Latent Genetic Backgrounds and Molecular Pathogenesis in Drug-induced Long QT Syndrome

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Genetic backgrounds in drug-induced long-QT syndrome (MS ID#: 2009/862649)

Abstract

**Background:** Drugs with $I_{Kr}$ blocking action cause secondary long QT syndrome. Several cases have been associated with mutations of genes coding cardiac ion channels, but their frequency among patients affected by drug-induced long QT syndrome (dLQTS) and the resultant molecular effects remain unknown.

**Methods and Results:** Genetic testing was carried out for long QT syndrome-related genes in 20 subjects with dLQTS and 176 subjects with congenital long QT syndrome (cLQTS); electrophysiological characteristics of dLQTS-associated mutations were analyzed using a heterologous expression system with Chinese Hamster Ovary (CHO) cells together with a computer simulation model. The positive-mutation rate in dLQTS was similar to cLQTS (dLQTS vs. cLQTS, 8 of 20 [40%] vs. 91 of 176 [52%] subjects, $p=0.32$). The incidence of mutations was higher in patients with *torsades de pointes* induced by non-antiarrhythmic drugs than by antiarrhythmic drugs (antiarrhythmic vs. others, 3 of 14 [21%] vs. 5 of 6 [83%] subjects, $p<0.05$). When reconstituted in CHO cells, $KCNQ1$ and $KCNH2$ mutant channels showed complex gating defects without dominant negative effects or a relatively-mild decreased current density. Drug sensitivity for mutant channels was similar to that of the wild type channel (WT). With the Luo-Rudy simulation model of action potentials, action potential durations of most mutant channels were between those of WT and cLQTS.

**Conclusions:** dLQTS had a similar positive-mutation rate compared to cLQTS while the functional changes of these mutations identified in dLQTS were mild. When $I_{Kr}$ blocking agents produce excessive QT prolongation (dLQTS), the underlying genetic background of the dLQTS subject should also be taken into consideration, as would be the case with cLQTS; dLQTS can be regarded as a latent form of long QT syndrome.

**Key Words:** long QT syndrome, secondary, drug, electrophysiology, ion channel
INTRODUCTION

Congenital long QT syndrome (cLQTS) is characterized by abnormally prolonged ventricular repolarization and familial inheritance, leading to polymorphic ventricular tachycardia (torsades de pointes, TdP) causing sudden cardiac death.\(^1,2\) In contrast, secondary long QT syndrome can be induced by a variety of commercially-available drugs, including antiarrhythmic drugs, antihistamines, antibiotics and major tranquilizers.\(^3\) In patients with drug-induced long QT syndrome (dLQTS), after a washout period of the culprit drugs, the QT interval usually returns to within normal range. Genetic factors may underlie the susceptibility to drug-induced serious adverse reactions such as a long QT interval and TdP. Sesti et al\(^4\) demonstrated that a polymorphism of the KCNE2 gene (T8A) is present in 1.6% of the population and is associated with drug-induced TdP related to quinidine and to sulfamethoxazole/trimethoprim administration. We have also previously reported that a mutant SCN5A channel (L1825P), found in an elderly woman with cisapride-induced TdP, appeared to have unique electrophysiological characteristics with both loss and gain of functions for the cardiac sodium current.\(^5\) In addition, there have been several case reports with long QT syndrome-associated gene mutations in dLQTS.\(^6-10\) The accurate prevalence of long QT syndrome-related gene mutations in a larger dLQTS cohort, however, remains unknown and the relationship between genotypes and cellular electrophysiology has not been fully examined. The present study therefore aimed to survey mutations in long QT syndrome-related genes responsible for dLQTS in 20 patients who had been referred to our institutes consecutively over the past 11 years and analyze the functional effects induced by these mutations.
METHODS

Subjects

Blood samples of 305 long QT syndrome subjects, comprising 196 long QT syndrome probands and 109 family members were referred to Kyoto University Graduate School of Medicine and Shiga University of Medical Sciences from March 1996 to January 2006 for genetic analysis. A diagnosis of dLQTS was made in those subjects who had not previously been diagnosed with long QT syndrome and who only developed typical electrocardiographic features of QT prolongation after administration of culprit drugs. A diagnosis of cLQTS was made in those subjects with clinical phenotypes of long QT syndrome, but without the involvement of secondary factors (eg, drugs, hypokalemia or bradycardia). Among the subjects, 20 probands had drug-induced cardiac events (10.2% of long QT syndrome probands). Their clinical information was collected, including family history of sudden death aged 30 years or younger and long QT syndrome members, previous syncope, electrocardiograms (ECG) and serum electrolyte levels at the time of cardiac events. TdP was defined as either non-sustained or sustained ventricular tachycardia showing variation in the electronic polarity of the QRS complex and a “short-long-short” initiating sequence. Written informed consent was obtained from all subjects in accordance with the guidelines approved by our institutional review board. QT intervals were measured in lead II or V5 using Bazett’s formula, before and after allowing sufficient time for the complete washout of drugs.

Schwartz’s scores were calculated in all probands. A Schwartz score ≥4 points indicates that long QT syndrome is definitely present, a score of 2 or 3 points indicates there is a strong possibility that long QT syndrome is present, while a score ≤1 point indicates a low probability of long QT syndrome, respectively.

Mutation analysis

The protocol for genetic analysis was approved by and performed under the guidelines of
Genetic backgrounds in drug-induced long-QT syndrome (MS ID#: 2009/862649)

The Institutional Ethics Committee at Shiga University of Medical Science. Genomic DNA was isolated from peripheral white blood cells using conventional methods. Genetic screening was performed for KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2 and KCNJ2 using polymerase chain reaction/single-strand conformational polymorphism (PCR-DHPLC, WAVE system, Transgenomic Inc, NE, USA) analysis. For the abnormal DHPLC patterns, we determined the DNA sequences on both strands with an automated sequencer (PRISM 3130 Sequencer, Perkin Elmer, CA, USA).

Expression plasmids

The expression plasmids, pIRES2-EGFP/KCNQ1 (wild type [WT]/KCNQ1) and pRc-CMV/KCNH2 (wild type [WT]/KCNH2) were kindly provided by Dr. J. Barhanin (UMR 6097 CNRS and Université de Nice Sophia Antipolis, Valbonne, France) and Dr. M. Sanguinetti (University of Utah, Salt Lake City, UT, USA), respectively. The mutations were introduced using overlap PCR. The mutant plasmids were constructed by substituting the 857-bp XhoI-BglII for R231C and R243H mutants, 287-bp EagI-BstEII for the D342V mutant, 794-bp BstEII-BglII for the H492Y mutant or 392-bp BglII-SphI fragments for S706F and M756V mutants, respectively, for the corresponding fragments of WT/KCNQ1 or WT/KCNH2.

Cell transfection

Functional potassium channels were expressed transiently in Chinese Hamster Ovary (CHO) cells by transfecting the same amount of α subunit plasmids (1 µg/ml KCNQ1 cDNA or 2 µg/ml KCNH2 cDNA). For the analysis of I_Ks currents, the same amount of pIRES/CD8-KCNE1 was coexpressed. Cells were trypsinized, diluted with Dulbecco’s Modified Eagle’s Medium (DMEM, Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum, 30 units/ml penicillin and 30 µg/ml streptomycin. The DMEM used for cell culture dishes was changed to OptiMEM (Invitrogen, CA, USA) for transfection and, after the addition of 10 µl lipofectamine (Invitrogen, CA, USA) and cDNA, the cells were
incubated at 37°C for 3 hours, unless otherwise described. OptiMEM was then replaced by DMEM and the cells were subjected to electrophysiological measurements 48-72 hours after transfection. Cells expressing the potassium channels were selected through detection of green fluorescent protein and by decoration with anti-CD8 antibody-coated beads.

**Electrophysiology**

Whole-cell patch-clamp recordings were made at 37°C using an EPC-8 patch-clamp amplifier (HEKA, Lambrecht, Germany) with pipettes filled with (in mM):

- 70 aspartate, 70 KOH, 10 K2HPO4, 1 MgSO4, 3 Na2-ATP, 0.1 Li2P-GTP, 0.1 GTP6S, 5 HEPES, and 5 EGTA (pH 7.3 with 1N KOH), with a resistance of 2.0 to 4.0MΩ. The external superfusate contained (in mM): NaCl 140, KCl 5.4, MgCl2 0.5, CaCl2 1.8, NaH2PO4 0.33, glucose 5.5, and HEPES 5 (pH 7.4 with NaOH). Data were filtered at 2 kHz. Data acquisition was performed using a PatchMaster acquisition software (HEKA). The holding potential was set at -80 mV. Current densities (pA/pF) were calculated for each cell studied, by normalizing peak tail current amplitude to cell capacitance. Current-voltage relations were fitted with Boltzmann’s function:

\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + \exp((V_{1/2} - V_m)/k)}
\]

where \(V_{1/2}\) indicates the potential at which the activation or inactivation is half-maximal, \(V_m\) the test potential and \(k\) the slope factor.

Drug sensitivities were examined by various concentrations for erythromycin, disopyramide and pirmenol (kind gift from Pfizer Inc. MI, USA). Depolarizing pulses were applied every 15 s and peak tail currents at -60 mV after +20 mV test potential were recorded in the absence or presence of various concentrations of agent. Percent inhibition was calculated by dividing the peak amplitude in the presence of drug by control. Drug concentration-inhibition relations were fitted to Hill’s equation:

\[
\text{Fractional drug inhibition} = 1/(1+(IC_{50}/[\text{drug}])^n)
\]

where IC\(_{50}\) is the amount of drug necessary to produce the half-maximal inhibition of \(I_{Kr}\) tail.
currents, and \( n \) is the Hill coefficient for the fit.

**Computer simulation**

The dynamic Luo-Rudy model (Clancy & Rudy 2001 model) of a ventricular cell was used, with recent modifications and action potentials were simulated using a previously reported model.\(^{16} \) The ratio of \( I_{Kr} \) and \( I_{Ks} \) conductance of M cell layer was set at 23:7. Based on the experimental data of voltage-clamp recordings of \( KCNQ1 \) and \( KCNH2 \) channels heterologously expressed in CHO cells, we constructed Markov or Hodgkin-Huxley models for simulated mutant channels as compared to mutants associated with cLQTS (see supplemental data 1). To make the mutant channel models, we decreased the conductance of each channel as appropriate for the decreased current density, and looked for adequate changes for mutant channels by changing each coefficient value, in turn, for gating states associated with impaired gating defects. The simulation for voltage-clamp experiments was calculated using the fourth-order Runge-Kutta method with a fixed-time step of 0.020 ms. The simulation programs (see supplemental data 1) were coded in C++ and implemented for personal computers.

**Statistical analysis**

Experimental data are expressed as means ± SE while other clinical data as means ± SD, and the statistical comparisons were made using the unpaired Student’s \( t \)-test. Differences in the positive-mutation rate between 2 groups were analyzed by \( \chi^2 \) and Fisher’s exact probability test. Statistical significance was considered as \( p<0.05 \).
RESULTS

Molecular genetics of dLQTS

Table 1 summarizes the clinical characteristics of 20 subjects with dLQTS (14 females and 6 males, mean age 65 ± 16 years). Nineteen subjects developed TdP with marked QT prolongation and one had syncope without documented TdP after taking one of the drugs listed in Table 1. The average QTc interval before taking drugs, available for 15 subjects, was 446 ± 29 ms. The QTc interval was significantly prolonged to 616 ± 91 ms after taking one of the culprit drugs (vs. QTc interval before taking drugs, p<0.001) and significantly shortened to 441 ± 33 ms after washout of drugs (vs. QTc interval after taking drugs, p<0.001). However, in 3 patients (Cases 8, 13, 18 in Table 1) a prolonged QT interval of over 480 ms was maintained after washout. The average RR interval immediately before TdP was 1.2 ± 0.4 sec and the average serum potassium level after TdP was 3.9 ± 0.6 mEq/ml. In the majority of subjects (N=14, 70%), dLQTS was induced by antiarrhythmic drugs (disopyramide, pirmenol, cibenzoline, procainamide and aprindine in 8, 3, 1, 1 and 1 subjects, respectively; Cases 1-14); the remaining cases were induced by antihistamines, antibiotics, psychiatric or miscellaneous drugs (Cases 15-20 in Table 1). None had a family history of long QT syndrome while 3 subjects (Cases 4, 14 and 20) had unexplained syncope and another subject (Case 17) had a family member with sudden cardiac death. During a mean follow-up period of 52 ± 44 months after discontinuing drugs, 1 subject without any gene mutations (Case 9) had recurrent ventricular fibrillation. Compared to cLQTS, the age at first cardiac event in subjects diagnosed with dLQTS was significantly older (dLQTS vs. cLQTS; 65 ± 16 vs. 19 ± 18 years, p<0.001). No subject in the dLQTS group had a family member with long QT syndrome (dLQTS vs. cLQTS; 0 vs. 24% subjects, p<0.01). The QTc interval in dLQTS subjects was significantly shorter than in cLQTS subjects (dLQTS vs. cLQTS; 446 ± 29 vs. 507 ± 71 ms, p<0.001); the Schwartz score was significantly lower in dLQTS than in cLQTS (dLQTS vs.
Genetic backgrounds in drug-induced long-QT syndrome (MS ID#: 2009/862649)

cLQTS; 0.9 ± 1.4 vs. 4.1 ± 2.1, p<0.001).

