Simulation of Transmural Repolarization Time

Simulation of transmural ECGs was performed for each of the mutants using mutant basic electrophysiological characteristics as previously determined [8], producing the simulated transmural electrical potentials shown in Figure 1A and 1B. We refer to the change in simulated transmural repolarization time for mutant channels compared to wild-type as the transmural repolarization prolongation (TRP). Transmural repolarization was defined as the difference of wild type and mutant pseudo transmural QT measured at the end of the T wave. The maximum slope intercept method was used, defining the end of the T wave as the intercept between the isoelectric line with the tangent drawn through the maximum down slope of the T wave (Fig. 1C). TRP values associated with each mutant channel are shown in Fig 1A and 1D. For one of the mutant channels tested (D611Y), repolarization time was predicted to be shorter (-7 ms) than the one produced by the presence of the wild-type channel, while other mutations caused changes ranging from +4 ms (R591H) to +49 ms (G314S). The mean TRP for all mutations was 27.5 ms. Mutations with the largest effects on TRP (top quartile [>36 ms]) were present in the transmembrane domain of the channel, one in the S4-S5 cytoplasmic loop (V254M), one in the S5 membrane spanning domain (L266P) and two in the pore loop (T312I and G314S). Figure 1E shows a schematic drawing of the KCNQ1 channel protein with the location of the mutations in the study population. Mutations resulting in TRP in the top quartile are shown in red and others are shown in green.

Baseline Patient Characteristics by TRP

A simulated value of prolonged transmural repolarization (TRP upper quartile) among all mutations studied was present in 186 patients (29%). The mean QTc of these patients was 502 ± 53 ms, significantly prolonged when compared to patients with a simulated lower quartiles TRP (475 ± 49 ms; $p<0.001$) . Other baseline characteristics of patients with upper quartile TRP are shown in Table 1, demonstrating no significant difference in heart rate (RR interval), gender, or β-blocker usage among patients with a prolonged TRP. In addition, patients with upper quartile TRP had a higher frequency of cardiac events during follow-up including syncope, ACA, and SCD (Table 1).

Correlation between TRP and Measured QTc

To measure the correlation between TRP and individual patient QTc, we plot TRP for each patient against the patients' measured baseline QTc (Figure 1D). This plot illustrates the broad variation in QTc measurements from patients with the same mutation. Simple linear regression (shown as red solid line), demonstrates a weak association between QTc and TRP ($R=0.27$, $p<0.0001$). Although the values were correlated, there was a wide distribution of individual QTcs for each modeled mutation. The baseline QTc measurements variation amongst patients with the same IKs mutation also is consistent with the significant variation observed among multiple measurements taken from a single individual. [7]

TRP is an independent risk factor for cardiac events in LQT1

In a multivariate Cox regression model, mutant-specific prolongation of transmural repolarization was significantly associated with increased rate of cardiac events both as a continuous and as a dichotomized variable independent from other clinical variables and after adjustment for the patient's individual QTc (Table 2). For every additional 10 msec increment in simulated TRP there was a corresponding significant 35% ($p<0.001$) increase risk for the occurrence of cardiac events (Table 2A). Kaplan Meier survival analysis dividing TRP in four quartile subgroups show that the top quartile of TRP has an increased cumulative probability of cardiac events when compared to the three lower quartiles (Fig S4), indicating that probability of cardiac event is associated with a threshold level of TRP. For patients with mutations identified to have upper quartile with a simulated TRP experienced nearly a 3-fold ($p<0.001$) increased risk for cardiac events. Consistent with these findings, Kaplan Meier survival analysis (Fig 2A, middle panel) showed that the cumulative probability of cardiac events from birth through age 40 years was significantly higher among patients with upper quartile simulated TRP as compared with patients with lower TRP values.

Simulation of transmural ECG prolongation is an independent risk factor for cardiac events in LQT1 in patient with QTc<500ms.

In a secondary analysis, we evaluated risk factors for cardiac events among patients with only mild to moderate QTc prolongation (<500 ms) since in this patient subset individual QTc

provides less prognostic information. Among these patients, each additional 10 ms of simulated TRP was associated with a significant 36% ($p<0.001$) increased risk for cardiac events (Table 3B). Upper quartile TRP was associated with nearly a 3-fold increased risk [2.97 (95% CI 2.00-4.40]) after adjustment for the patient's individual QTc. Consistent with these findings, Kaplan-Meier curves including only patients with QTc<500 ms demonstrate early separation of event-free survival rates when patients were grouped into the upper vs. lower TRP quartiles (Figure 2B). When patients were stratified by both individual baseline QTc and TRP, Kaplan-Meier survival analysis showed that patients who had upper TRP experienced a significantly higher event rate throughout follow-up regardless of the patient's individual QTc (Figure 2C). Results from multivariate Cox proportional hazard regression analysis corresponding to the groups in Figure 2C are shown in Table 2S.

