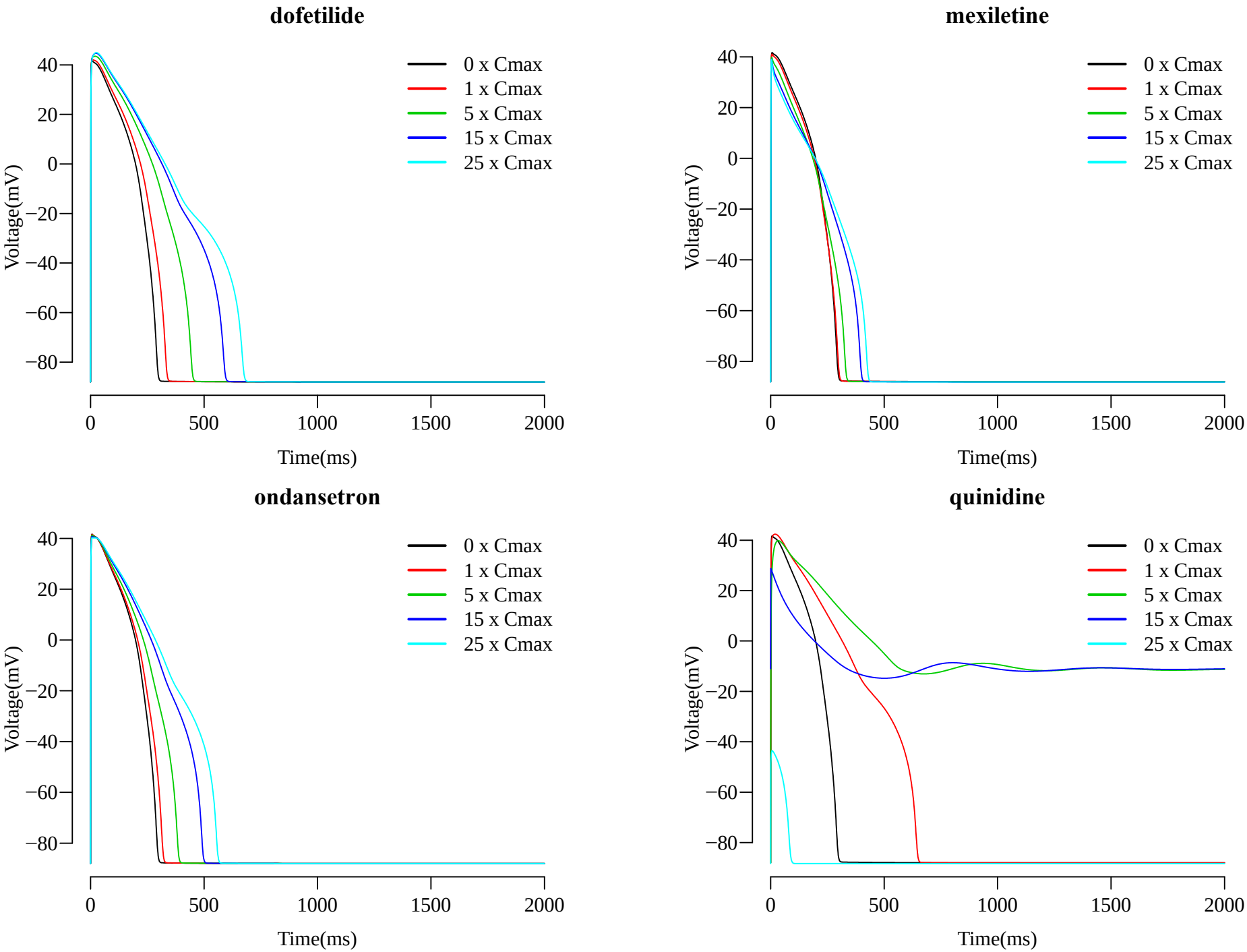
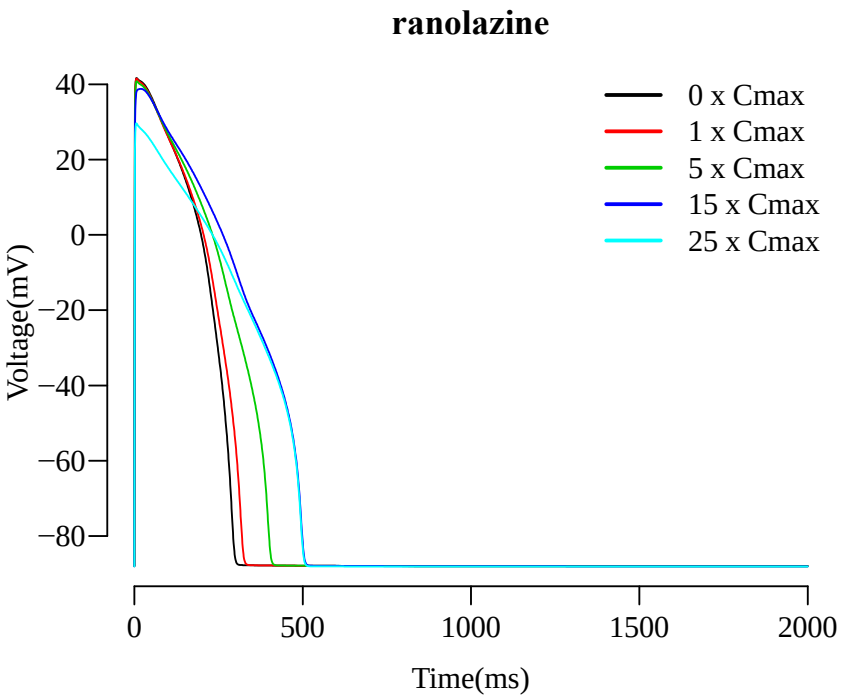