In 8 (40%) dLQTS subjects, the genetic analysis identified 8 mutations in LQTS-related genes; 2 KCNQ1, 5 KCNH2 and 1 SCN5A (Table 1). These variants were not observed in the controls (220 chromosomes from non-cLQTS and non-dLQTS subjects), suggesting that they represented disease-related mutations. The 91 cLQTS patients also had gene mutations such as 33 KCNQ1, 36 KCNH2, 12 SCN5A, 2 KCNE2, and 8 compound mutations, and the positive-mutation rate was similar between dLQTS and cLQTS subjects (8 of 20 [40%] vs. 91 of 176 [52%] subjects, respectively, p=0.32, Figure 1A). These mutations were found in only 3 of 14 (21%) subjects with TdP induced by antiarrhythmic drugs. In contrast, 5 of 6 subjects with TdP induced by non-antiarrhythmic drugs had gene mutations (83% vs. patients with TdP induced by antiarrhythmic drugs, p<0.05) (Figure 1B). Seven of the 8 mutations were located in the non-pore regions (red circles in Figure 1C); except the A614V-KCNH2 mutation in a case of hydroxyzine-induced TdP17. The green circles in Figure 1C indicate the location of 8 mutations previously-reported in drug-induced LQTS.5-10

Clinical characteristics of genotyped dLQTS subjects

Detailed subject characteristics are presented in Table 1 and the supplemental data 2. Case 2 (M756V/KCNH2) was a 63-year-old man who was admitted with syncope after taking pirmenol (300 mg/day). Case 4 (H492Y/KCNH2) was a 52-year-old woman who experienced syncope while taking disopyramide (300 mg/day). She had previously had one episode of unexplained syncope. Case 14 (R243H/KCNQ1) was a 52-year-old woman who suffered from repetitive syncope after taking aprindine (60mg/day). Case 15 (S706F/KCNH2) was a 21-year-old woman who complained of sudden onset of palpitations and dyspnea after taking amphetamine and methamphetamine. An SCN5A mutation (L1825P/SCN5A) (Case 16), has been reported previously5, this concerned a 70-year-old woman who had TdP and prolongation of the QT interval after taking cisapride (5mg/day) and pirmenol (200mg/day). Case 17 (D342V/KCNH2) was a 70-year-old woman who experienced repetitive syncope due to
erythromycin intake (1200 mg/day). This subject had a 24-year-old sister who had died suddenly, but it is not unknown if her sister had suffered from LQTS. Case 18 (R231C/KCNQ1) was a 72-year-old woman who experienced pre-syncope while taking probucol (250mg/day). Case 20 concerned a 34-year-old woman (A614V/KCNH2 according to Table 1) who experienced syncope and QT prolongation induced by hydroxyzine (3mg/day).\textsuperscript{17} None of the 20 subjects in the study had structural heart disease or a family history of documented LQTS. In genotyped families, a genetic test for 5 members revealed one R243H/KCNQ1 mutation carrier. This carrier had no syncope, and a normal QT interval (QTc 400ms). The Schwartz scores in the 8 subjects with mutations were 1.0 ± 1.5 points (range; 0 to 4 points). Thus, among the genotyped dLQTS subjects in this current study there was only a low or moderate possibility of LQTS being present, both before taking the drugs or after their withdrawal.

**Electrophysiological characteristics of mutations associated with dLQTS**

The biophysical effects of the respective KCNQ1 and KCNH2 mutations were analyzed using a heterologous expression system with the CHO cell line. The upper panel of Figure 2A shows representative current traces for wild type (WT) and 2 mutant KCNQ1 channels and the lower panel shows those recorded from cells cotransfected with WT and each mutant. On their own, both mutants displayed smaller currents compared with WT while R231C channel was a slightly open K\(^{+}\) leak channel. Under heterozygous condition, however, they displayed currents comparable to those of WT without dominant negative effects.

At the end of the depolarizing pulse to +40 mV, current densities were smaller than those of WT channels (77.0 ± 10.6 pA/pF) in R231C/WT (39.6 ± 11.9 pA/pF, p<0.05 vs. WT) but not in R243H/WT (61.9 ± 10.7 pA/pF) (Figure 2B). On the other hand, the R243H channels showed a significant positive shift of steady-state activation curve (Figure 2C). Half activation voltages (V\(_{1/2}\)) and \(k\) were -8.2 ± 3.1 mV and 12.6 ± 0.6 for WT, -12.8 ± 3.6 mV and 13.6 ± 1.3 for R231C/WT, 1.7 ± 3.1 mV and 12.9 ± 0.6 for R243H/WT, respectively (V\(_{1/2}\): WT vs.
R243H/WT, p<0.05). Figure 2D shows representative families of current traces (left panel) and time constants (right panel) of deactivation in each channel. The time course of deactivating kinetics could be fitted by a single-exponential function. The R243H/WT channel had faster deactivation process over -60 mV while this process in the R231C/WT channel was slower than WT under -90 mV.

Figure 3A shows representative families of current traces recorded during depolarizing pulses from CHO cells transfected with KCNH2 cDNAs as indicated in the graph. The left column depicts current traces from cells transfected with each construct alone and the right column those recorded under heterozygous conditions except for the upper most traces (WT 1 μg). When expressed alone, D342V was non-functional and 3 other mutations displayed functional channels. When co-expressed with WT, D342V showed weak dominant negative effects while the other 3 channels showed non-dominant negative effects. The functional outcome of the remaining mutation, A614V-KCNH2\(^{17}\), has recently been reported. Using an oocyte expression system, Nakajima et al.\(^{18}\) reported that the A614V channel showed loss of function in a dominant negative manner, and the results from the present study were almost identical (18% current density of WT).

Current densities at the end of a 2-s depolarization pulse were calculated in multiple cells and plotted as a function of test potential in Figure 3B. At the end of the depolarizing pulse to +20 mV, the densities were smaller than those of WT channels (36.2 ± 6.8 pA/pF) in D342V/WT (18.7 ± 4.5 pA/pF, p<0.05 vs. WT) and S706F/WT (21.9 ± 2.7 pA/pF, p=0.055 vs. WT). Those in H492Y/WT and M756V/WT were also smaller than in the WT, but the difference did not reach statistical significance.

The mean peak amplitudes of tail currents at -60 mV on repolarization from various test potentials (2-s duration) plotted as a function of test potentials are displayed in figure 3C. H492Y and M756V channels are displayed amplitudes of peak tail currents similar to WT. For example, the peak tail current densities after a test pulse to +20 mV were 66.2 ± 10.5 pA/pF
for WT, 62.1 ± 13.0 pA/pF for H492Y/WT, and 58.4 ± 7.7 pA/pF for M756V/WT. On the other hand, the current densities of D342V/WT (1.5 ± 0.9 pA/pF, p<0.001 vs. WT) and S706F/WT (38.9 ± 7.3 pA/pF, p<0.05 vs. WT) channels were significantly smaller than WT. D342V/WT channel displayed currents smaller than WT 1μg (indicated by dotted line), whereas S706F/WT currents were similar to WT 1μg in size. Thus, D342V channels had weakly dominant negative suppression effects on reconstituted $I_{Kr}$-like currents, whereas S706F had no dominant negative suppression effect. In order to examine the voltage dependence for activation, Boltzmann’s function curves were fitted to the relationship between peak tail currents and test voltages under respective conditions and are represented by solid lines in Figure 3C. Half inactivation voltages ($V_{1/2}$) were -23.2 ± 1.6 mV for WT, -19.8 ± 3.0 mV for D342V/WT, -24.6 ± 1.4 mV for H492Y/WT, -26.1 ± 1.5 mV for S706F/WT, and -19.8 ± 1.7 mV for M756V/WT, respectively (Figure 3D). Therefore, the mutations did not affect the voltage-dependent activation of the reconstituted $I_{Kr}$-like channel.

Whether the mutations affected the inactivation kinetics of $KCNH2$ channels was then assessed. Figure 4A shows the voltage dependence of availability of WT and mutant channels measured by a brief repolarization method (inset). With a 1 s depolarizing pulse, peak tail current amplitudes (arrow in the inset) following short pre-conditioning voltage pulses (5 ms) are plotted against the voltage of conditioning pulse. Mutant channels caused significant voltage shift of channel availability to the hyperpolarizing direction compared with WT ($V_{1/2}$ of -58.3 ± 4.7 mV for WT, $V_{1/2}$ of -61.4 ± 6.5 mV for D342V/WT, -77.8 ± 4.7 mV for H492Y/WT, -70.1 ± 3.2 mV for S706F/WT, and -71.1 ± 4.6 mV for M756V/WT, respectively). The time course to recovery from or the development of the inactivation (recovery from inactivation at hyperpolarized potentials and development of inactivation at >-70 mV) was analyzed by double or triple pulse protocols. The time course of inactivating kinetics could be fitted by a single-exponential function. Time constants thus calculated were significantly smaller than WT and mutant channels over a wide range of voltage (between -50 and +40 mV.
for D342V/WT and H492Y/WT; between -30 and +20 mV for S706F/WT; between -40 and 0 mV for M756V/WT, Figure 4B). These results demonstrate that drug-induced LQTS mutants have accelerated inactivation kinetics. Figure 4C shows representative families of current traces (left panel) and time constants (right panel) of deactivation in each channel. When the time course of deactivating kinetics was fitted by a double-exponential function, the S706F/WT channel slightly accelerated the deactivation process (Figure 4C).

Most of the drugs that induced LQTS in the study subjects have been known to block $I_{Kr}$ in a concentration-dependent manner\textsuperscript{8,17,19-22} while $I_{Kr}$ channel with $KCNH2$ mutations may have different drug sensitivities compared to the WT channel. Figure 5 shows three sets of drug concentration-current inhibition relationships associated with $KCNH2$ mutations with respect to erythromycin (A), disopyramide (B) and pirmenol (C). In each case of a drug-induced LQTS, an electrophysiological assay of current inhibition by the respective culprit drug was performed, i.e., hydroxyzine\textsuperscript{17}, erythromycin, disopyramide and pirmenol. IC\textsubscript{50}S were not significantly different between WT and the respective mutant channels, suggesting that a change in drug sensitivity was not involved in causing the drug-induced TdP in the study subjects.

**Computer simulation of ventricular action potentials.**

In order to compare how the functional changes caused by mutants affect ventricular action potentials, a simulation study was conducted using the Luo-Rudy computer model which incorporated the Markov\textsuperscript{16} or Hodgkin-Huxley\textsuperscript{23} process gating for the mutant channels (Figure 6A). Table 2 shows parameters of simulation which have been changed to fit to experimental results. Firstly, the $I_{Kr}$ or $I_{Ks}$ conductance was reduced to the level observed in the D342V/WT, A614V/WT and R231C/WT channels. The deactivation time course for R231C/WT was also fitted by modifying a parameter. Secondly, the transition rate was changed accordingly from inactivation to open states (ii) and fitted with the experimental data seen in H492Y/WT and M756V/WT channels. Acceleration of inactivation induced by
Genetic backgrounds in drug-induced long-QT syndrome (MS ID#: 2009/862649)

modifying the transition rate $\alpha_i$ reproduced smaller amplitudes for $I-V$ relationships and negative shift of steady-state inactivation curve (Figure 4A); these findings were compatible with the experimental results (Figures 3A-C). Thirdly, some parameters were altered to simulate the S706F/WT model, by reducing the $I_{Kr}$ conductance, modifying $\alpha_i$, and increasing the transition rate from open to deactivation states. This was followed by change to parameters associated with the activation and deactivation rates for the R243H/WT model. Finally, not only was $I_{Na}$ conductance decreased in the subjects, but the burst mode was also added to simulate the sustained current for the L1825P model. The L1825P/WT channel was heterogeneously simulated to equally mix WT and L1825P models.

In the simulated M cells using the Luo-Rudy model, the order of increase in magnitude of action potential duration (APD) was A614V/WT > R231C/WT > D342V/WT > S706S/WT > R243H/WT = L1825P/WT > H492Y/WT > M756V/WT for drug-induced LQTS mutations (Figure 6B, middle panel). Typically, for congenital LQTS mutations, the simulated APD was longer than for WT or drug-induced models, while APDs in drug-induced models were intermediate between those in WT and those in congenital LQTS (clinical information for simulated congenital LQTS are presented in supplemental Table 1).

Finally, an effort was made to reproduce action potentials in the presence of $I_{Kr}$-blocking drugs (Figure 6C). Early afterdepolarizations (EADs) appeared in all mutants where there were smaller reductions in the $I_{Kr}$ conductance compared with the corresponding WT. Because drug sensitivities for WT and mutant channels were not different (Figure 5), the same inhibition rate was employed in both the WT and mutant models and the $I_{Kr}$ conductance was gradually reduced at the cycle length of 1200 ms. As shown in Figure 6C, typically, when the $I_{Kr}$ conductance was theoretically decreased to 89% of the basal conductance for each channel, the D342V/WT model began to develop EADs, while the WT model produced only a 2.9% increase in APD.
Discussion

There were three major findings. (1) In 8 out of 20 consecutive dLQTS subjects, $5 \text{KCNH2}$, 2 $\text{KCNQ1}$ and 1 $\text{SCN5A}$ heterozygous missense mutations were identified; there was a similar positive-mutation rate with dLQTS compared to cLQTS. (2) Both $\text{KCNQ1}$ and $\text{KCNH2}$ mutants possessed loss of function effects on reconstituted $I_{Ks}$– or $I_{Kr}$–like channels. (3) The functional changes in mutant channels reconstituted by the computer simulation resulted in a mildly prolonged APD, suggesting that the dLQTS may partially have a genetic background, especially mild or latent long QT syndrome-associated mutations.

Mutations in dLQTS

Potential torsadegenic drugs are used in the clinical setting, and include antiarrhythmic drugs, antibiotics, antihistamines, psychiatric drugs, cholinergic antagonists. These might induce TdP which may lead to the sudden cardiac death of individuals whose QT intervals were within normal range before taking the drug. Several drugs such as cisapride and terfenadine have been withdrawn from the market because of these possible side effects.\textsuperscript{25,26} The incidence of dLQTS is not high and the drugs lead to TdP in only a small percentage of individuals, suggesting that there may be an underlying genetic background that predisposes these individuals to the risk.

In the medical literature, 13 $\text{KCNQ1}$ or $\text{KCNH2}$ mutations (13 of 15 mutations, 87%) associated with dLQTS, including 6 of the mutations identified in this present study, have been located in non-pore regions (Figure 1B). Mutations in non-transmembrane regions have been shown to cause either mild long QT syndrome or benign clinical phenotypes.\textsuperscript{27} Mutation sites may influence the clinical and basic electrophysiological characteristics of patients with dLQTS. It is of interest that the functional assay of our 7 mutations resulted in various levels of loss-of-function, but most of them showed no dominant negative suppression, which is
usually observed in the classical cLQTS. Clinical characteristics during the drug intake were not significantly distinct from those of cLQTS, which shares a similar genetic background with the dLQTS. Several polymorphisms have been shown to be associated with dLQTS.\textsuperscript{4,28} Abbott et al\textsuperscript{4} identified a polymorphism (T8A) of the \textit{KCNE2} gene encoding MiRP, a \( \beta \) subunit for the \( I_{Kr} \) channel, which is present in 1.6\% of the population and is associated with TdP induced by quinidine or sulfamethoxazole/trimethoprim administration. Splawski and coworkers\textsuperscript{28} also found a heterozygous polymorphism involving substitution of serine with tyrosine (S1102Y) in the sodium channel gene \textit{SCN5A} among African Americans that increased the risk for drug-induced TdP. The polymorphism was present in 57\% of 23 patients with proarrhythmic episodes, but in only 13\% of control subjects. These findings suggested that common genetic variations may increase the risk for development of drug-related arrhythmias.