To test whether TRP simulation parameters further improved clinical risk stratification when compared to previously identified risk factor related to ion channel characteristics we run a secondary analysis adding slow rate of channel activation as a co-variate. Our previous work showed that that channels with slow rate of activation (over 20% slower than wild-type channels) are associated with increased risk for cardiac events [8]. Our results show that for both the population as a whole and for patients with QTc<500ms slower channel activation and TRP top quartile are independent risk factors (Table 3A and 3B). Additionally, because we found QTc≥480ms to be a very poor predictor of event risk in this population (HR=0.96, 95% CI 0.63-1.46, p=0.85), we performed a secondary analysis to evaluate the association of QTc≥450ms to event risk in this population. This lower threshold is a similarly poor predictor of risk (HR=1.09, 95% CI 0.71-1.67, p=0.71) (Table S3).

Simulation of transmural ECG prolongation is an independent risk factor for life threatening cardiac events (ACA/SCD) in LQT1.

In a multivariate Cox regression model, mutant-specific prolongation of transmural repolarization was also significantly associated with increased risk of live threatening cardiac events (defined as first occurrence of ACA or SCD). The risk for ACA/SCD associated with a prolonged TRP was consistent when this parameter was assessed as both a continuous measure and

dichotomized at the upper quartile (>36 ms), after adjustment for the patient's individual's QTc (Table 4). Thus, for every additional 10 ms of added simulated transmural repolarization time, there was a corresponding significant 37% increase in the risk of ACA/SCD (Table 4A). Furthermore, patients carrying a mutation with upper quartile TRP experienced >2-fold (p=0.002) increased risk of ACA/SCD after adjustment for the patient's individual QTc (Table 2B). This is also illustrated by Kaplan-Meier curves (Figure 3) with early separation of event free survival rates for the population of patients with upper quartile TRP.

DISCUSSION

In this study, we describe a method of simulating transmural myocardial repolarization based on wild-type and mutant channel characteristics determined in cellular electrophysiology. We show that this transmural repolarization parameter is an independent predictor for occurrence of cardiac events and life-threatening events in LQT1 patients. The risk associated with simulated prolonged transmural repolarization was shown to be independent of patient's baseline QTc. These results regarding mutation-specific risk are particularly important for the subpopulation with normal-to moderate QTc prolongation (QTc<500ms), where clinical risk factors provide less prognostic value.

Several experimental and computation models have been developed on the premise that transmural ECGs are a surrogate for the QT interval as measured by body surface ECGs. Transmural repolarization is thought to be particularly important in generating inhomogeneities of repolarization that lead to cardiac arrhythmias [21, 22]. IKs channels expression changes across the ventricular wall, contributing to transmural dispersion of repolarization [23-25]. This is consistent with our results that indicate a significant increase in cardiac risk associated with mutation-specific changes in IKs and transmural repolarization in LQT1. The use of TRP as an index for cardiac risk in other inherited and acquired LQT syndrome is promising, but needs further study.

We showed previously that a slow activation rate is an independent risk factor in LQT1. Our results indicate that prolonged TRP is independent from channel slow activation rate (Table 4). Notable is that all four mutant channels in the top quartile of TRP show also slow activation kinetics [8]. Nonetheless, TRP combines slower activation, changes in voltage dependence of activation and conductance of the channel in relevant cardiac cell types to provide an overall effect of the mutation regarding transmural repolarization.

The simulation model proposed here consists of electrically coupled cardiac cells with heterogeneous electrophysiological (EP) properties that produce waveforms that are similar to those measured experimentally [26-28]. The development of heterogeneous models depends both on accurately representing the action potential of the different cell populations and realistic coupling between the cells. At the single cell level, the exact difference in membrane currents and Ca^{2+} handling that produce differences across the ventricular wall is controversial and very likely both region and species dependent. Based on the three standard cell types (Epi-, Endo- and Mid-myocardial) in Flaim et al. [14] we continually varied parameters to interpolate differences in IKs, I_{to}, I_{NaL} and Ca^{2+}-handling in an attempt to match the experimentally measured transmural profiles [27]. The conductivity between cells is assumed to be fixed so that the conduction velocity is constant across the ventricular wall, as seen experimentally[26, 27]. This assumption of constant conductivity is consistent with the limited available experimental data that show resistance is uniform except for a layer with higher resistivity [28]. This layer, situated to roughly 30% depth, involves a systematic change in cellular orientation. The discontinuity in cellular orientation cannot be directly represented in the 192 equally spaced point models comprising our 1-D cable. However, future extensions to the model could incorporate this additional level of detail.

A recent study [29] used Markov models, action potential and transmural ECG simulations to infer the mechanism of arrhythmia generation associated with a LQT1 associated mutation present in one patient with normal QTc and a history of syncope (Q357R, not included in the present study). The predisposition to arrhythmia was demonstrated by the propensity to generate EADs when combined with IKr blockage and β-adrenergic drive, whereas the effect on

the simulated transmural repolarization prolongation alone (without IKr blockage and β-adrenergic drive) was mild (13ms). This TRP value is comparable to the ones associated with mutations in this study with TRP in the first quartile (Fig.1), a range associated with 35% risk of cardiac event by age 40 (Fig.S3). In contrast to the previous study, our studied focuses on how transmural repolarization prolongation relates to a clinical risk that may be revealed over the time frame of up to 40 years for a patient population. Because our study included a large number mutants, we chose a simpler Hodgkin-Huxley model of IKs that could be systematically constrained by our *in vitro* data. Although Markov models can potentially capture additional mechanistic details of ion channel function, identification of states and constraining parameters are difficult and complex with typical electrophysiological data [30].