bepridil
0 x Cmax
1 x Cmax
5 x Cmax
15 x Cmax
25 x Cmax
Voltage(mV)
40
20
0
−20
−40
−60
−80
0
500
1000
1500
2000
Time(ms)

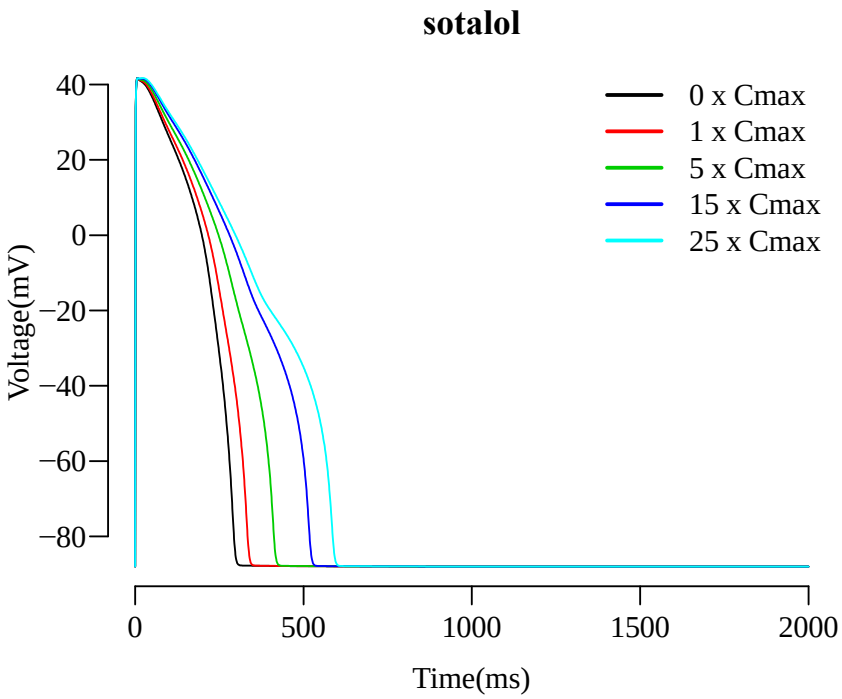

chlorpromazine
0 x Cmax
1 x Cmax
5 x Cmax
15 x Cmax
25 x Cmax
Voltage(mV)
40
20
0
−20
−40
−60
−80
0
500
1000
1500
2000
Time(ms)