Roden et al.\textsuperscript{29} reported that cisapride could rescue trafficking of L1825P channel with a potentially sustained current and revealed a new mechanism of dLQTS with the Luo-Rudy model. However, in the genotyped subjects in the present study, dLQTS mainly resulted from the \( I_{Kr} \) blocking effect of culprit drugs in the presence of latent genetic backgrounds. Cardiac repolarization reserve may protect subjects against the drug-induced QT prolongation by \( I_{Kr} \) blocking drugs.\textsuperscript{30,31} In the presence of latent genetic backgrounds, however, reduction in the repolarization reserve unveils the presence of so-called “concealed” long QT syndrome when drugs with \( I_{Kr} \) blocking effects are administered. The presence of borderline prolongation of QT interval, together with personal information such as unexplained previous syncope and family history of premature sudden death may help to prevent drug-induced arrhythmia even if a subject’s Schwartz score is low, because they could have a potential risk of TdP. Special attention should be paid to family members of the index subject with drug-induced QT prolongation because ~30\% of family members were found to have a pre-disposing genetic background in the present study. Indeed, they may have inherited the risk for being susceptible
Limitations of the study

This study has some limitations. Because of the small cohort of dLQTS subjects, this study was not powered to quantify the overall prevalence of ion channel mutations in the group of subjects with drug-induced TdP; there was also a possible selection bias in the population. As for the causative agents, we were unable to test the action of amphetamine and methamphetamine because it was impossible to obtain these illegal drugs for clinical study. However a previous report has shown that 3,4-methylenedioxy methamphetamine (ecstasy, NMDA) prolongs the APD of hippocampal neurons by blocking the conductance of a resting K+ channel.32 It is quite possible that these drugs also suppressed cardiac K+ currents and induced QT prolongation and TdP in one subject in our study, based on her medical records. Regarding protein trafficking, 2 mutations in this study, A614V in KCNH217 and L1825P in SCN5A29, had been reported to be trafficking-deficient mutations. Though protein trafficking of other mutants remains unclear, especially R243H in KCNQ1, and H492Y, S706F and M756V in KCNH2, would be not trafficking-deficient because, under heterozygous conditions, these mutants showed adequate current density compared to WT. In the simulation study, the parameter settings could mimic mutant channels. In addition, the setting of the parameters might have innumerable patterns, and it therefore remains possible that other combinations of patterns could also simulate mutant channels.

Acknowledgements

We thank Ms. Arisa Ikeda for excellent technical assistance.

Sources of Funding

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Genetic backgrounds in drug-induced long-QT syndrome (MS ID#: 2009/862649)

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Disclosures

None
Genetic backgrounds in drug-induced long-QT syndrome (MS ID#: 2009/862649)

References


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Genetic backgrounds in drug-induced long-QT syndrome (MS ID#: 2009/862649)


Table 1: Clinical Characteristics and Gene Mutations of Probands with Drug-Induced Arrhythmic Events

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Drug types</th>
<th>Cubrit drugs, doses/day</th>
<th>Cubrit drugs, doses/day</th>
<th>QTC (ms) before taking drugs</th>
<th>QTC (ms) after events</th>
<th>QTC (ms) after washout of drug</th>
<th>Serum K(+/Eql) after TdP</th>
<th>RR interval (sec) before TdP</th>
<th>Nucleotide change</th>
<th>Mutation/Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68</td>
<td>M</td>
<td>antiarrhythmic drug</td>
<td>disopyramide, 300mg</td>
<td>TdP</td>
<td>-/-</td>
<td>620</td>
<td>425</td>
<td>4.4</td>
<td>0.7</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>63</td>
<td>M</td>
<td>antiarrhythmic drug</td>
<td>pimened, 160mg</td>
<td>TdP</td>
<td>-/-</td>
<td>632</td>
<td>normal</td>
<td>NA</td>
<td>1.1</td>
<td>a226G &gt; N</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>F</td>
<td>antiarrhythmic drug</td>
<td>disopyramide, 300mg</td>
<td>TdP</td>
<td>-/-</td>
<td>447</td>
<td>563</td>
<td>456</td>
<td>2.3</td>
<td>2.3</td>
<td></td>
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<td>4</td>
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<td>disopyramide, 300mg</td>
<td>TdP</td>
<td>-/-</td>
<td>49</td>
<td>585</td>
<td>447</td>
<td>3.2</td>
<td>1.1</td>
<td>c.1474G &gt; A</td>
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<td>antiarrhythmic drug</td>
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<td>-/-</td>
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<td>553</td>
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<td></td>
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<td>cibergeline, 100mg</td>
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<td>600</td>
<td>430</td>
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<td></td>
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<td>7</td>
<td>56</td>
<td>F</td>
<td>antiarrhythmic drug</td>
<td>disopyramide, 300mg</td>
<td>TdP</td>
<td>-/-</td>
<td>449</td>
<td>577</td>
<td>363</td>
<td>NA</td>
<td>1.2</td>
<td></td>
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<tr>
<td>8</td>
<td>79</td>
<td>F</td>
<td>antiarrhythmic drug</td>
<td>disopyramide, 300mg</td>
<td>TdP</td>
<td>-/-</td>
<td>453</td>
<td>538</td>
<td>495</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>71</td>
<td>N</td>
<td>antiarrhythmic drug</td>
<td>pimened, 200mg</td>
<td>TdP</td>
<td>-/-</td>
<td>453</td>
<td>538</td>
<td>449</td>
<td>4.3</td>
<td>2.1</td>
<td></td>
</tr>
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<td>antiarrhythmic drug</td>
<td>disopyramide, 150mg</td>
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<td>-/-</td>
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<td>11</td>
<td>87</td>
<td>F</td>
<td>antiarrhythmic drug</td>
<td>disopyramide, 50mg*</td>
<td>TdP</td>
<td>-/-</td>
<td>464</td>
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<td>pimened, 200mg</td>
<td>TdP</td>
<td>-/-</td>
<td>463</td>
<td>464</td>
<td>424</td>
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<td>13</td>
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<td>antiarrhythmic drug</td>
<td>procainamide, 200mg*</td>
<td>TdP</td>
<td>-/-</td>
<td>461</td>
<td>705</td>
<td>496</td>
<td>4.9</td>
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<td>14</td>
<td>72</td>
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<td>antiarrhythmic drug</td>
<td>Acripette, 80mg</td>
<td>TdP</td>
<td>-/-</td>
<td>NA</td>
<td>657</td>
<td>469</td>
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<td>TdP on adn.</td>
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<tr>
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<td>61</td>
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<td>non-antiarrhythmic drug</td>
<td>3,4-methylenedioxymethamphetamine, 10tabs</td>
<td>TdP</td>
<td>-/-</td>
<td>478</td>
<td>700</td>
<td>401</td>
<td>3.3</td>
<td>1.5</td>
<td>L102F &gt; P</td>
</tr>
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<td>79</td>
<td>F</td>
<td>non-antiarrhythmic drug</td>
<td>cisapride, 5mg</td>
<td>TdP</td>
<td>-/-</td>
<td>463</td>
<td>700</td>
<td>401</td>
<td>3.2</td>
<td>1.6</td>
<td>L102F &gt; P</td>
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<td>70</td>
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<td>non-antiarrhythmic drug</td>
<td>erythromycin, 1200mg</td>
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<td>-/-</td>
<td>459</td>
<td>775</td>
<td>440 (AF)</td>
<td>3.3</td>
<td>0.9</td>
<td>a1025T &gt; C</td>
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<tr>
<td>18</td>
<td>72</td>
<td>F</td>
<td>non-antiarrhythmic drug</td>
<td>propranolol, 200mg</td>
<td>TdP</td>
<td>-/-</td>
<td>459 (AF)</td>
<td>575</td>
<td>419 (AF)</td>
<td>3.3</td>
<td>0.9</td>
<td>a1025T &gt; C</td>
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<tr>
<td>19</td>
<td>77</td>
<td>F</td>
<td>non-antiarrhythmic drug</td>
<td>haloperidol, 5mg</td>
<td>TdP</td>
<td>-/-</td>
<td>NA</td>
<td>574</td>
<td>447</td>
<td>4.6</td>
<td>4.6</td>
<td></td>
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<td>34</td>
<td>F</td>
<td>non-antiarrhythmic drug</td>
<td>hydrocortisone, 3mg</td>
<td>TdP</td>
<td>-/-</td>
<td>49</td>
<td>836</td>
<td>449</td>
<td>4.3</td>
<td>1.1</td>
<td></td>
</tr>
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</table>

Ave ± SD: 62 ± 16

N = male, F = female, TdP = torsades de pointes, NA = not available, AF = atrial fibrillation, K(+/Eql) = type A WPW syndrome, prolonged QT = ECG > 350ms, normal QT = ECG > 350ms, normal QT interval but unspecified QT interval, normal = normal QT interval but unspecified QT interval, ± = intravenous administration.
Genetic backgrounds in drug-induced long-QT syndrome (MS ID#: 2009/862649)

Table 2 Parameters of Simulation data in LQTS

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Gene</th>
<th>Mutant</th>
<th>WT basal parameters</th>
<th>Mutant changed parameters</th>
</tr>
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<tr>
<td>dLQTS</td>
<td>KCNQ1</td>
<td>R231C</td>
<td>gk=0.202/(1+0.6/(1+pow((0.0000038/cal), 1.4)))</td>
<td>gk=0.103/(1+0.6/(1+pow((0.0000038/cal), 1.4)))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tauex=1/((0.0000071*(v+30))/(1-exp(-0.148...))</td>
<td>tauex=1/((0.0000071*(v+30))/(1-exp(-0.148...))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>x1ex=1/((1+exp(-v/16.7))</td>
<td>x1ex=1/((1+exp(-v/16.7))</td>
</tr>
<tr>
<td>dLQTS</td>
<td>KCNQ1</td>
<td>R243H</td>
<td>taux=1/((0.0000000071*(v+30))/(1-exp(-0.148...))</td>
<td>taux=1/((0.0000000071*(v+30))/(1-exp(-0.148...))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>gher=0.135*pow(Kout, 0.59)</td>
<td>gher=0.0943*pow(Kout, 0.59)</td>
</tr>
<tr>
<td>dLQTS</td>
<td>KCNQ2</td>
<td>H492Y</td>
<td>gher=0.135<em>exp(-0.02352</em>(v+25))/4.5/Kout</td>
<td>gher=0.135<em>exp(-0.02352</em>(v+25))/4.5/Kout</td>
</tr>
<tr>
<td>dLQTS</td>
<td>KCNQ2</td>
<td>S706F</td>
<td>aL=0.439<em>exp(-0.02352</em>(v+25))/4.5/Kout</td>
<td>aL=0.439<em>exp(-0.02352</em>(v+25))/4.5/Kout</td>
</tr>
<tr>
<td>dLQTS</td>
<td>KCNQ2</td>
<td>M756V</td>
<td>Gm=16</td>
<td>Gm=16</td>
</tr>
<tr>
<td>dLQTS</td>
<td>KCNQ2</td>
<td>A614V</td>
<td>no burst mode</td>
<td>no burst mode</td>
</tr>
<tr>
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<td>SCN5A</td>
<td>L1825P</td>
<td>Gm=16</td>
<td>Gm=16</td>
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<tr>
<td>cLQTS</td>
<td>KCNQ1</td>
<td>A341V</td>
<td>gk=0.202/(1+0.6/(1+pow((0.0000038/cal), 1.4)))</td>
<td>gk=0.103/(1+0.6/(1+pow((0.0000038/cal), 1.4)))</td>
</tr>
<tr>
<td>cLQTS</td>
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<td>A344Aspl</td>
<td>gk=0.202/(1+0.6/(1+pow((0.0000038/cal), 1.4)))</td>
<td>gk=0.103/(1+0.6/(1+pow((0.0000038/cal), 1.4)))</td>
</tr>
<tr>
<td>cLQTS</td>
<td>KCNQ2</td>
<td>A561V</td>
<td>gher=0.135*pow(Kout, 0.59)</td>
<td>gher=0.0943*pow(Kout, 0.59)</td>
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<tr>
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<td>KCNQ2</td>
<td>G604S</td>
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<td>aL=0.439<em>exp(-0.02352</em>(v+25))/4.5/Kout</td>
</tr>
<tr>
<td>cLQTS</td>
<td>KCNQ2</td>
<td>A614V</td>
<td>gher=0.135*pow(Kout, 0.59)</td>
<td>gher=0.0943*pow(Kout, 0.59)</td>
</tr>
<tr>
<td>cLQTS</td>
<td>SCN5A</td>
<td>L819F</td>
<td>Gm=16</td>
<td>Gm=16</td>
</tr>
<tr>
<td>cLQTS</td>
<td>SCN5A</td>
<td>L1330P</td>
<td>Gm=16</td>
<td>Gm=16</td>
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</tbody>
</table>

dLQTS = drug induced long QT syndrome, cLQTS = congenital long QT syndrome.
Figure Legends

Figure 1. Molecular genetics of gene mutations in subjects with drug-induced long QT syndrome (dLQTS). A, Bar graph showing positive-mutation rates of congenital LQTS (cLQTS) and drug-induced LQTS (dLQTS). B, Schemes indicating the location associated with dLQTS. Red circles indicate the sites of mutations detected in this study while green circles indicate previously-reported mutations.