Simulated repolarization prolongation weakly correlated with baseline QTc measurements in our study population, which supports physiologic relevance of the simulation method. However, QTc measurements can vary widely amongst patients with a particular mutation and over time for the same patient [7]. In addition, QTc has been shown to change during exercise [31], with age [32] and has been suggested to depend on patient emotional state[33]. The simulated transmural repolarization parameter described here may reflect an overall life time risk for patients with each specific mutation, a risk that may not be reflected in a single determination of patient's QTc.

Here we identify mutations associated with high risk of cardiac events in patients in LQT1. Four of these mutations, the ones included in the top quartile of repolarization dysfunction, were identified as of particular high risk: V254M, L266P, T312I and G314S. These are all highly conserved residues among voltage gated potassium channels suggesting an important physiological role [8, 34]. We recently showed that V254M has impaired β-adrenergic activation, which would contribute to an increase in risk for patients with this mutation at high adrenergic states; the other mutations have not been identified previously as of particular high risk.

Limitations: Although our findings regarding the use of simulated TRP in risk stratification are novel, these results are based on a single population study of 633 LQT1 patients, and

therefore need to be further validated in larger populations, comprising also patients with recently identified novel mutations in the KCNQ1 gene.

Conclusion

Identification of mutations conferring high risk of cardiac events can help guide treatment decisions by identifying those patients that will benefit most from therapies including pharmacologic agents (i.e. beta blockers) and implantable defibrillators. In particular, patients with moderate QTc prolongation (i.e. QTc <500 ms) are a challenge for clinicians since their risk of cardiac events remains significantly elevated compared to the general population, although markers of risk are relatively unknown [9]. We have shown that simulated TRP is a particularly strong marker of risk for cardiac events in this population which may translate into changes in treatment decision for identifying high-risk LQTS patients independently of traditional ECG markers. It would be recommended that patients with the identified prolonged TRP mutations (V254M, G314S, T312I and L266P) should be considered to be at a high risk for cardiac events even in the absence of QTc prologation or other clinical risk factors. This patient subset should be routinely treated beta-blocker therapy at the maximum tolerated dosage and carefully followed up for residual symptoms during medical therapy.

FIGURE CAPTIONS:

Figure 1: Transmural Repolarization Prolongation (TRP) determination for ion channels associated with Long QT type 1.

(A) Schematic diagram depicting the 192 cell array used in the computational simulation. Action potential determination for wild-type channels at the different cell types is shown. (B) Diagram showing determination of pseudo transmural ECG based on the wild-type channel action potential duration across the array of cells. (C) Pseudo-transmural ECG simulated for mutant channels in the study with lower (left) and higher

(right) prolongation observed. A unique TRP parameter was determined for each mutant ion channel as indicated. (D) Correlation of TRP determined from simulation with patient QTc for all patients that are genotype positive for each mutantion. (E) Location of mutations in the study. Mutations associated with the top quartile TRP are marked in red. Circle size indicates number of patients with the mutation.

Figure 2: Rate of cardiac event during follow up

Cumulative probability of the first cardiac event (syncope, aborted cardiac arrest or death) during follow up dichotomized by patients with the highest quartile TRP (Q4) and all other quartiles (Q13). (A) among all study patients. (B) among the subset of patients with QTc<500ms. (C) among all patients with combined assessment of TRP and QTc.

Figure 3: Rate of life threatening cardiac event during follow up

Cumulative probability of aborted cardiac arrest or sudden cardiac death during follow up among all study patients dichotomized by patients with the highest quartile TRP (Q4) and all other quartiles (Q13).

Table 1. Characteristics of the Study Population

Characteristic	TRP: upper quartile N=186	TRP: lower quartiles N=447	P Value
ECG Parameters			
QTc, msec (mean±SD)			
Overall	502 ± 53	475 ± 49	<0.001
QTc > 500 msec, %	49	26	<0.001
RR, msec (mean±SD)	804 ± 232	847 ± 200	0.04
LQTS-Therapies during Follow-Up			
Beta blockers, %	47	44	0.49
Pacemaker, %	1.1	2.2	0.49
ICD, %	6	8	0.39
Cardiac Events during Follow-Up			
Syncope, %	59	29	<0.001
ACA, %	7	2	0.007
SCD, %	18	7	<0.001
Appropriate ICD shocks, %	0	0.2	
ACA or SCD, %*	23	9	<0.001

Plus – minus values are means ± SD.
ACA = aborted cardiac arrest; ICD = implantable cardioverter defibrillator; LCSD = left cervical sympathetic denervation; SCD = sudden cardiac death; MS = membrane spanning domain
*Only the first event for each patient was considered.