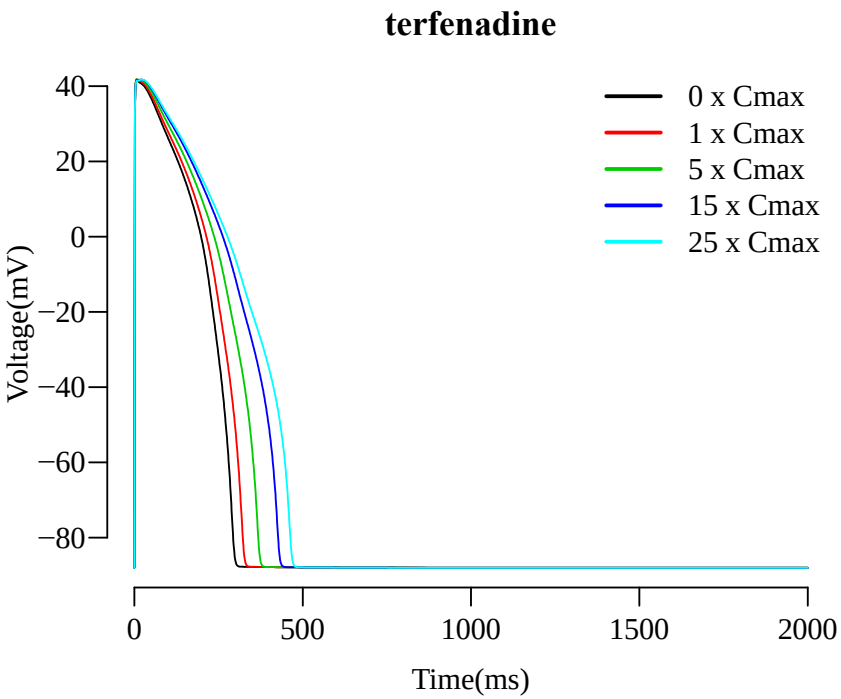

cisapride
0 x Cmax
1 x Cmax
5 x Cmax
15 x Cmax
25 x Cmax
Voltage(mV)
40
20
0
−20
−40
−60
−80
0
500
1000
1500
2000
Time(ms)