Figure 2. Mutations associated with non-dominant negative effects of KCNQ1 channels.
A, Current traces reconstituted in Chinese Hamster Ovary cells. Current amplitude was normalized by respective cell capacitance and was indicated as the current density. B, Current-voltage (I-V) relationships for amplitudes of steady-state currents at the end of 4-sec depolarizing pulses. Currents were elicited by depolarizing pulses from a holding potential of -80 mV to test potential between -50 to +50 mV (with a 10-mV step increment), followed by repolarization to -50 mV in order to monitor tail current amplitude. The voltage-clamp protocol is shown in the inset. Open circles, wild type (WT) 1 μg; filled squares, mutant 1 μg; filled circles, WT 0.5 μg plus mutant 0.5 μg; and dotted lines, WT 0.5 μg. All data were recorded from 10-25 cells. C, Steady-state activation curves for WT and WT plus mutants. D. The KCNQ1 mutants modify deactivation time course. The left column presents the time course of deactivation for each channel. Each inset illustrates scale bars of 2nA and 1-sec times. To examine the deactivation time course, a conditioning pulse to +40 mV for 4 sec from a holding potential of -80 mV was followed by hyperpolarizing test pulses between -120 mV and -20 mV in 10-mV increments (inset in graph on right). Currents were not leak-subtracted. Deactivation time constants (tau) were measured by fitting deactivating currents during test pulses at each potential with a single exponential. *p<0.05 vs. WT.

Figure 3. KCNH2 mutations identified in drug-induced LQT subjects produced various levels of functional effects. A, Current traces of I_Kr reconstituted in Chinese Hamster Ovary cells. Expression of the respective clones, indicated above each graph, all displayed I_Kr-like currents except D342V. Current amplitude was normalized by respective cell capacitance and was indicated as the current density. B, Current-voltage (I-V) relationships for amplitudes of steady-state currents at the end of 2-sec depolarizing pulses. Currents were elicited by depolarizing pulses from a holding potential of -80 mV to test potential between -60 to +50 mV (with a 10-mV step increment), followed by repolarization to -60 mV in order to monitor tail current amplitude. The voltage-clamp protocol is shown in the inset. C, I-V relationships for
amplitudes of peak tail currents measured at –60 mV. Open circles, wild type (WT) 2 μg; filled squares, mutant 2 μg; filled circles, WT 1 μg plus mutant 1 μg; and dotted lines, WT 1 μg. All data were recorded from 10-25 cells. D, Voltage-dependence of activation for each channel. The data were fitted to a Boltzmann function.

Figure 4. Multiple gating defects associated with KCNH2 mutations. A, Mutations associated with drug-induced arrhythmia caused a negative shift of inactivation gate of KCNH2 channels. Steady-state channel availability as a function of membrane potential was measured by using a double step method as shown in the insets: the voltage-clamp protocol and original current traces from a representative cell expressing wild type (WT) KCNH2 channels. Open circles indicate the inactivation calculated from cells expressing cDNA with WT 2 μg, closed triangles D342V/WT, closed squares H492Y/WT, open triangles S706F/WT, and closed circles M756V/WT. All data were taken from 14-20 cells except for D342V/WT. B, To examine the inactivation time course, a conditioning pulse to +40 mV for 900 ms from a holding potential of -80 mV was followed by a hyperpolarizing pulse to -120 mV for 5 ms, and subsequent depolarizing test pulses between -50 and +40 mV in 10 mV steps were applied. In addition, a conditioning pulse to +40 mV for 750 ms was applied from a holding potential of -80 mV, followed by test pulses to various potentials between -130 and -60 mV in 10 mV increments. The inset illustrates the voltage protocol. Inactivation time constants were measured by fitting inactivating currents during test pulses at each potential with a single exponential function. C, The S706F/KCNH2 mutant channel slightly accelerates deactivation time course. The left column, time course of deactivation for each channel. To examine the deactivation time course, a conditioning pulse to +40 mV for 1.6 sec from a holding potential of -80 mV was followed by hyperpolarizing test pulses between -70 mV and -40 mV in 10-mV increments for 16 seconds (inset). Currents were not leak-subtracted. Each inset illustrates scale bars of 200 pA and 5-sec times. Deactivation time constants (τ) were measured by fitting deactivating currents during test pulses at each potential with double exponentials. The slow components of τ for the S706F/WT channel were smaller than that of wild type (WT); *p<0.05.

Figure 5. Both wild type (WT) and mutant channels showed similar drug sensitivities to the culprit agents. A, Fractional blockade by micromolar erythromycin was recorded with regard to D342V/WT or WT as described in the methods and is plotted against the drug concentrations. B, Similarly, the relationship between the fractional block by disopyramide and its concentration are shown. C, The relationship between the fractional pirmenol block of M756V/WT or WT channels and its concentration. IC50s
Genetic backgrounds in drug-induced long-QT syndrome (MS ID#: 2009/862649)

for erythromycin, disopyramide and pirmenol were 327 μM (WT) and 248 μM (D342V/WT), 13.9 μM (WT) and 8.4 μM (H492Y/WT), 16.6 μM (WT) and 13.4 μM (M756V/WT), respectively; n=4 or 5 cells per condition.

Figure 6. Simulation study of congenital and drug-induced LQTS-associated channels.

A. A scheme showing a Markov model for $I_{Kr}$ channels and simulation results for voltage-clamp protocols with a modified the transition rate $\alpha_i = 0.439 \times \exp(-0.02352 \times (V+40))/K_{out}$. The three panels illustrate the result on the M756V/WT model, (a); the I-V relationship, (b); the I-V relationship for amplitudes of peak tail currents and (c); the steady-state inactivation curves. Open circles, wild type (WT) and filled circles mutant. B, Simulation study of action potential durations (APD). The parameters used for simulation were changed and matched the experimental results for voltage-clamp protocols. Myocardium models were stimulated at the cycle length of 600 ms for 5 minutes. C, Simulated APDs with $I_{Kr}$ blocking effects. When $I_{Kr}$ conductance was decreased to 11% in each of the models, the D342V model showed early afterdepolarization whereas the WT model had only slight prolonged APD. Bold lines, controls and dotted lines, models with $I_{Kr}$ blocking effects.
Figure 4, Itoh et al.
Figure 6. Itoh et al.
Latent Genetic Backgrounds and Molecular Pathogenesis in Drug-induced Long QT Syndrome

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Supplemental data 1.

Header Files (LRc.h)

```
//
#define WINDOWS 1
#define DISPLAY 1  // 0: no display, 1: display trace (only for Windows)
#define DATA_FILE 1  // 0: No file output, 1: Text file, 2: Binary file
#define NTRACES 1

#define NUMBER_OF CELLS 1  // Number of cells

#define TIMESTEP 0.002  // in ms
#define BEATS 500  // Number of beats
#define BCL 600  // Basic Cycle Length (ms)

#define STIM -120  // Stimulation intensity

#define MARKOV NA 0  // 0: conventional HH-model, 1: Markov model
#define MARKOV HERG 1  // 0: conventional HH-model, 1: Markov model

#define WT NA CHANNEL G (16.0 * 0)  //WT NA CHANNEL G (8.0 * 1)
#define MT NA CHANNEL G (16.0 * 0)
```

//include files

```
#include <stdlib.h>
#include <stdio.h>
#include <fcntl.h>
#ifndef WINDOWS
#include <process.h>
#include <io.h>
#endif
#include <string.h>
#include <math.h>

#include "MarkovNaChannel.h"
#include "MarkovHERGChannel.h"
#include "LRModel.h"
```
class CMarkovNaChannel;

class CMycocyte
{
public:
    CMycocyte();
    ~CMycocyte();

public:
    void setStepTime(double ut);
    bool checkChanges(double t);
    void calculateChanges(double t);
    bool advanceStep(double t);

public:
    CMycocyte* pCellArray;
    int cellIndex;
    int nCells;
    double v;        // Membrane voltage (mV)
    double bcl;
    double st;       // Constant Stimulus (uA/cm^2)
//   double cai;     // Intracellular Ca Concentration (mM)
    double irelcicr; // Ca release from JSR to myo. due to CICR (mM/ms)

    double ikr;
    double ikr2;

    double dvdtmax:
#if MARKOV_NA == 1
    CMarkovNaChannel wtNaChannel;
    CMarkovNaChannel mutantNaChannel;
#endif

#if MARKOV_HERG == 1
    CMarkovHERGChannel HERGChannel;
#endif

private: // constant values
    double R:  // 8314: Universal Gas Constant (J/kmol*K)
    double frdy: // 96485: Faraday’s Constant (C/mol)
    double temp: // 310: Temperature (K)
    double pi: // 3.141592: Pi

    // Ion Valences
    double zna: // 1: Na valence
    double zk: // 1: K valence
    double zca: // 2: Ca valence

private:
    CMyocyte* getPrevCell(int ci);
    CMyocyte* getNextCell(int ci):

    // Ion Current Functions
    void comp_ina (): // Calculates Fast Na Current
    void comp_ical (): // Calculates Currents through L-Type Ca Channel
    void comp_icat (): // Calculates Currents through T-Type Ca Channel
    void comp_ikr (): // Calculates Rapidly Activating K Current
    void comp_iks (): // Calculates Slowly Activating K Current
    void comp_i ki (): // Calculates Time-Independant K Current
    void comp_ikp (): // Calculates Plateau K Current
    void comp_i kna (): // Calculates Na-activated K Current
    void comp_ikatp (): // Calculates ATP-Sensitive K Current
    void comp_ito (): // Calculates Transient Outward Current
    void comp_inaca (): // Calculates Na-Ca Exchanger Current
    void comp_inak (): // Calculates Na-K Pump Current
    void comp_insca (): // Calculates Non-Specific ca-Activated Current
    void comp_ipca (): // Calculates Sarcolemmal Ca Pump Current
    void comp_icab (): // Calculates Ca Background Current
    void comp_inab (): // Calculates Na Background Current
    void comp_gap (): // Calculates gap-junction current
    void comp_it (double t): // Calculates Total Current

    // Ion Concentration Functions
    void conc_nai (): // Calculates new myoplasmic Na ion concentration
    void conc_ki (): // Calculates new myoplasmic K ion concentration
    void conc_nsr (): // Calculates new NSR Ca ion concentration
    void conc_jsr (): // Calculates new JSR Ca ion concentration
void calc_itr();    // Calculates Translocation of Ca from NSR to JSR
void conc_cai();    // Calculates new myoplasmic Ca ion concentration
void conc_cleft();  // Calculates new cleft ion concentrations

private:
    // Time Step
    double dt;       // Time step (ms), used for calculating current changes
    double udt;      // Universal Time Step, used for calculating the voltage change
    int utsc;        // Universal Time Step Counter
    int nxstep;      // Interval Between Calculating Ion Currents

    // int steps;      // Number of Steps
    // int increment;  // Loop Control Variable

    int stimCounter;  // Stimulus Counter
    int printdata;
    int printval;
    bool printFlag;

    // cell geometry
    double l;       // = 0.01;       // Length of the cell (cm)
    double a;       // = 0.0011;     // Radius of the cell (cm)
    double vcell;   // Cell volume (uL)
    double ageo;    // Geometric membrane area (cm^2)
    double acap;    // Capacitive membrane area (cm^2)
    double vmym;    // Myoplasm volume (uL)
    double vmymo;   // Mitochondria volume (uL)
    double vsr;     // SR volume (uL)
    double vnsr;    // NSR volume (uL)
    double vjsr;    // JSR volume (uL)
    double vcleft;  // Cleft volume (uL)

    // Total Current and Stimulus
    double st;       // Constant Stimulus (uA/cm^2)
    double tstim;    // Time Stimulus is Applied (ms)
    double stimtime; // Time period during which stimulus is applied (ms)
    double it;      // Total current (uA/cm^2)

    // Voltage
    double v;       // Membrane voltage (mV)
    double vnew;    // New Voltage (mV)
    double dvdt;    // Change in Voltage / Change in Time (mV/ms)
    bool triggerCICR;  // triggerCICR condition to test for dvdtmax

    double dvdtnew; // New dv/dt (mV/ms)

public:

    // Ion Concentrations
    double nai;    // Intracellular Na Concentration (mM)
    double nao;    // Extracellular Na Concentration (mM)
    double nabm;   // Bulk Medium Na Concentration (mM)
double dnao;  // Change in Cleft Na Concentration (mM)
double ki;   // Intracellular K Concentration (mM)
double ko;   // Extracellular K Concentration (mM)
double kbm;  // Bulk Medium K Concentration (mM)
double dko;  // Change in Cleft K Concentration (mM)
double cai;  // Intracellular Ca Concentration (mM)
double cao;  // Extracellular Ca Concentration (mM)
double cabm; // Bulk Medium Ca Concentration (mM)
double dcao; // Change in Cleft Ca Concentration (mM)
double cmdn; // Calmodulin Buffered Ca Concentration (mM)
double trpn; // Troponin Buffered Ca Concentration (mM)
double nsr;  // NSR Ca Concentration (mM)
double jsr;  // JSR Ca Concentration (mM)
double csqn; // Calsequestrin Buffered Ca Concentration (mM)
double taudiff; // = 1000; // Diffusion Constant for Ion Movement from Bulk Medium to Cleft Space

// Myoplasmic Na Ion Concentration Changes
double naiont; // Total Na Ion Flow (uA/uF)
double dnai;  // Change in Intracellular Na Concentration (mM)

// Myoplasmic K Ion Concentration Changes
double kiont; // Total K Ion Flow (uA/uF)
double dki;   // Change in Intracellular K Concentration (mM)

// NSR Ca Ion Concentration Changes
double dnsr;  // Change in [Ca] in the NSR (mM)
double iup;   // Ca uptake from myo. to NSR (mM/ms)
double ileak; // Ca leakage from NSR to myo. (mM/ms)
double kleak; // Rate constant of Ca leakage from NSR (ms^-1)
double kmup;:// = 0.00092; // Half-saturation concentration of iup (mM)
double iupbar;:// = 0.00875; // Max. current through iup channel (mM/ms)
double nsrbar;:// = 15; // Max. [Ca] in NSR (mM)

// JSR Ca Ion Concentration Changes
double djsr;  // Change in [Ca] in the JSR (mM)
double tauon;:// = 2; // Time constant of activation of Ca release from JSR (ms)
double taoff;:// = 2; // Time constant of deactivation of Ca release from JSR (ms)
double tjc;   // t=0 at time of CICR (ms)