Table 2 - Multivariate analysis: Risk factors for cardiac events (syncope, sudden death and aborted cardiac arrest) among II LQT1 patients.*

A. TRP assessed as a continuous measure

Parameter	*Hazard ratio*	*95% CI*	*p-value*
TRP per 10 ms	**1.35**	**1.18 – 1.56**	**<0.001**
QTc ≥ 500 ms vs. <500ms	1.78	1.34 - 2.38	<0.001
Male<13 years old	1.58	1.16 - 2.14	0.003
Female >14 years old	1.47	0.98 – 2.19	0.06
Time-dependent β-blocker	0.37	0.20 – 0.66	<0.001

B. TRP dichotomized at the upper quartile

Parameter	*Hazard ratio*	*95% CI*	*p-value*
TRP Q4 vs. Q1-3	**2.80**	**1.96 - 4.01**	**<0.001**
QTc ≥ 500 ms vs. <500ms	1.78	1.37 - 2.32	<0.001
Male<13 years old	1.60	1.19 - 2.15	0.002
Female >14 years old	1.38	0.91 - 2.09	0.13
Time dependent β-blocker	0.32	0.18 – 0.58	<0.001

*Multivariate analysis was carried out using Cox proportional hazards regression modelling; separate models were developed for parts A and B of the Table, adjusting for the 5 covariates in each part.

QTc = QT interval corrected for heart rate; TRP = simulated pseudo-transmural ECG prolongation

Table 3 - Multivariate analysis: Risk factors for cardiac events (syncope, sudden death and aborted cardiac arrest) among 369 LQT1 patients with QTc < 500 ms.*

A. TRP assessed as a continuous measure

Parameter	*Hazard ratio*	*95% CI*	*p-value*
TRP per 10 ms	**1.36**	**1.18 – 1.56**	**<0.001**
QTc ≥ 480 ms vs ≤480ms	0.99	0.67 – 1.46	0.97
Male < 13 years old	1.42	0.87 - 2.33	0.16
Female > 14 years old	1.86	0.96 – 3.60	0.06
Time-dependent β-blocker	0.48	0.23 – 1.02	0.06

B. TRP dichotomized at the upper quartile

Parameter	*Hazard ratio*	*95% CI*	*p-value*
TRP Q4 vs. Q1-3	**2.97**	**2.00 - 4.40**	**<0.001**
QTc ≥ 480 ms vs ≤480ms	0.96	0.63 – 1.46	0.85
Male<13 years old	1.38	0.86 – 2.21	0.19
Female >14 years old	1.90	0.98 – 3.67	0.06
Time-dependent β-blocker	0.45	0.22 – 0.58	0.04

Table 4 - Multivariate analysis: Risk factors for cardiac events (syncope, sudden death and aborted cardiac arrest).*

A. TRP dichotomized at the upper quartile for the whole population

Parameter	*Hazard ratio*	*95% CI*	*p-value*
TRP Q4 vs. Q1-3	**2.25**	**1.49 – 3.39**	**<0.001**
τ_{act} > 1.20	**1.42**	**1.00 – 2.03**	**0.05**
QTc ≥ 500 ms vs. <500ms	1.73	1.32 – 2.27	<0.001
Male<13 years old	1.60	1.17 - 2.18	0.003
Female >14 years old	1.39	0.981– 2.11	0.12
Time-dependent β-blocker	0.33	0.18 – 0.59	<0.001

B. TRP dichotomized at the upper quartile for patients with QTc<500ms

Parameter	*Hazard ratio*	*95% CI*	*p-value*
TRP Q4 vs. Q1-3	**2.11**	**1.23 – 3.61**	**<0.01**
τ_{act} > 1.20	**1.70**	**1.03 – 2.80**	**0.04**
QTc ≥ 480 ms vs. <480ms	0.94	0.62 – 1.43	0.78
Male<13 years old	1.39	0.84 - 2.28	0.19
Female >14 years old	1.86	0.95 – 3.65	0.07
Time dependent β-blocker	0.44	0.21 – 0.92	0.03

*Multivariate analysis was carried out using Cox proportional hazards regression modelling; separate models were developed for parts A and B of the Table, adjusting for the covariates in each part.

QTc = QT interval corrected for heart rate; TRP = simulated pseudo-transmural ECG prolongation

Table 5 - Multivariate analysis: Risk factors for SCD/ACA among all LQT1 patients. Channel prolongation of transmural repolarization was: **A**: used as a continuous variable; **B:** dichotomized at top quartile.

A.