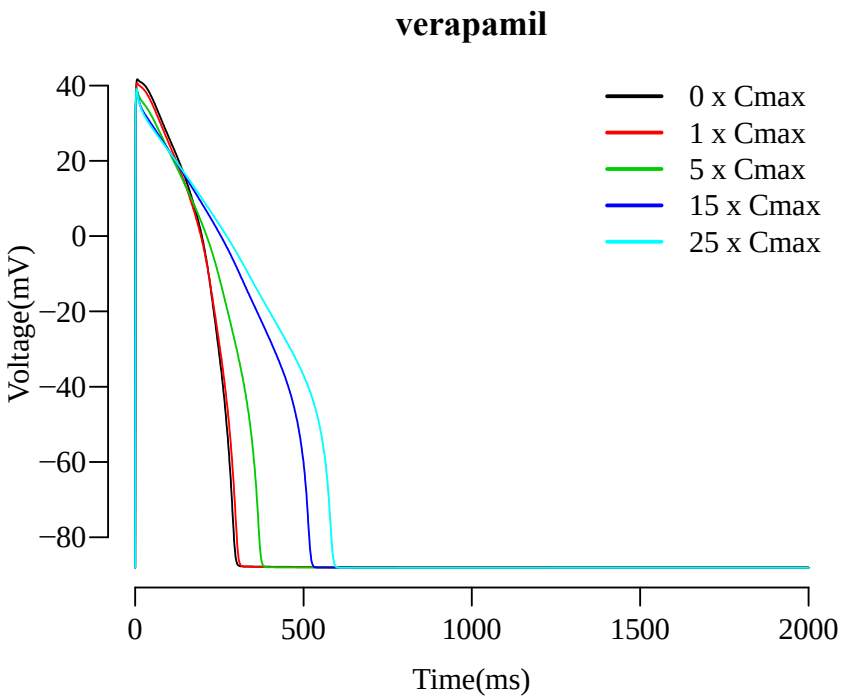

diltiazem
0 x Cmax
1 x Cmax
5 x Cmax
15 x Cmax
25 x Cmax
Voltage(mV)
40
20
0
−20
−40
−60
−80
0
500
1000
1500
2000
Time(ms)


dofetilide
mexiletine
ondansetron
quinidine
0 x Cmax
1 x Cmax
5 x Cmax
15 x Cmax
25 x Cmax
Voltage(mV)
Time(ms)
40
20
0
−20
−40
−60
−80
0
500
1000
1500
2000

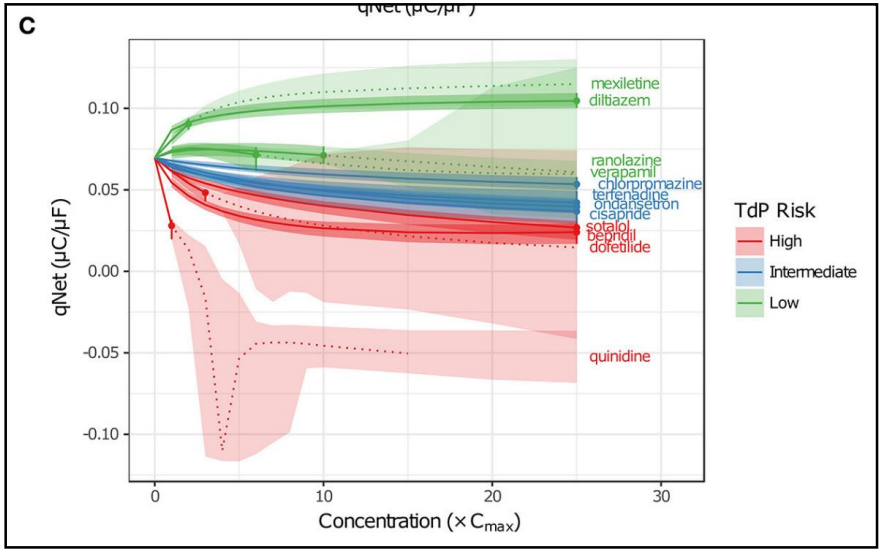

ranolazine
0 x Cmax
1 x Cmax
5 x Cmax
15 x Cmax
25 x Cmax
Voltage(mV)
40
20
0
−20
−40
−60
−80
0
500
1000
1500
2000
Time(ms)


sotalol
0 x Cmax
1 x Cmax
5 x Cmax
15 x Cmax
25 x Cmax
Voltage(mV)
40
20
0
−20
−40
−60
−80
0
500
1000
1500
2000
Time(ms)


terfenadine
0 x Cmax
1 x Cmax
5 x Cmax
15 x Cmax
25 x Cmax
Voltage(mV)
40
20
0
−20
−40
−60
−80
0
500
1000
1500
2000
Time(ms)


verapamil
0 x Cmax
1 x Cmax
5 x Cmax
15 x Cmax
25 x Cmax
Voltage(mV)
40
20
0
−20
−40
−60
−80
0
500
1000
1500
2000
Time(ms)