// Ca release from JSR to myo. due to CICR (mM/ms)
double ireljsrol;:// = 0; // Rate constant of Ca release from JSR due to CICR (ms^-1)
double greljsrol; // Rate constant of Ca release from JSR due to overload (ms^-1)
double tjsrol;  // t=0 at time of JSR overload (ms)
double ireljsrol; // Ca release from JSR to myo. due to JSR overload (mM/ms)
double csqnth;:// = 8.75; // Threshold for release of Ca from CSQN due to JSR overload (mM)
double gmaxrel;:// = 150; // Max. rate constant of Ca release from JSR due to overload (ms^-1)
double grelbarjsrol; // Rate constant of Ca release from JSR due to overload (ms^-1)
double grelbarjsrol; // Rate constant of Ca release from JSR due to overload (ms^-1)
double tjcsrol;  // t=0 at time of JSR overload (ms)
double ireljsrol; // Ca release from JSR to myo. due to JSR overload (mM/ms)
double csqnbar;:// = 10; // Max. [Ca] buffered in CSQN (mM)
double kmcsqn;:// = 0.8; // Equilibrium constant of buffering for CSQN (mM)

// b Variable for analytical computation of [Ca] in JSR (mM)
double bjjsr;  // c Variable for analytical computation of [Ca] in JSR (mM)
double cjsr;   // c Variable for analytical computation of [Ca] in JSR (mM)
double RyRopen; // Time constant of activation of Ca release from JSR (ms)
double RyRclose; // Time constant of deactivation of Ca release from JSR (ms)
double grel; // Magnitude of Ca release
double dcaiont; // Rate of change of Ca entry
double dcaiontnew; // New rate of change of Ca entry
double caiontold; // Old rate of change of Ca entry

// Translocation of Ca Ions from NSR to JSR
double itr; // Translocation current of Ca ions from NSR to JSR (mM/ms)
double taur; // = 180; // Time constant of Ca transfer from NSR to JSR (ms)

// Myoplasmic Ca Ion Concentration Changes
double caiont; // Total Ca Ion Flow (uA/uF)
double dcai; // Change in myoplasmic Ca concentration (mM)
double catotal; // Total myoplasmic Ca concentration (mM)
double bm; // Variable for analytical computation of [Ca] in myoplasm (mM)
double cm; // Variable for analytical computation of [Ca] in myoplasm (mM)
double dm; // Variable for analytical computation of [Ca] in myoplasm (mM)
double gpig; // Tribute to all the guinea pigs killed for the advancement of knowledge

double cmdnbar; // = 0.050; // Max. [Ca] buffered in CMDN (mM)
double trpnbar; // = 0.070; // Max. [Ca] buffered in TRPN (mM)
double kmcmdn; // = 0.00238; // Equilibrium constant of buffering for CMDN (mM)
double kmtrpn; // = 0.0005; // Equilibrium constant of buffering for TRPN (mM)

// Fast Sodium Current (time dependant)
double ina; // Fast Na Current (uA/uF)
double gna; // Max. Conductance of the Na Channel (mS/uF)
double ena; // Reversal Potential of Na (mV)
double am; // Na alpha-m rate constant (ms^-1)
double bm; // Na beta-m rate constant (ms^-1)
double ah; // Na alpha-h rate constant (ms^-1)
double bh; // Na beta-h rate constant (ms^-1)
double aj; // Na alpha-j rate constant (ms^-1)
double bj; // Na beta-j rate constant (ms^-1)
double m; // Na activation
double h; // Na inactivation
double j; // Na inactivation

// Current through L-type Ca Channel
double ilca; // Ca current through L-type Ca channel (uA/uF)
double ilicana; // Na current through L-type Ca channel (uA/uF)
double ilcak; // K current through L-type Ca channel (uA/uF)
double ilcatot; // Total current through the L-type Ca channel (uA/uF)
double ibarca; // Max. Ca current through Ca channel (uA/uF)
double ibarna; // Max. Na current through Ca channel (uA/uF)
double ibark; // Max. K current through Ca channel (uA/uF)
double d; // Voltage dependant activation gate
double dss; // Steady-state value of activation gate d
double taud; // Time constant of gate d (ms^-1)
double f; // Voltage dependant inactivation gate
double fss; // Steady-state value of inactivation gate f
double tauf; // Time constant of gate f (ms^-1)
double fca; // Ca dependant inactivation gate
double kmca; // = 0.0006: // Half-saturation concentration of Ca channel (mM)
double pca; // = 0.00054: // Permiability of membrane to Ca (cm/s)
double gacai; // = 1: // Activity coefficient of Ca
double gacao; // = 0.341: // Activity coefficient of Ca
double pna; // = 0.000000675: // Permiability of membrane to Na (cm/s)
double ganai; // = 0.75: // Activity coefficient of Na
double gako; // = 0.75: // Activity coefficient of K

double icat; // Ca current through T-type Ca channel (uA/uF)
double gcat; // Max. Conductance of the T-type Ca channel (mS/uF)
double eca; // Reversal Potential of the T-type Ca channel (mV)
double b; // Voltage dependant activation gate
double bss; // Steady-state value of activation gate b
double taub; // Time constant of gate b (ms^-1)
double g; // Voltage dependant inactivation gate
double gss; // Steady-state value of inactivation gate g
double taug; // Time constant of gate g (ms^-1)

double ikr; // Rapidly Activating K Current (uA/uF)
double ekr; // Reversal Potential of Rapidly Activating K Current (mV)
double xr; // Rapidly Activating K time-dependant activation
double xrss; // Steady-state value of inactivation gate xr
double tauxr; // Time constant of gate xr (ms^-1)
double r; // K time-independant inactivation

double iks; // Slowly Activating K Current (uA/uF)
double eks; // Reversal Potential of Slowly Activating K Current (mV)
double xs1; // Slowly Activating K time-dependant activation
double xs1ss; // Steady-state value of inactivation gate xs1
double tauxs1; // Time constant of gate xs1 (ms^-1)
double xs2; // Slowly Activating K time-dependant activation
double xs2ss; // Steady-state value of inactivation gate xs2
double tauxs2; // Time constant of gate xs2 (ms^-1)
double prnak; // = 0.01833: // Na/K Permiability Ratio

double iki; // Time-independant K current (uA/uF)
double gki;  // Channel Conductance of Time Independent K Current (mS/µF)
double eki;  // Reversal Potential of Time Independent K Current (mV)
double aki;  // K alpha-ki rate constant (ms^-1)
double bki;  // K beta-ki rate constant (ms^-1)
double kin;  // K inactivation

// Plateau Potassium Current
double ikp;  // Plateau K current (µA/µF)
double gkp;  // Channel Conductance of Plateau K Current (mS/µF)
double ekp;  // Reversal Potential of Plateau K Current (mV)
double kp;  // K plateau factor

// Na-Activated K Channel
double ikna; // Na activated K channel
double pona; // Open probability dependant on Nai
double pov; // Open probability dependant on Voltage
double ekna; // Reversal potential
double gkna; = 0.12848; // Maximum conductance (mS/µF)
double nkna; = 2.8; // Hill coefficient for Na dependance
double kdkna; = 66; // Dissociation constant for Na dependance (mM)

// ATP-Sensitive K Channel
double ikatp; // ATP-sensitive K current (µA/µF)
double ekatp; // K reversal potential (mV)
double gkbaratp; // Conductance of the ATP-sensitive K channel (mS/µF)
double gkatp; // Maximum conductance of the ATP-sensitive K channel (mS/µF)
double patp; // Percentage availability of open channels
double natp; = 0.24; // K dependence of ATP-sensitive K current
double nicholsarea; = 0.00005; // Nichol’s area (cm^2)
double atpi; = 3; // Intracellular ATP concentration (mM)
double hapt; = 2; // Hill coefficient
double katp; = 0.250; // Half-maximal saturation point of ATP-sensitive K current (mM)

double ito;  // Transient outward current
double gitodv; // Maximum conductance of Ito
double ekdv; // Reversal Potential of Ito
double rvdv; // Time independant voltage dependence of Ito
double zdv;  // Ito activation
double azdv; // Ito alpha-z rate constant
double bzdv; // Ito beta-z rate constant
double tauzdv; // Time constant of z gate
double zssdv; // Steady-state value of z gate
double ydv;  // Ito inactivation
double aydv; // Ito alpha-y rate constant
double bydv; // Ito beta-y rate constant
double tauydv; // Time constant of y gate
double yssdv; // Steady-state value of y gate
// Sodium-Calcium Exchanger V-S
double inaca; // NaCa exchanger current (uA/uF)
double c1:// = .00025: // Scaling factor for inaca (uA/uF)
double c2:// = 0.0001: // Half-saturation concentration of NaCa exchanger (mM)
double gammas:// = .15: // Position of energy barrier controlling voltage dependence of inaca

// Sodium-Potassium Pump
double inak; // NaK pump current (uA/uF)
double fnak; // Voltage-dependence parameter of inak
double sigma; // [Na] dependance factor of fnak
double ibarnak:// = 2.25: // Max. current through Na-K pump (uA/uF)
double kmnai:// = 10: // Half-saturation concentration of NaK pump (mM)
double kmko:// = 1.5: // Half-saturation concentration of NaK pump (mM)

// Nonspecific Ca-activated Current
double insna; // Non-specific Na current (uA/uF)
double insk; // Non-specific K current (uA/uF)
double ibarnsnsa; // Max. Na current through NSCa channel (uA/uF)
double ibarnsnsk: // Max. K current through NSCa channel (uA/uF)
double pnsca:// = 0.000000175: // Permiability of channel to Na and K (cm/s)
double kmnsca:// = 0.0012: // Half-saturation concentration of NSCa channel (mM)

// Sarcolemmal Ca Pump
double ipca; // Sarcolemmal Ca pump current (uA/uF)
double ibarpca:// = 1.15: // Max. Ca current through sarcolemmal Ca pump (uA/uF)
double kmpca:// = 0.0005: // Half-saturation concentration of sarcolemmal Ca pump (mM)

// Ca Background Current
double icab; // Ca background current (uA/uF)
double gcab: // Max. conductance of Ca background (mS/uF)
double ecan: // Nernst potential for Ca (mV)

// Na Background Current
double inab; // Na background current (uA/uF)
double gnab: // Max. conductance of Na background (mS/uF)
double enan: // Nernst potential for Na (mV)

// gap junction current
double igap;
double ggap;
}:
Header Files (MarkovHERGChannel.h)

// Na channel Markov process model
//
// IC3 - IC2 - IF - IM1 - IM2
//    |     | /   |
// C3 - C2 - C1 - O
//
// xd[0]  C3
// xd[1]  C2
// xd[2]  C1
// xd[3]  0
// xd[4]  IC3
// xd[5]  IC2
// xd[6]  IF
// xd[7]  IM1
// xd[8]  IM2
//
// integration methods
// modified from Colleen Clancy's method
//
#define HERG_CHANNEL_PARAM_NUM 4
#define MARKOV_HERG_C3   0
#define MARKOV_HERG_C2   1
#define MARKOV_HERG_C1   2
#define MARKOV_HERG_O    3
#define MARKOV_HERG_I    4
#define OPEN_STATE 3
#define NUM_OF_HERG_STATES 5

class CMarkovHERGChannel
{

public:
    CMarkovHERGChannel();
    ~CMarkovHERGChannel();

public:
    double compCurrent(double t, double dt, double v, double ena, double ko);
private:
    void getdy(double dt, double* dy);
    void getRateConstants(double v);

public:
    double gherg;
    double pHERG[NUM_OF_HERG_STATES];

private:
    int ix, i4;
    int nStates;
    bool done;

    double sum;
    double Kout;
    double aa, ain, a, b, bin, bb, ai, bi, mu;
    double pC3, pC2, pC1;
    double pO;
    double pI;

    double y[NUM_OF_HERG_STATES];
    double yn[NUM_OF_HERG_STATES];
    double k[NUM_OF_HERG_STATES];
    double dny[NUM_OF_HERG_STATES];
    double dny1[NUM_OF_HERG_STATES];

};
Header Files (MarkovNaChannel.h)

    //
    //   Na channel Markov process model
    //
    //
    //               IC3 - IC2 - IF - IM1 - IM2
    //               |       |       /   |
    //                C3 - C2 - C1 - 0
    //
    //
    //    xd[0]    C3
    //    xd[1]    C2
    //    xd[2]    C1
    //    xd[3]    0
    //    xd[4]    IC3
    //    xd[5]    IC2
    //    xd[6]    IF
    //    xd[7]    IM1
    //    xd[8]    IM2
    //
    //
    // integration methods
    //    modified from Colleen Clancy’s method
    //

#define OPEN_STATE 3
#define OPEN_STATE_B 12
#define NUM_OF_NA_STATES 9
#define NUM_OF_NA_BURST_STATES 4

class CMarkovNaChannel
{

public:
    CMarkovNaChannel();
    ~CMarkovNaChannel();

public:
    double compCurrent(double t, double dt, double v, double ena);

private:
    void getdy(double dt, double* dy);
    void getRateConstants(double v);
public:
    bool bBurstMode;
    double gna;
    double pNa[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
private:
    int ix, i4;
    int nStates;
    bool done;
    double sum;
    double a11, a12, a13, a2, a3, a4, a5;
    double b11, b12, b13, b2, b3, b4, b5;
    double a6, b6;
    double y[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
    double yn[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
    double k[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
    double dny[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
    double dny1[NUM_OF_NA_STATES+NUM_OF_NA_BURST_STATES];
};
#include "LRc.h"

void flashData(double t, int nFileCounter, int stepCounter, double **a, int nTraces);

int main (int argc, char** argv) 
{
  int i,j;

  int bcl = BCL;
  int beats = BEATS;
  int nCells = NUMBER_OF_CELLS;
  int nTraces = NTRACES;
  int steps, stepCounter;

  double udt = TIMESTEP;      // time (ms)
  double t;                   // time (ms)
  bool checkFlag;

  int nFileCounter = 0;
  int nFileDataSize = 20*1024; // 100K

#if MARKOV_NA == 1
  printf("Markov Na channel model\n");
#else
  printf("Markov Na channel model\n");
#endif
printf("Conventional Na channel model\n");
#endif
#if MARKOV_HERG == 1
printf("Markov HERG channel model\n");
#else
printf("Conventional HERG channel model\n");
#endif
#if DATA_FILE == 1  // text file
printf("Ascii output file\n");
#elif DATA_FILE == 2  // binary file
printf("Binary output file\n");
#endif
printf("%d cells, BCL = %d, BEATS = %d\n", nCells, bcl, beats);
steps = (int) ((bcl * beats) / udt);  // Number of steps.