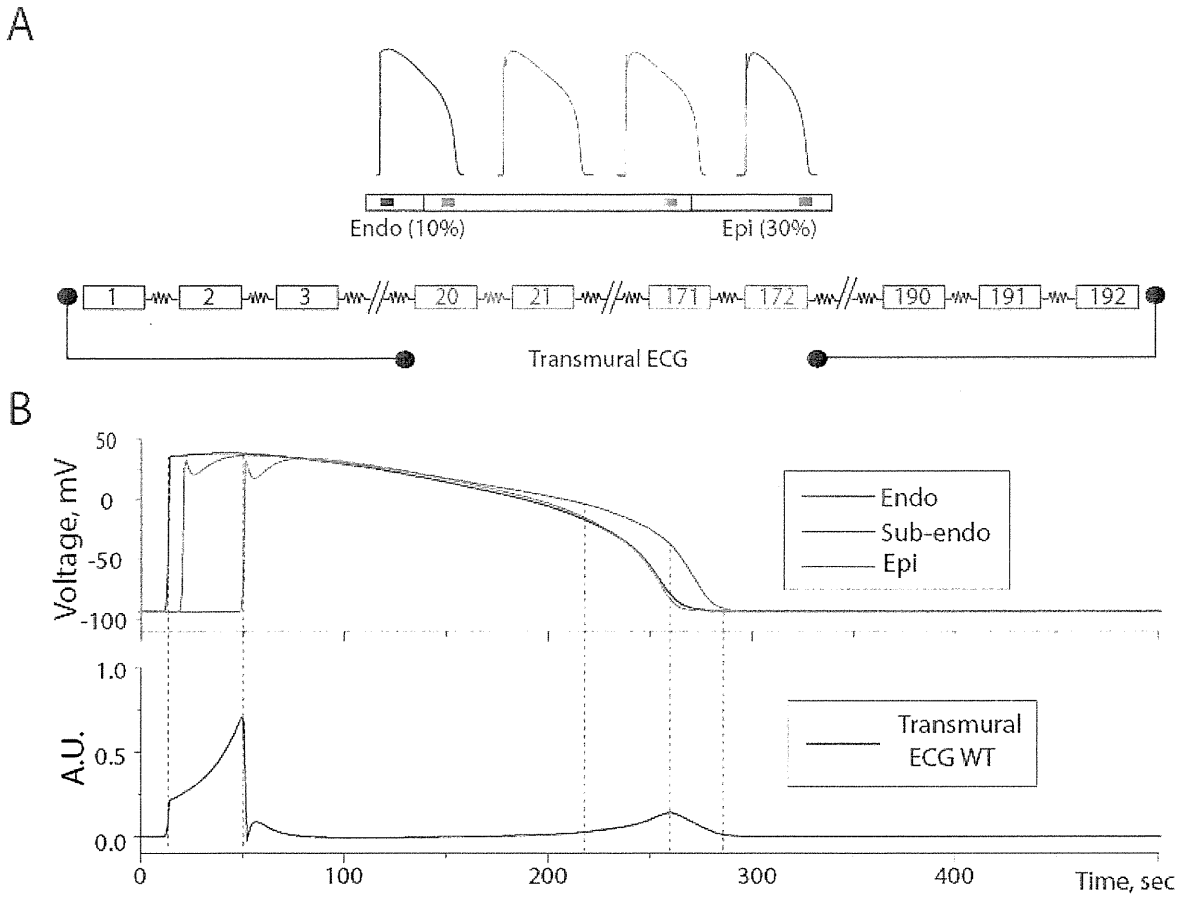
Parameter	*Hazard ratio*	*95% CI*	*p-value*
TRP: per 10 ms increment	**1.27**	**1.02 – 1.59**	**0.03**
QTc ≥ 500 ms	3.93	1.96 - 7.87	<0.001
Male<13 years old	2.16	1.22 – 3.84	0.008
Female >14 years old	1.19	0.63 – 2.26	0.59
Time dependent β-blocker	0.40	0.16 – 0.98	0.046

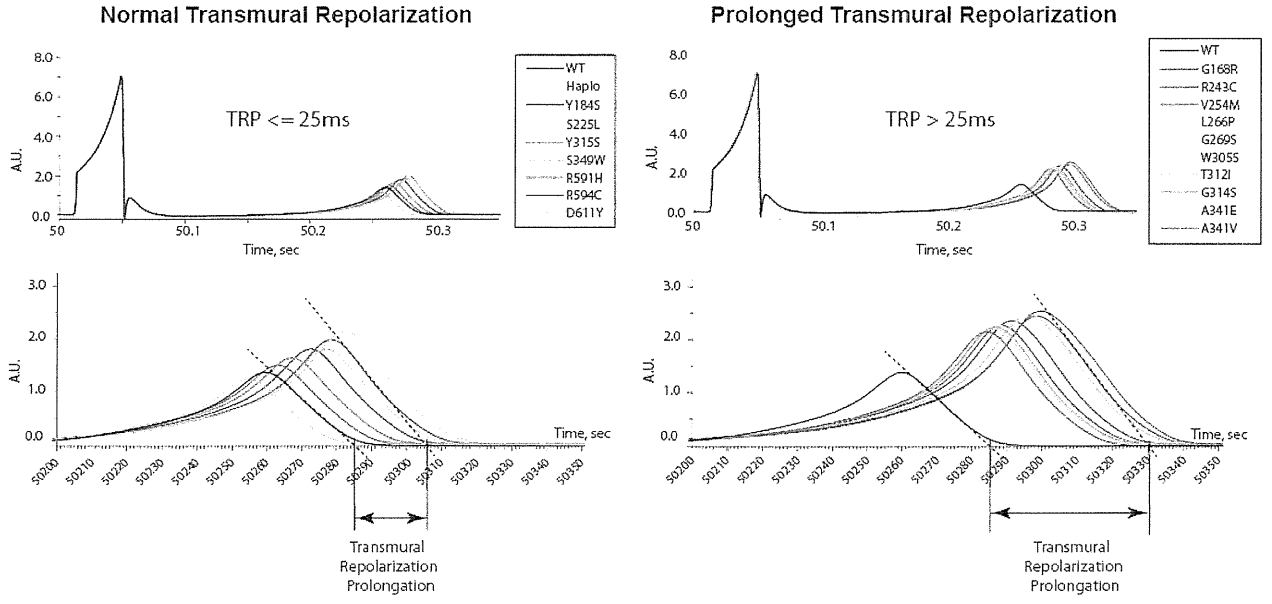
B.