qNet score



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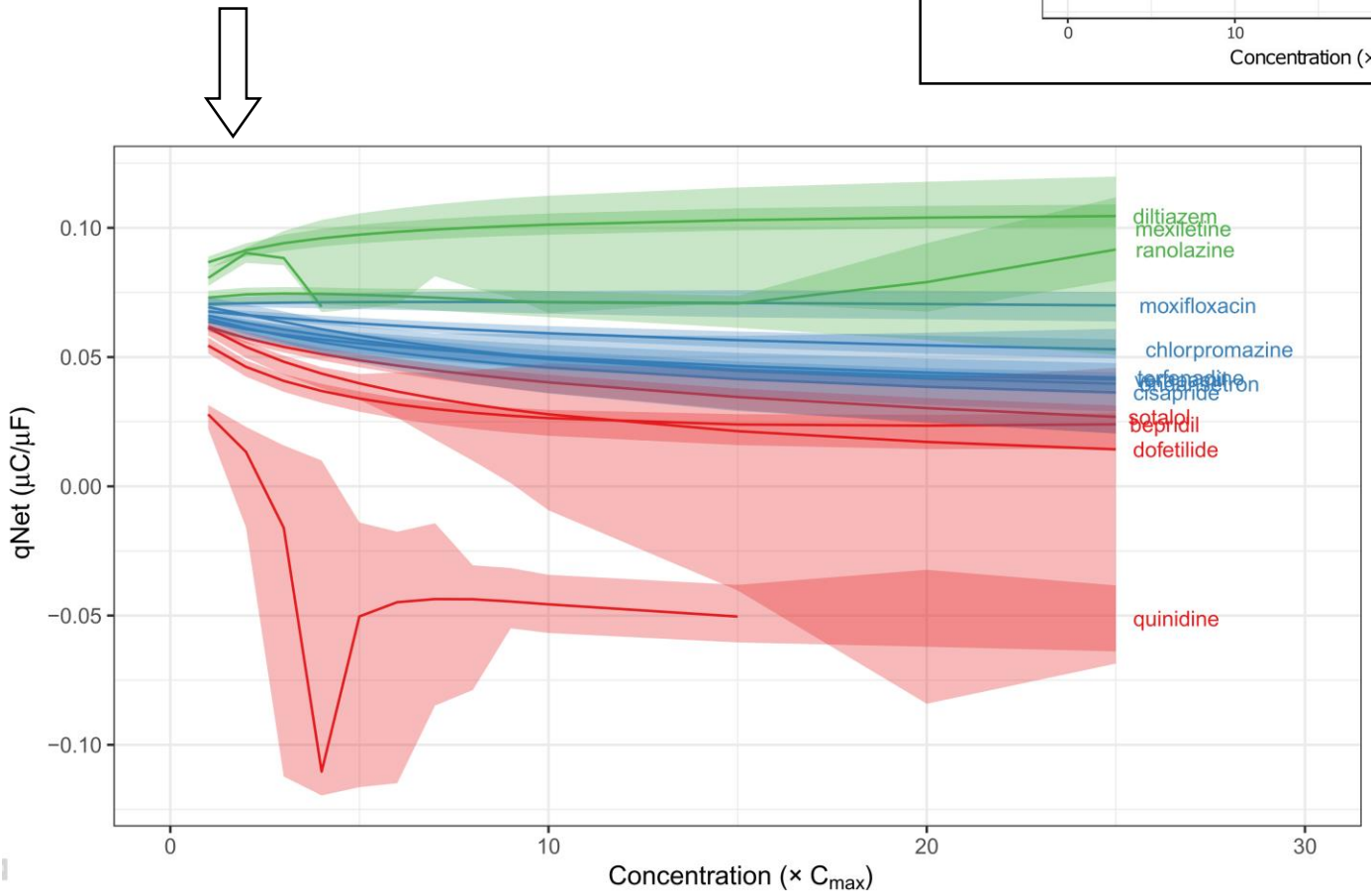

Perspectives of CiPA technology

- **qNet score may be very useful for prediction of arrhythmic outcome with a drug administration at therapeutic concentration.**
- **However, it requires huge amount of experimental data (voltage-clamp experiments by Mines protocol).**
- **Calculation of uncertainty propagation requires huge amount computing resources.**
- **Measurement of net inward current without and with drugs in action potential clamp mode might be more practical to predict proarrhythmic risk of drugs.**

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