// initialization of cells
CMyocyte* pCells = new CMyocyte[nCells];

    for (j = 0; j < nCells; j++) {
        pCells[j].pCellArray = pCells;
        pCells[j].cellIndex = j;
        pCells[j].nCells = nCells;
        pCells[j].bcl = BCL;
        pCells[j].setStepTime (udt);
#if MARKOV_NA == 1
        pCells[j].wtNaChannel.gna = WT_NA_CHANNEL_G;     //16.0 * 0.5;
        pCells[j].wtNaChannel.bBurstMode = false;
        pCells[j].mutantNaChannel.gna = MT_NA_CHANNEL_G; // 16.0 * 0.5;
        pCells[j].mutantNaChannel.bBurstMode = true;
#endif
    }
    pCells[0].st = STIM;

// trace buffer
    double** a = new double *[nTraces+1];
    for (i = 0; i < nTraces+1; i++) a[i] = new double[nFileDataSize];

// main loop
    t = 0;
    stepCounter=0;
    for (i = 0; i < steps; i++) {
        t = i * udt;
        // printf("t=%g\n", t);
        for (j = 0; j < nCells; j++) {
            if (pCells[j].checkChanges (t)) {
                pCells[j].calculateChanges (t);
            }
        }
    }
if (checkFlag) {
    a[0][stepCounter] = t;
    //for (j = 0; j < nCells; j++) a[j+1][stepCounter] = pCells[j].v;
    a[1][stepCounter] = pCells[0].v;
    //a[1][stepCounter] = pCells[0].fca;//fca
    //a[2][stepCounter] = pCells[0].ikr;
    //a[3][stepCounter] = pCells[0].cai;
    //a[4][stepCounter] = pCells[0].ilca;
    //a[5][stepCounter] = pCells[0].ina;
    //a[6][stepCounter] = pCells[0].jsr;
    //a[7][stepCounter] = pCells[0].iks;
    //a[8][stepCounter] = pCells[0].HERGChannel.pHERG[MARKOV_HERG_C3];
    //a[9][stepCounter] = pCells[0].HERGChannel.pHERG[MARKOV_HERG_C2];
    //a[10][stepCounter] = pCells[0].HERGChannel.pHERG[MARKOV_HERG_C1];
    //a[11][stepCounter] = pCells[0].HERGChannel.pHERG[MARKOV_HERG_I];
    //a[12][stepCounter] = pCells[0].HERGChannel.pHERG[MARKOV_HERG_O];
    stepCounter++;

    if (stepCounter >= nFileDataSize) {
        flashData(t, nFileCounter, stepCounter, a, nTraces);
        nFileCounter++;
        stepCounter = 0;
    }
}
} // end of main loop

flashData(t, nFileCounter, stepCounter, a, nTraces):

]*)
for (j=0; j<nTraces; j++) fprintf(fap,"%lf", a[j][i]);
fprintf(fap,"\n");
}
fclose(fap);
#endif

dataFile == 2 // binary file
FILE* fap:
    char fname[]="ap.dat";
    printf("Binary output file\n");
fap = fopen(fname,"wb");
    double*ax = new double[nTraces+1];
for (i=0; i<stepCounter; i++) {
    for (j=0; j<nTraces+1; j++) ax[j] = a[j][i];
    fwrite(ax, sizeof(double), nTraces+1, fap);
}
fclose(fap);
#endif
*

// clean up
delete [] pCells;
for (j=0; j<nCells+1; j++) delete [] a[j];
delete [] a;

#if DISPLAY == 1
    char fname[] = "ap001.dat";
    char s[10];
    sprintf(s,"%d", nTraces):
    _spawnl(_P_NOWAIT, "gc", "gc", fname, s, NULL):
#endif

return 0:
}

//--------Data output ------------------------------

void flashData(double t, int nFileCounter, int stepCounter, double **a, int nTraces) {
    FILE *fap:
    int i, j;
    char fname[32];

    nFileCounter++:
#if DATA_FILE == 1  // text file
    printf(fname,"ap%03d.txt", nFileCounter);
    fap = fopen(fname,"w");
    for(i=0;i<stepCounter;i++) {
        fprintf(fap,"%lf", a[0][i]);
        for(j=0;j<nTraces;j++) fprintf(fap,"%lf", a[j+1][i]);
        fprintf(fap,"\n");
    }
#else
    printf(fname,"ap%03d.dat", nFileCounter);
    fap = fopen(fname,"wb");
    double*ax = new double[nTraces+1];
    for(i=0;i<stepCounter;i++) {
        for(j=0;j<nTraces+1;j++) ax[j] = a[j][i];
        fwrite(ax, sizeof(double), nTraces+1, fap);
    }
#endif
fclose(fap);
printf("t=%lf: %d points -> file <%s>\n", t, stepCounter, fname);
return;
#include "LRc.h"

CMyocyte::CMyocyte()
{
    // constant values
    R = 8314; // Universal Gas Constant (J/kmol*K)
    frdy = 96485; // Faraday's Constant (C/mol)
    temp = 310; // Temperature (K)
    pi = 3.141592; // Pi

    // Ion Valences
    zna = 1; // Na valence
    zk = 1; // K valence
    zca = 2; // Ca valence

    // Cell Geometry
    l = 0.01; // Length of the cell (cm)
    a = 0.0011; // Radius of the cell (cm)
    vcell = 1000*pi*a*a*l; // 3.801e-5 uL
    ageo = 2*pi*a*a+2*pi*a*l; // 7.671e-5 cm^2
    acap = ageo*2; // 1.534e-4 cm^2
    vmyo = vcell*0.68;
    vmito = vcell*0.26;
    vsr = vcell*0.06;
    vnsr = vcell*0.0552;
    vjsr = vcell*0.0048;
    vcleft = vcell*0.12/0.88;

    //=======================================
    // time
    dt = udt;
    utsc = 50;

    // output control
printdata = 0;
printval = 0;

// Ion Concentrations
// -- bulk medium
nabm = 140;   // Initial Bulk Medium Na (mM)
kbm = 4.5;    // Initial Bulk Medium K (mM)
//   kbm = 2.0;    // Initial Bulk Medium K (mM)
cabm = 1.8;   // Initial Bulk Medium Ca (mM)
taudiff = 1000; // Diffusion Constant for Ion Movement from Bulk Medium to Cleft Space
// -- external
nao = 140;    // Initial Extracellular Na (mM)

<<<<<<<<<<<
// Change ko for external K concentration
// The normal default value is 4.5.
// ko = 4.5;    // Initial Extracellular K (mM)
ko = 4.5;     // Initial Extracellular K (mM)
cao = 1.8;    // Initial Extracellular Ca (mM)
dnao = 0;
dko = 0;
dcao = 0;
// -- internal
nai = 9;      // Initial Intracellular Na (mM)
ki = 141.2;   // Initial Intracellular K (mM)
cai = 0.00006; // Initial Intracellular Ca (mM)

cmdn = 0.00257849; // Calmodulin Buffered Ca Concentration (mM)
trpn = 0.0143923;  // Troponin Buffered Ca Concentration (mM)
nsr = 1.838;      // NSR Ca Concentration (mM)
jsr = 1.838;      // JSR Ca Concentration (mM)
csqn = 6.97978;   // Calsequestrin Buffered Ca Concentration (mM)

naiont = 0;     // Total Na Ion Flow (uA/uF)
dnai = 0;       // Change in Intracellular Na Concentration (mM)
kiont = 0;      // Total K Ion Flow (uA/uF)
dki = 0;        // Change in Intracellular K Concentration (mM)

// stimulation
st = 0;        // Stimulus
tstim = 10;    // Time to begin stimulus
stimtime = 10; // Initial Condition for Stimulus
bcl = 0;       // BCL:

//Voltage
v = -90;       // Initial Voltage (mv)
it = 0;        // total current
dvdt = 0;      // force to calculate currents at the beginning
dvdtmax = dvdt;
triggerCICR = false; // flag for Ca release

// Fast Sodium Current (time dependant)
gna = 16;
m = 0.0008; // Na activation
h = 0.993771; // Na inactivation
j = 0.995727; // Na inactivation

// Current through L-type Ca Channel
kmca = 0.0006; // Half-saturation concentration of Ca channel (mM)
pca = 0.000054; // Permiability of membrane to Ca (cm/s)
gacai = 1; // Activity coefficient of Ca
gacao = 0.341; // Activity coefficient of Ca
pna = 0.000000675; // Permiability of membrane to Na (cm/s)
ganai = 0.75; // Activity coefficient of Na
ganao = 0.75; // Activity coefficient of Na
pk = 0.000000193; // Permiability of membrane to K (cm/s)
gaki = 0.75; // Activity coefficient of K
gako = 0.75; // Activity coefficient of K

prnak = 0.01833; // Na/K Permiability Ratio
gkna = 0.12848; // Maximum conductance (mS/uF)
nkna = 2.8; // Hill coefficient for Na dependance
kdkna = 66; // Dissociation constant for Na dependance (mM)
natp = 0.24; // K dependence of ATP-sensitive K current
nicholsarea = 0.00005; // Nichol's ares (cm^2)
atpi = 3; // Intracellular ATP concenatraion (mM)
hatp = 2; // Hill coefficient
katp = 0.250; // Half-maximal saturation point of ATP-sensitive K current (mM)

//*** PUMPS ***
c1 = .00025; // Scaling factor for inaca (uA/uF)
c2 = 0.0001; // Half-saturation concentration of NaCa exhanger (mM)
gammas = .15; // Position of energy barrier controlling voltage dependance of inaca

ibarnak = 2.25; // Max. current through Na-K pump (uA/uF)
kmnai = 10; // Half-saturation concentration of NaK pump (mM)
kmko = 1.5; // Half-saturation concentration of NaK pump (mM)
pnsca = 0.000000175; // Permiability of channel to Na and K (cm/s)
kmnsc = 0.0012; // Half-saturation concentration of NSCa channel (mM)
ibarpca = 1.15; // Max. Ca current through sarcollemal Ca pump (uA/uF)
kmpca = 0.0005; // Half-saturation concentration of sarcolemmal Ca pump (mM)
ggap = 0.1;

// Initial Gate Conditions
d = 3.210618e-06:
f = 0.999837;
xs1 = 0.00445683;
xs2 = 0.00445683;
xr = 0.000124042;
b = 0.000970231;
g = 0.994305;
zdv = 0.0120892;
ydv = 0.999978;

// *** SR ***
// NSR Ca Ion Concentration Changes
kmup = 0.00092;  // Half-saturation concentration of iup (mM)
iupbar = 0.00875;  // Max. current through iup channel (mM/ms)
srbar = 15;  // Max. [Ca] in NSR (mM)

// JSR Ca Ion Concentration Changes
tauon = 2;  // Time constant of activation of Ca release from JSR (ms)
tauoff = 2;  // Time constant of deactivation of Ca release from JSR (ms)
csqnth = 8.75;  // Threshold for release of Ca from CSQN due to JSR overload (mM)
gmaxrel = 150;  // Max. rate constant of Ca release from JSR due to overload (ms⁻¹)
csqnbar = 10;  // Max. [Ca] buffered in CSQN (mM)
kmcseq = 0.8;  // Equilibrium constant of buffering for CSQN (mM)

// Translocation of Ca Ions from NSR to JSR
itr = 0;  // Translocation current of Ca ions from NSR to JSR (mM/ms)
tautr = 180;  // Time constant of Ca transfer from NSR to JSR (ms)

// Myoplasmic Ca Ion Concentration Changes
cmdnbar = 0.050;  // Max. [Ca] buffered in CMDN (mM)
trpbar = 0.070;  // Max. [Ca] buffered in TRPN (mM)
kmcmdn = 0.00238;  // Equilibrium constant of buffering for CMDN (mM)
kmtrpn = 0.0005;  // Equilibrium constant of buffering for TRPN (mM)

// Ca release
grelbarjsrol = 0;
tjsrol = 25;
tcicr = 25;
dcaiont = 0;
}

void CMyocyte::setStepTime(double ut)
{
    udt = ut;
    dt = udt;
}

CMyocyte* CMyocyte::getPrevCell(int ci)
{
}
if (ci==0) return NULL;
return &pCellArray[ci-1];
}

CMyocyte* CMyocyte::getNextCell(int ci)
{
    if (ci>=nCells-1) return NULL;
    return &pCellArray[ci+1];
}

bool CMyocyte::checkChanges(double t)
{
    //  nxstep = (fabs(dvdt)<0.25 && v<0) ? 500 : 50:  
    nxstep = (fabs(dvdt)<0.25 && v<0) ? 50 : 25;
    if (utsc>=nxstep || dvdt>5 || irelcicr>0.01 || fabs(t-tstim)<=udt ||
        (stimtime>=0 && stimtime<0.5)){
        return true;
    }
    return false;
}

void CMyocyte::calculateChanges(double t)
{
    ena = ((R*temp)/frdy)*log(nao/nai);
    ekr = ((R*temp)/frdy)*log(ko/ki);

    #if MARKOV_NA == 1
        ina = wtNaChannel.compCurrent(t, dt, v, ena);
        ina += mutantNaChannel.compCurrent(t, dt, v, ena);
    #else
        comp_ina ();
    #endif

    comp_ical ()
    comp_icat ();

    #if MARKOV_HERG == 1
        ikr = HERGChannel.compCurrent(t,dt,v,ekr,ko);
    #else
        comp_ikr ();
    #endif

    comp_iks ()
    comp_iki ()
    comp_ikp ()
    ikna = 0; //comp_ikna ();
    ikatp = 0;//comp_ikatp ();
ito = 0; // comp_ito();
comp_inaca();
comp_inak();
insna = 0; insk = 0; // comp_insca();
comp_ipca();
comp_icab();
comp_inab();
comp_gap();

comp_it(t);
conc_nai();
conc_ki();
conc_nsr();
conc_jsr();
calc_itr();
conc_cai();

//conc_cleft(); // Cleft Space disabled, if you want to use cleft space, make sure the initial
conditions of ion concentrations in the bulk medium are the same as the extracellular concentrations
utsc = 0;
dt = 0; // dt : dt used for calculating current changes
}

bool CMMyocyte::advanceStep(double t)
{
    printval = (dvdt>3 || irelcicr>.01) ? 50 : 750;
    printFlag = false;
    if(printdata>=printval){
        printFlag = true;
        printdata = 0;
    }
    printdata++;

    if(csqn>=csqnth && tjsrol>50){
        grelbarjsrol = 4;
        tjsrol = 0;
        printf("Spontaneous Release occured at time %lf\n", t);
    }

    vnew = v-it*udt;
    dvdtnew = (vnew-v)/udt;
    v = vnew;
    dvdt = dvdtnew;

    v -= it*udt; // get new voltage
dvdt = -it;