Parameter	*Hazard ratio*	*95% CI*	*p-value*
TRP: Q4 vs. Q1-3	**2.24**	**1.96 - 4.01**	**0.002**
QTc ≥ 500 ms vs. <500 msec	3.95	1.37 - 2.32	<0.001
Male<13 years old	2.24	1.19 - 2.15	0.005
Female >14 years old	1.12	0.91 - 2.09	0.74
Time dependent β-blocker	0.38	0.15 – 0.94	0.04

*Multivariate analysis was carried out using Cox proportional hazards regression modelling; separate models were developed for parts A and B of the Table, adjusting for the 5 covariates in each part.

QTc = QT interval corrected for heart rate; TRP = simulated pseudo-transmural ECG prolongation


A
Endo (10%)
Epi (30%)
1
2
3
20
21
171
172
190
191
192
Transmural ECG
B
Voltage, mV
50
0
-50
-100
Endo
Sub-endo
Epi
A.U.
1.0
0.5
0.0
Transmural
ECG WT
0
100
200
300
400
Time, sec


C
Normal Transmural Repolarization
TRP <= 25ms
A.U.
Time, sec
WT
Haplo
Y184S
S225L
Y315S
S349W
R591H
R594C
D611Y
Transmural
Repolarization
Prolongation
Prolonged Transmural Repolarization
TRP > 25ms
A.U.
Time, sec
WT
G168R
R243C
V254M
L266P
G269S
W305S
T312I
G314S
A341E
A341V
Transmural
Repolarization
Prolongation

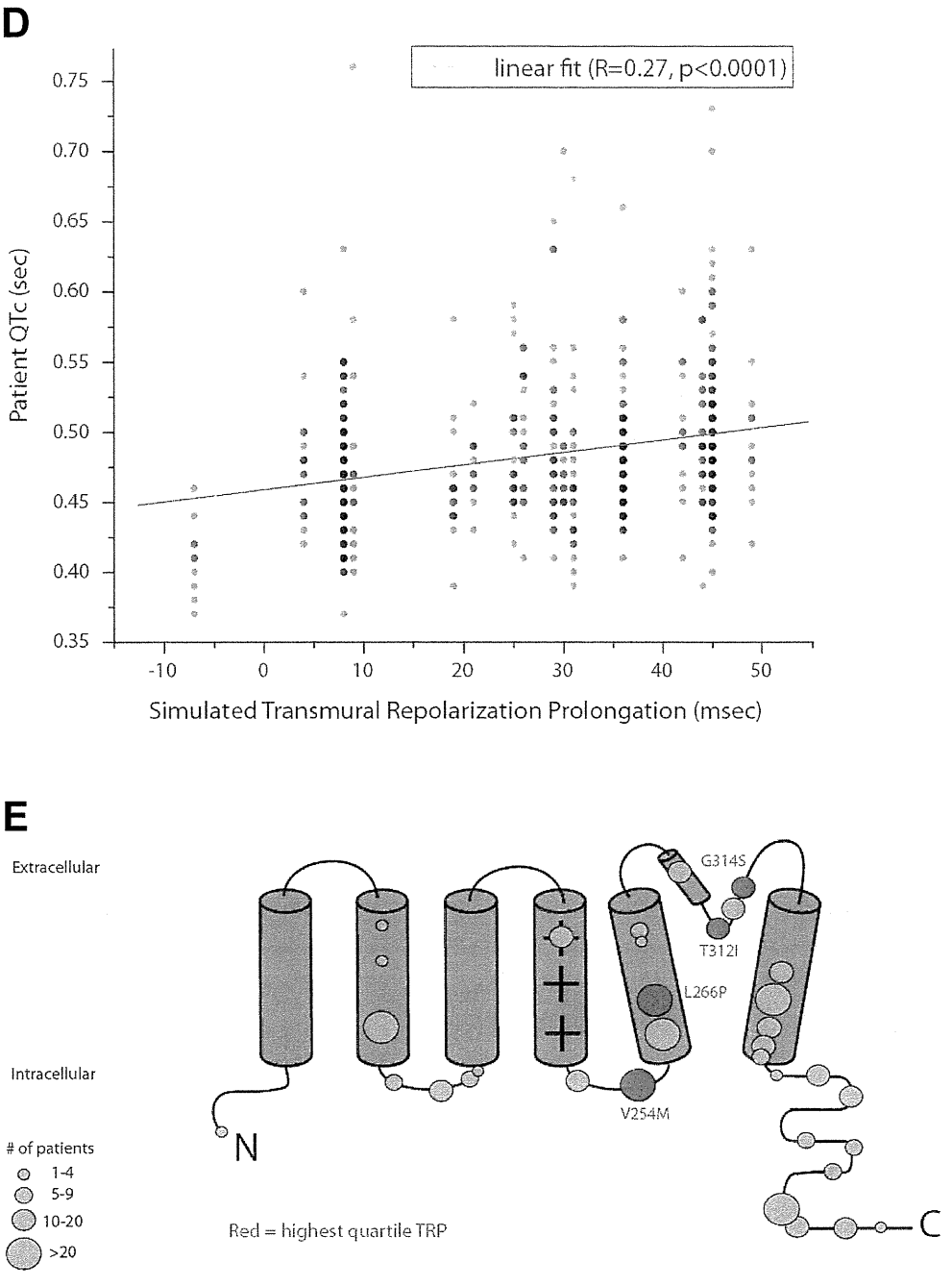
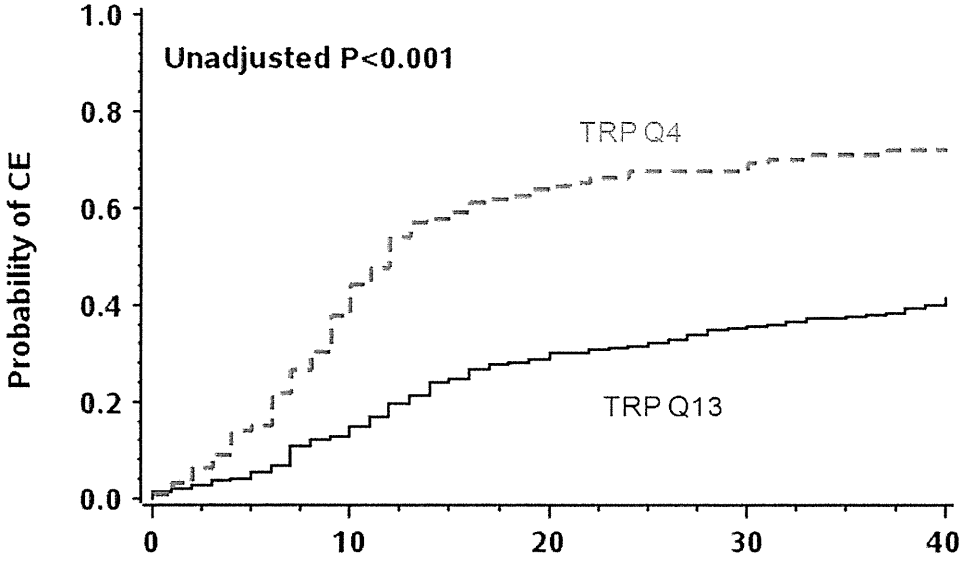