    //### added 10-Jan-2004
    if(dvdt >= dvdtmax)dvdtmax = dvdt;
if(dvdt<dvdtmax && dvdtmax > 100) triggerCICR = true;
//###

dt += udt;
utsc++;

caiontold = caiont;
dcaiont = dcaiontnew;

return printFlag;
}

//________________________________________________
// Total sum of currents is calculated here.
// if the time is between stimtime = 0 and stimtime = 0.5,
// a stimulus is applied

void CMyocyte::comp_it (double t)
{

    naiont = ina+inab+ilcana+insna+3*inak+3*inaca;
    kiont = ikr+iks+iki+ikp+ilcak+insk-2*inak+ito+ikna+ikatp+igap;
    caiont = ilca+icab+ipca-2*inaca+icat;

    it = naiont+kiont+caiont;

    if(st == 0) return;

    if (t>=tstim && t<(tstim+dt)){
        stimtime = 0;
        tstim = tstim + bcl;
        triggerCICR = true;
    }

    if(stimtime>=0 && stimtime<0.5 && t > bcl) it += st;

    stimtime += dt;

}

//******************************************************************************
// Functions that describe the currents begin here
//
// gap junction:
void CMyocyte::comp_gap ()
{
    CMyocyte* pCell:
pCell = getPrevCell(cellIndex);
igap = (pCell==NULL) ? 0 : ggap*(v - pCell->v);

pCell = getNextCell(cellIndex);
igap += (pCell==NULL) ? 0 : ggap*(v - pCell->v);
}

void CMyocyte::comp_ina ()
{
    ena = ((R*temp)/frdy)*log(nao/nai);
    am = 0.32*(v+47.13)/(1-exp(-0.1*(v+47.13)));
    bm = 0.08*exp(-v/11);

    if (v < -40) {
        ah = 0.135*exp((80+v)/-6.8);
        bh = 3.56*exp(0.079*v)+310000*exp(0.35*v);
        aj = (-127140*exp(0.2444+v)-0.00003474*exp(-0.04391*v))*(v+37.78)/(1+exp(0.311*(v+79.23)));
        bj = (0.1212*exp(-0.01052*v))/(1+exp(-0.1378*(v+40.14)));
    } else {
        ah = 0;
        bh = 1/(0.13*(1+exp((v+10.66)/-11.1)));
        aj = 0;
        bj = (0.3*exp(-0.0000002535*v))/(1+exp(-0.1*(v+32)));
    }

    h = ah/(ah+bh)-((ah/(ah+bh))-h)*exp(-dt/(1/(ah+bh)));
    j = aj/(aj+bj)-((aj/(aj+bj))-j)*exp(-dt/(1/(aj+bj)));
    m = am/(am+bm)-((am/(am+bm))-m)*exp(-dt/(1/(am+bm)));

    ina = gna*m*m*h*j*(v-ena);
}

void CMyocyte::comp_ical ()
{
    dss = 1/(1+exp(-(v+10)/6.24));
    taud = dss*1-exp(-(v+10)/6.24)/(0.035*(v+10));

    fss = (1/(1+exp((v+32)/8)))+(0.6/(1+exp((50-v)/20)));
    tauf = 1/(0.0197*exp(-pow(0.0337*(v+10), 2))+0.02);

    d = dss-(dss-d)*exp(-dt/taud);
    f = fss-(fss-f)*exp(-dt/tauf);

    ibarca = pca*zca*zca*((v*frdy*frdy)/(R*temp))*((gacai*cai*exp((zca*v*frdy)/(R*temp))-gacao*cao)/(exp((zca*v*frdy)/(R*temp))-1));
ibarna = 
pn*a*n*a*n*a*((v*frdy*frdy)/(R*temp))*((ganai*nai*exp((zna*v*frdy)/(R*temp)) - ganao*nao)/(exp((zna*v*frdy)/(R*temp)) - 1));

ibark = 
 pk*zk*zk*((v*frdy*frdy)/(R*temp))*((gaki*ki*exp((zk*v*frdy)/(R*temp)) - gako*ko)/(exp((zk*v*frdy)/(R*temp)) - 1));

fca = 1/(1+cai/kmca);

ilca = d*f*fca*ibarca;
ilcana = d*f*fca*ibarna;
ilcak = d*f*fca*ibark;
ilcatot = ilca+ilcana+ilcak;

} void CMyocyte::comp_icat () 
{
 bss = 1/(1+exp(-(v+14)/10.8));
taub = 3.7+6.1/(1+exp((v+25)/4.5));
gss = 1/(1+exp((v+60)/5.6));
 if (v<=0) {
 taug = -0.875*v+12;
 } else {
 taug = 12;
 }

 b = bss-(bss-b)*exp(-dt/taub);
g = gss-(gss-g)*exp(-dt/taug);
gcat = 0.05;
eca = (R*temp/(2*frdy))*log(cao/cai);
icat = gcat*b*b*g*(v-eca);
}

void CMyocyte::comp_ikr ()
{
gkr = 0.02614*sqrt(ko/5.4);
ekr = ((R*temp)/frdy)*log(ko/ki);
xr = 1/(1+exp(-(v+21.5)/7.5));

 tauxr = 1/(0.00138*(v+14.2)/(1-exp(-0.123*(v+14.2)))+0.00061*(v+38.9)/(exp(0.145*(v+38.9))-1));
exr = xrss-(xrss-xr)*exp(-dt/tauxr);
r = 1/(1+exp((v+9)/22.4));
ikr = gkr*xr*r*(v-ekr);

void CMyocyte::comp_iks ()
{
    gks = 0.202*(1+0.6/(1+pow((0.000038/cai), 1.4)));
    eks = ((R*temp)/frdy)*log((ko+prnak*nao)/(ki+prnak*nai));

    xs1ss = 1/(1+exp(-(v-1.5)/16.7));
    xs2ss = xs1ss;

    tauxs1 = 1/(0.0000719*(v+30)/(1-exp(-0.148*(v+30))) + 0.000131*(v+30)/(exp(0.0687*(v+30))-1));
    tauxs2 = 4*tauxs1;

    xs1 = xs1ss-(xs1ss-xs1)*exp(-dt/tauxs1);
    xs2 = xs2ss-(xs2ss-xs2)*exp(-dt/tauxs2);

    iks = gks*xs1*xs2*(v-eks);
}

void CMyocyte::comp_iki ()
{
    gki = 0.75*(sqrt(ko/5.4));
    eki = ((R*temp)/frdy)*log(ko/ki);

    aki = 1.02/(1-exp(0.2385*(v-eki-59.215)));
    bki = (0.49124*exp(0.08032*(v-eki+5.476)) + exp(0.06175*(v-eki-594.31)))/(1+exp(-0.5143*(v-eki+4.753)));

    kin = aki/(aki+bki);
    iki = gki*kin*(v-eki);
}

void CMyocyte::comp_ikp ()
{
    gkp = 0.00552;
    ekp = eki;

    kp = 1/(1+exp((7.488-v)/5.98));

    ikp = gkp*kp*(v-ekp);
void CMyocyte::comp_ikna ()
{
    ekna = ((R*temp)/frdy)*log(ko/ki);
    pona = 0.85/(1+pow((kdkna/nai),2.8));
    pov = 0.8-0.65/(1+exp((v+125)/15));
    ikna = gkna*pona*pov*(v-ekna);
}

void CMyocyte::comp_ikatp ()
{
    // Note: If you wish to use this current in your simulations, there are additional
    // changes which must be made to the code as detailed in Cardiovasc Res 1997;35:256-272
    ekatp = ((R*temp)/frdy)*log(ko/ki);
    gkatp = 0.000195/nicholsarea;
    patp = 1/(1+(pow((atpi/katp),hatp)));
    gkbaratp = gkatp*patp*(pow((ko/4),natp));
    ikatp = gkbaratp*(v-ekatp);
}

void CMyocyte::comp_ito ()
{
    gitodv = 1.1;
    ekdv = ((R*temp)/frdy)*log((ko)/(ki));
    rvdv = exp(v/100);
    azdv = (10*exp((v-40)/25))/(1+exp((v-40)/25));
    bzdv = (10*exp(-(v+90)/25))/(1+exp(-(v+90)/25));
    tauzdv = 1/(azdv+bzdv);
    zssdv = azdv/(azdv+bzdv);
    zdv = zssdv-(zssdv-zdv)*exp(-dt/tauzdv);
    aydv = 0.015/(1+exp((v+60)/5));
    bydv = (0.1*exp((v+25)/5))/(1+exp((v+25)/5));
    tauydv = 1/(aydv+bydv);
    yssdv = aydv/(aydv+bydv);
    ydv = yssdv-(yssdv-ydv)*exp(-dt/tauydv);
    ito = gitodv*zdv*zdv*zdv*ydv*rvdv*(v-ekdv);
}

void CMyocyte::comp_inaca()
{
    inaca =
        c1*exp((gammas-1)*v*frdy/(R*temp))*((exp(v*frdy/(R*temp))*nai*nai*nai*cao-nao*nao*nao*cai)/(1+
        c2*exp((gammas-1)*v*frdy/(R*temp))*exp(v*frdy/(R*temp))*nai*nai*nai*cao+nao*nao*nao*cai));
}

void CMyocyte::comp_inak()
{
    sigma = (exp(nao/67.3)-1)/7;
    fnak = 1/(1+0.1245*exp((-0.1*v*frdy)/(R*temp))+0.0365*sigma*exp((-v*frdy)/(R*temp)));
    inak = ibarnak*fnak*(1/(1+pow(kmnai/nai,2)))*(ko/(ko+kmko));
}

void CMyocyte::comp_insca()
{
    ibarnsna =
        pnsca*zna*zna*((v*frdy*frdy)/(R*temp))*((ganai*nai*exp((zna*v*frdy)/(R*temp))-
        ganao*nao)/(exp((zna*v*frdy)/(R*temp))-1));
    ibarnsk =
        pnsca*zk*zk*((v*frdy*frdy)/(R*temp))*((gaki*ki*exp((zk*v*frdy)/(R*temp))-
        gako*k)/exp((zk*v*frdy)/(R*temp)))*1);;
    insna = ibarnsna/(1+pow(kmnsca/cai,3));
    insk = ibarnsk/(1+pow(kmnsca/cai,3));
}

void CMyocyte::comp_ipca()
{
    ipca = (ibarpca*cai)/(kmpca+cai);
}

void CMyocyte::comp_icab()
{
    gcab = 0.003016;
    ecan = ((R*temp)/(2*frdy))*log(cao/cai);
    icab = gcab*(v-ecan);
}

void CMyocyte::comp_inab()
{
    gnab = 0.004;
    enan = ena;
    inab = gnab*(v-enan);
}

// Functions that calculate intracellular ion concentrations begins here
// The units of dnai is in mM. Note that naiont should be multiplied by the
// cell capacitance to get the correct units. Since cell capacitance = 1 uF/cm^2,
// it doesn't explicitly appear in the equation below.
// This holds true for the calculation of dki and dcai.

void CMyocyte::conc_nai ()
{
  dnai = -dt*(naiont*acap)/(vmyo*zna*frdy);
  nai = dnai + nai;
}

void CMyocyte::conc_ki ()
{
  if(stimtime>=0 && stimtime<0.5){
    dki = -dt*((kiont+st)*acap)/(vmyo*zk*frdy);
  }else{
    dki = -dt*(kiont*acap)/(vmyo*zk*frdy);
  }
  ki = dki + ki;
}

// NSR Ca Ion Concentration Changes
//  kmup = 0.00092;    // Half-saturation concentration of iup (mM)
//  iupbar = 0.00875;  // Max. current through iup channel (mM/ms)
//  nsrbar = 15;       // Max. [Ca] in NSR (mM)
//  kleak;  Rate constant of Ca leakage from NSR (ms^-1)
//  nsr     NSR Ca Concentration (mM)
void CMyocyte::conc_nsr ()
{
  kleak = iupbar/nsrbar;
  ileak = kleak*nsr;
  iup = iupbar*cai/(cai+kmup);
  dnsr = dt*(iup-ileak-itr*vjsr/vnsr);
  nsr = nsr+dnsr;
}

//
void CMyocyte::conc_jsr ()
{
  dcaiontnew = (caiont-caiontold)/dt;

  if(v>-35 && dcaiontnew<dcaiont && triggerCICR){
    dvdtmax = 0;    //### added 10-Jan-2004
    triggerCICR = false;
    tcicr = 0;
  }

  // Release due to CICR
}
RyRopen = 1/(1+exp((-tcicr+4)/0.5));
RyRclose = (1-1/(1+exp((-tcicr+4)/0.5)));
grel = gmaxrel/(1+exp(((iloa+icab+ipca-2*inaca+icat)+5)/0.9));
irelcicr = grel * RyRopen * RyRclose * (jsr-cai);
tcicr = tcicr+dt;

// Release due to overload

greljsrol = grelbarjsrol*(1-exp(-tjsrol/tauon))*exp(-tjsrol/tauoff);
ireljsrol = greljsrol*(jsr-cai);
tjsrol = tjsrol+dt;

csqn = csqnbar*(jsr/(jsr+kmcsqn));
djsr = dt*(itr-irelcicr-ireljsrol);
bjsr = csqnbar-csqn-djsr-jsr+kmcsqn;
cjsr = kmcsqn*(csqn+djsr+jsr);
jsr = (sqrt(bjsr*bjsr+4*cjsr)-bjsr)/2;
}

void CMyocyte::calc_itr()
{
    itr = (nsr-jsr)/tautr;
}

void CMyocyte::conc_cai()
{
    dcai =
-dt*(((caiont*acap)/(vmyo*zca*frdy))+(iup-ileak)*vnsr/vmyo)-(irelcicr*vjsr/vmyo)-(ireljsrol*vjsr/vmyo));
    trpn = trpnbar*(cai/(cai+kmtrpn));
    cmdn = cmdnbar*(cai/(cai+kmcmdn));
    catotal = trpn+cmdn+dcai+cai;
    bmyo = cmdnbar+trpnbar-catotal+kmtrpn+kmcmdn;
    cmyo = (kmcmdn*kmtrpn)-(catotal*(kmtrpn+kmcmdn))+(trpnbar*kmcmdn)+(cmdnbar*kmtrpn);
    dmyo = -kmtrpn*kmcmdn*catotal;
    gpig = sqrt(bmyo*bmyo-3*cmyo);
    cai =
(2*gpig/3)*cos(acos((9*bmyo*cmyo-2*bmyo*bmyo*bmyo-27*dmyo)/(2*pow((bmyo*bmyo-3*cmyo),1.5))))/3
-(bmyo/3);
}

void CMyocyte::conc_cleft()
{
    dnao = dt*((nabm-nao)/taudiff+naiont*acap/(vcleft*frdy));
    nao = dnao+nao;
    dko = dt*((kbm-ko)/taudiff+kiont*acap/(vcleft*frdy));
    ko = dko+ko;
    dcao = dt*((cabm-cao)/taudiff+caiont*acap/(vcleft*frdy*2));
    cao = dcao+cao;
}
Source Files (MarkovHERGChannel.cpp)