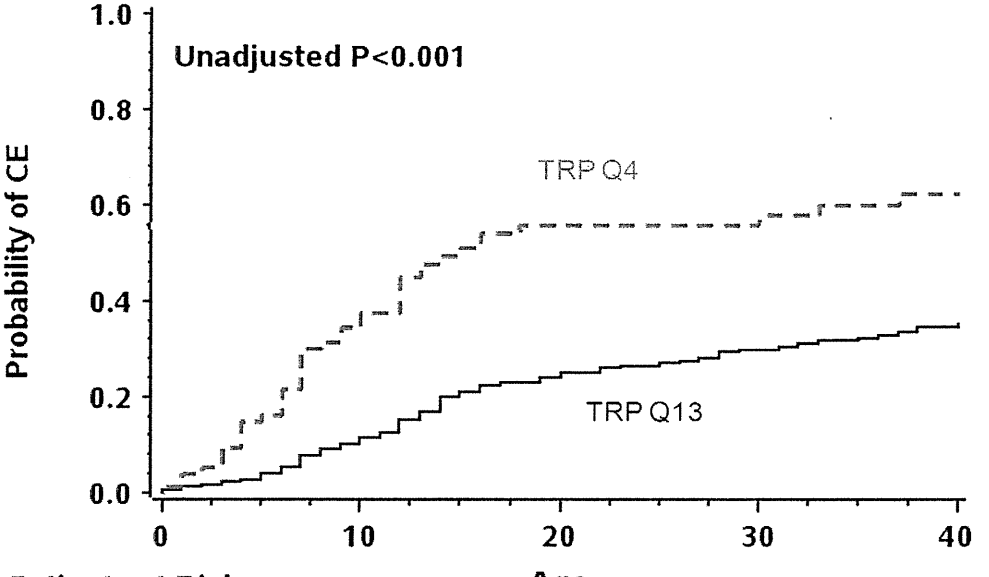
Figure 1: Transmural Repolarization Prolongation (TRP) determination for ion channels associated with Long QT type 1.
(A) Schematic diagram depicting the 192 cell array used in the computational simulation. Action potential determination for wild-type channels at the different cell types is shown. (B) Diagram showing determination of pseudo transmural ECG based on the wild-type channel action potential duration across the array of cells. (C) Pseudo-transmural ECG simulated for mutant channels in the study with lower (left) and higher (right) prolongation observed. A unique TRP parameter was determined for each mutant ion channel as indicated. (D) Correlation of TRP determined from simulation with patient QTc for all patients that are genotype positive for each mutation. (E) Location of mutations in the study. Mutations associated with the top quartile TRP are marked in red. Circle size indicates number of patients.