//
//      C3  y[0]
//      C2  y[1]
//      C1  y[2]
//      O   y[3]
//      I   y[4]

#include "LRc.h"

CMarkovHERGChannel::CMarkovHERGChannel()
{
    nStates = NUM_OF_HERG_STATES;
    gherg = 0;
    for(ix=0;ix<nStates;ix++)pHERG[ix] = 0;
    pHERG[0] = 1;

    for(ix=0;ix<nStates;ix++){
        y[ix]=0;
        yn[ix]=0;
        k[ix]=0;
        dny[ix]=0;
        dny1[ix]=0;
    }
}

CMarkovHERGChannel::~CMarkovHERGChannel()
{
}

void CMarkovHERGChannel::getdy(double dt, double* dy)
{
    k[0] = -a*y[0] + b*y[1];
    k[1] = -(b + ain)*y[1] + a*y[0] + bin*y[2];

    for(int ix=0;ix<nStates;ix++) dy[ix]= k[ix]*dt;
}

void CMarkovHERGChannel::getRateConstants(double v)
{
    aa  = 65.5e-3 * exp(0.05547153*(v-36));  // C1->O, C1->I 65.5e-3 * exp(0.05547153*(v-36));
    ain = 2.172;  // C2->C1
\[
a = 55.5 \times 10^{-3} \times \exp(0.05547153 \times (v-12)); \quad \text{\(C3\to C2\)}
\]
\[
b = 2.357 \times 10^{-3} \times \exp(-0.036588 \times v); \quad \text{\(C2\to C3\)}
\]
\[
b = 1.077; \quad \text{\(C1\to C2\)}
\]
\[
b = 2.9357 \times 10^{-3} \times \exp(-0.02158 \times v); \quad \text{\(3^*0\to C1\)}
\]
\[
a = 0.439 \times \exp(-0.02352 \times (v+25)) \times 4.5/Kout; \quad \text{\(I\to O\)}
\]
\[
b = 0.656 \times \exp(0.000942 \times v) \times \text{pow}(4.5/Kout, 0.3); \quad \text{\(I\to C1\)}
\]
\[
\mu = (a \times b \times a)/(a \times b); \quad \text{\(I\to C1d\)}
\]

```c
}
```

double CMarkovHERGChannel::compCurrent(double t, double dt, double v, double ek, double Ko) // current function
{
    Kout = Ko;
    getRateConstants(v);
    i4=0;
    while(1){
        if(i4==0){
            for(ix=0;ix<nStates;ix++) y[ix] = pHERG[ix];
            //          memcpy(y,pHERG,sizeof(double)*nStates);
            getdy(dt, dny1);
            for(ix=0;ix<nStates;ix++) yn[ix] = y[ix] + dny1[ix];
        }else{
            for(ix=0;ix<nStates;ix++) y[ix] = yn[ix];
            //          memcpy(y,yn,sizeof(double)*nStates);
            getdy(dt, dny);
            for(ix=0;ix<nStates;ix++){
                dny[ix] = (dny[ix] + dny1[ix])/2;
                yn[ix] = pHERG[ix] + dny[ix];
                dny1[ix] = dny[ix];
            }
        }
        sum = 0;
        for(ix=0;ix<nStates-1;ix++) sum += yn[ix];
        yn[nStates-1] = 1-sum; // last element
        done = true;
        for(ix=0;ix<nStates;ix++){ // check difference
            if(fabs(yn[ix] - y[ix])>1e-5){
                done = false; // not done yet
                break;
            }
        }
        if(done)break; // really done
    }
    i4++;
    if(i4>=40){
        printf("? error in Markov Na at %g: i4 = %d, item=%d, \n", t, i4, ix);
    }
```
exit(0);
}
} // end of while

for (ix=0; ix<nStates; ix++) pHERG[ix] = yn[ix];
// memcpy(pHERG, yn, sizeof(double)*nStates);

gherg = 0.0135*pow(Kout, 0.59);

return gherg * pHERG[OPEN_STATE] * (v-ek);
Source Files (MarkovNaChannel.cpp)

//   Na channel Markov process model
//
//               IC3 - IC2 - IF - IM1 - IM2
//               |     |    /  |
//                C3 - C2 - C1 - 0
//
//    xd[0]    C3
//    xd[1]    C2
//    xd[2]    C1
//    xd[3]    0
//    xd[4]    IC3
//    xd[5]    IC2
//    xd[6]    IF
//    xd[7]    IM1
//    xd[8]    IM2
//
// Integration methods
//   modified from Colleen Clancy's method
//   http://www.cwru.edu/med/CBRTC/LRdOnline/markovina.htm
//

#include "LRc.h"

CMarkovNaChannel::CMarkovNaChannel()
{
    nStates = (bBurstMode)? NUM_OF_NA_STATES + NUM_OF_NA_BURST_STATES : NUM_OF_NA_STATES;

gna = 16;
for (ix=0; ix<nStates; ix++) pNa[ix] = 0;
pNa[0] = 1;
}

CMarkovNaChannel::~CMarkovNaChannel()
{
}

void CMarkovNaChannel::getdy(double dt, double* dy)
{
    if (bBurstMode)
    {
        k[0] = -(a11 + b3 + a6)*y[0] + b11*y[1] + a3*y[4] + b6*y[9];
    }
\[
  k[8] = -b5 * y[8] + a5 * y[7]; \\
  \]

//
\[
  \]

} else {
  \[
  k[0] = -(a11 + b3) * y[0] + b11 * y[1] + a3 * y[4]; \\
  k[8] = -b5 * y[8] + a5 * y[7]; \\
  \]
  }

for (int ix=0; ix<nStates; ix++) dy[ix] = k[ix]*dt;
}

void CMarkovNaChannel::getRateConstants(double v) {
  \[
  a11 = 3.802 / (0.1027 * \exp(-v/17.0) + 0.20 * \exp(-v/150)); \\
  a12 = 3.802 / (0.1027 * \exp(-v/15.0) + 0.23 * \exp(-v/150)); \\
  a13 = 3.802 / (0.1027 * \exp(-v/12.0) + 0.25 * \exp(-v/150)); \\
  b11 = 0.1917 * \exp(-v/20.3); \\
  b12 = 0.20 * \exp(-v-5)/20.3; \\
  b13 = 0.22 * \exp(-v-10)/20.3; \\
  a3 = (3.7933e-7) * \exp(-v/7.7); \quad // (3.7933e-7) * \exp(-v/7.7); \\
  b3 = 0.0084 + 0.00002 * v; \\
  a2 = 9.178 * \exp(v/29.68); \\
  b2 = (a13 * a2 * a3) / (b13 * b3); \\
  a4 = a2/100; \\
  b4 = a3; \\
  a5 = a2/(9.5e4); \quad // a2/(9.5e4); \\
  b5 = a3/50; // a3/50; \\
  \]
  if (bBurstMode) {
    a6 = 1000e-4;
  }
}
double CMarkovNaChannel::compCurrent(double t, double dt, double v, double ena)  // current function
{
    getRateConstants(v);
    i4=0;
    while(1){
        if(i4==0){
            for(ix=0;ix<nStates;ix++) y[ix] = pNa[ix];
            getdy(dt, dny1);
            for(ix=0;ix<nStates;ix++) yn[ix] = y[ix] + dny1[ix];
        }else{
            for(ix=0;ix<nStates;ix++) y[ix] = yn[ix];
            getdy(dt, dny);
            for(ix=0;ix<nStates;ix++){
                dny[ix] = (dny[ix] + dny1[ix])/2;
                yn[ix] = pNa[ix] + dny[ix];
                dny1[ix] = dny[ix];
            }
        }
        sum = 0;
        for(ix=0;ix<nStates-1;ix++) sum += yn[ix];
        yn[nStates-1] = 1-sum;  // last element
        done = true;
        for(ix=0;ix<nStates;ix++)  // check difference
            if(fabs(yn[ix] - y[ix])>1e-5){
                done = false;  // not done yet
                break;
            }
    }
    if(done)break;  // really done
    i4++;
    if (i4>=40){
        printf("%? error in Markov Na at %g: i4 = %d, item=%d, yn", t, i4, ix);
        exit(0);
    }
}  // end of while

for(ix=0;ix<nStates;ix++) pNa[ix] = yn[ix];

if(bBurstMode){
    return gna * (pNa[OPEN_STATE] + pNa[OPEN_STATE_B]) * (v-ena);
}else{
    return gna * (pNa[OPEN_STATE_B] + pNa[OPEN_STATE]) * (v-ena);
}
return gna * pNa[OPEN_STATE] * (v-ena);
Supplemental data 2.

Case 2 with M756V/KCNH2

A 63-year-old man was admitted because of syncope. He had no past history of syncope or family history of sudden cardiac death or LQTS. He had been taking pirmenol (300 mg per day) for several years for the treatment of paroximal supraventricular tachycardia. An ECG taken on admission showed repetitive TdP preceded by the prolongation of QTc interval (632 ms). Pirmenol intake was immediately stopped, and his TdP was successfully treated by an infusion of lidocaine and magnesium sulfate. Afterwards, he underwent catheter ablation for the accessory pathway (under the diagnosis of concealed Wolff-Parkinson-White [WPW] syndrome) and his QT interval shortened to 453 ms.

Case 4 with H492Y/KCNH2

A 52-year-old woman experienced syncope while taking disopyramide (300 mg/day) for the treatment of frequent premature ventricular contractions. She had no family history of premature sudden death or long QT syndrome (LQTS). Her ECG on admission showed a prolonged QTc interval (585 ms), followed by repetitive TdP. Serum potassium was low (3.2 mEq/ml). The combined infusion of magnesium sulfate and lidocaine failed to terminate TdP. However, TdP did not recur after she stopped taking disopyramide and started taking an oral potassium preparation. At that time, her QTc interval shortened to 447 ms. Treadmill exercise test, performed in the absence of disopyramide, induced neither the prolongation of QT interval nor ventricular arrhythmia.

Case 14 with R243H/KCNQ1

A 52-year-old woman was admitted to hospital because of repetitive syncope. She had been taking aprindine (60mg/day), mexiletine (400mg/day) and propranolol (30mg/day) for the treatment of ventricular arrhythmia. She experienced frequent episodes of TdP with prolonged QT interval (857 ms). However, the QT interval shortened to 469 ms after discontinuing aprindine. After a follow-up of 3 years there have been no further TdP attacks; only aprindine was permanently discontinued.

Case 15 with S706F/KCNH2

A 21-year-old woman was admitted for emergency care because of palpitations and dyspnea. She had no history of syncope. A previous ECG taken at age 15 years had shown normal QTc
interval (430 ms). There was no family history of sudden cardiac death or LQTS. Her symptoms appeared about 20 minutes after she took 10 combination tablets of amphetamine and methamphetamine. Her ECG showed repeated TdP with the prolongation of QTc interval (600 ms). She was treated firstly with an infusion of lidocaine (100 mg), followed by an infusion of magnesium sulfate (20mEq/day). However, TdP still occurred at a low heart rate (55 bpm), and temporary ventricular pacing was carried out to ensure a minimum heart rate of 70 bpm. The latter procedure suppressed her TdP. After washout of amphetamines, her QT interval shortened to 450 ms.

Case 16 with L1825P/SCN5A

This 70-year-old woman had TdP and the prolongation of QT interval to 731 ms after taking cisapride and pirmenol. This case had been reported in a previous manuscript.1

Case 17 with D342V/KCNH2

A 70-year-old woman was admitted to hospital because of syncope during lunch. She had been taking erythromycin (1200mg/day) for the treatment of acute bronchitis and bronchoectasis for 2 weeks, and had no history of syncope. Her sister had died suddenly at the age of 24. ECG recorded in the ambulance documented repetitive TdP and a prolonged QTc interval of 776 ms. Serum electrolytes were normal. TdP was terminated by an intravenous infusion of magnesium sulfate (80 mEq/day) with atrial pacing at a rate of 100 bpm. After stopping erythromycin, her QTc interval shortened to 440 ms.

Case 18 with R231C/KCNQ1

A 72-year-old woman was admitted to the hospital because of pre-syncope. TdP, with a prolonged QT interval of 754 ms, was apparent on her ECG. She had been taking probucol (250mg/day) for hypercholesterolemia. After discontinuing probucol, her QT interval did not normalize although it was shortened to 495 ms, and therefore the decision was taken to implant an implantable cardioverter defibrillator (ICD).

Case 20 with A614V/KCNH2

A 34-year-old woman experienced syncope, with unknown etiology, 3 days after starting hydroxyzine (3mg/day) for chronic urticaria; the QT interval was prolonged to 626 ms. There was no family history of LQTS or sudden cardiac death. She had syncope and the QT interval prolonged to 626 ms after 3 days. Three weeks after stopping hydroxyzine, the QT interval shortened to 449 ms. Detailed information on this case, including pharmacological characteristics
has been submitted in another paper.²

References


**Supplemental Table 1**  Clinical Characteristics of Simulated Congenital LQT5

<table>
<thead>
<tr>
<th>genes</th>
<th>mutations</th>
<th>symptomatic carriers/all carriers</th>
<th>mean age at onset (yrs)</th>
<th>mean QTc (ms)</th>
<th>references</th>
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<td>SCN5A</td>
<td>L619F</td>
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<td>0</td>
<td>538</td>
<td>Wehrens XH, et al. Hum Mutat 2003; 21: 552.</td>
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<td>SCN5A</td>
<td>A1330P</td>
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<td>0</td>
<td>600</td>
<td>Wedekind et al. Circulation 2001; 104: 1159–64.</td>
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