A



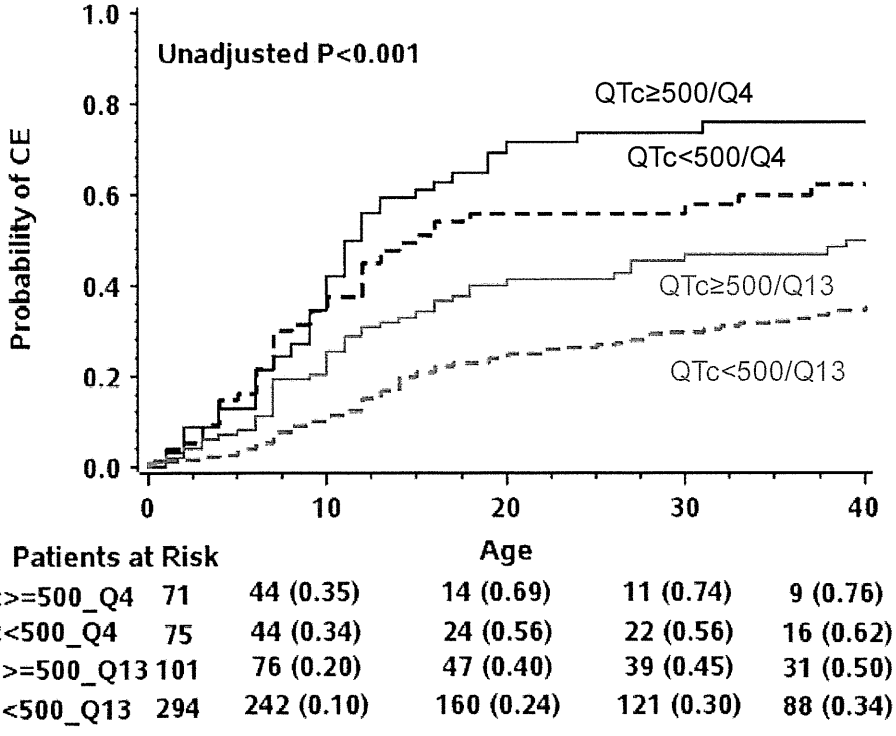
Patients at Risk					
TRP Q1-3	447	356 (0.13)	230 (0.29)	178 (0.35)	134 (0.40)
TRP Q4	186	107 (0.38)	48 (0.64)	39 (0.68)	28 (0.72)

B



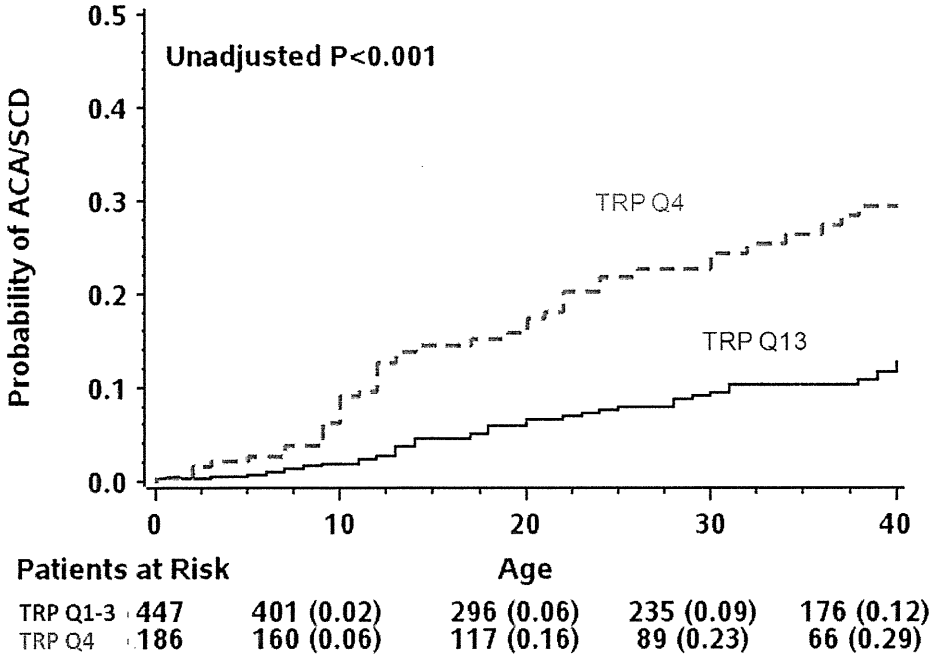
Patients at Risk					
TRP Q1-3	294	242 (0.10)	160 (0.24)	121 (0.30)	88 (0.34)
TRP Q4	75	44 (0.34)	24 (0.56)	22 (0.56)	16 (0.62)

C



Patients at Risk					
QTc>=500_Q4	71	44 (0.35)	14 (0.69)	11 (0.74)	9 (0.76)
QTc<500_Q4	75	44 (0.34)	24 (0.56)	22 (0.56)	16 (0.62)
QTc>=500_Q13	101	76 (0.20)	47 (0.40)	39 (0.45)	31 (0.50)
QTc<500_Q13	294	242 (0.10)	160 (0.24)	121 (0.30)	88 (0.34)

Figure 2: Rate of cardiac event during follow up
Cumulative probability of the first cardiac event (syncope, aborted cardiac arest or death) during follow up dichotomized by patients with the highest quartile TRP (Q4) and all other quartiles (Q13). (A) among all study patients. (B) among the subset of patients with QTc<500ms. (C) among all patients with combined assessment of TRP and QTc.



Patients at Risk					
TRP Q1-3	447	401 (0.02)	296 (0.06)	235 (0.09)	176 (0.12)
TRP Q4	186	160 (0.06)	117 (0.16)	89 (0.23)	66 (0.29)

Figure 3: Rate of life threatening cardiac event during follow up
Cumulative probability of aborted cardiac arrest or sudden cardiac death during follow up among all study patients dichotomized by patients with the highest quartile TRP (Q4) and all other quartiles (Q13).

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