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(54) Title: STRATIFICATION, THERAPIES, TARGETED TREATMENT AND PREVENTION OF LIFE-THREATENING VENTRICULAR TACHYIA

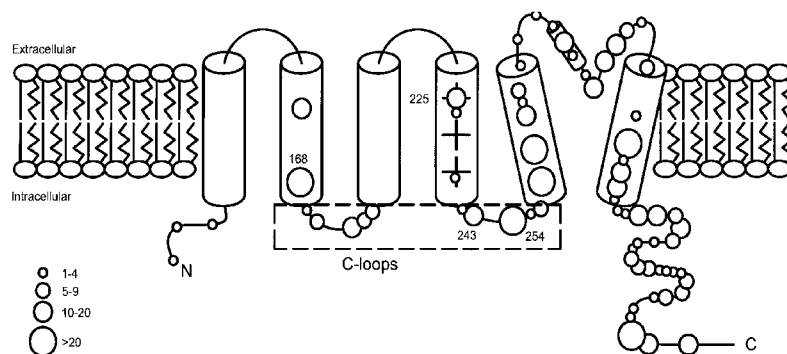


Fig. 1

(57) Abstract: Systems And Methods for risk stratification, therapies, targeted treatment and prevention of life-threatening ventricular tachyarrhythmias and sudden cardiac death for patients with congenital type-1 long QT syndrome (LQT1) is disclosed. Genetic testing to identify genetic mutations indicative of long QT syndrome is performed, with KCNQ1 channel C-loop missense mutations being separated from other mutations. The KCNQ1 channel C-loop missense mutations are an indicator of improved response to beta blocker therapy over other mutations responsible for genetic long QT syndrome. The use of such genetic testing data and targeted risk stratification is useful in determining the composition of a therapeutic agent, taking a therapeutic action, determining the optimal therapeutic efficacy of a drug for the treatment of long QT syndrome, determining a response to beta blocker therapy for patients with genetic long QT syndrome, and the like.



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**STRATIFICATION, THERAPIES, TARGETED TREATMENT AND
PREVENTION OF LIFE-THREATENING VENTRICULAR TACHYA**

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This application claims priority to United States Patent Application Serial No. 61/483,626 filed May 6, 2011 entitled "METHOD AND SYSTEM FOR RISK STRATIFICATION, THERAPIES, TARGETED TREATMENT AND PREVENTION OF LIFE-THREATENING VENTRICULAR TACHYARRHYTHMIAS AND SUDDEN CARDIAC DEATH FOR PATIENTS WITH CONGENITAL TYPE-1 LONG QT SYNDROME (LQTI) by Bar Sheshet, Goldenberg, Lopes, Moss, and Ouchi.

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STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with United States government support under HL-33843 and HL-51618

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awarded by National Institutes of Health. The government of the United States has certain rights in the invention.

TECHNICAL FIELD

This invention relates generally to systems and methods for risk stratification, therapies, targeted treatments and prevention of cardiac disorders, and more specifically to Systems And
5 Methods For Risk Stratification, Therapies, Targeted Treatment And Prevention of Life-Threatening Ventricular Tachyarrhythmias And Sudden Cardiac Death For Patients With Congenital Type-1 Long QT Syndrome (LQT1).

BACKGROUND ART

Congenital long-QT syndromes (LQTS) are inherited arrhythmogenic disorders that increase the risk for ventricular tachyarrhythmias and sudden cardiac death in young individuals without structural heart disease. LQTS type-1 (LQT1) is the most common genetic form of these disorders. Patients with LQT1 have increased risk for life-threatening ventricular tachyarrhythmias and sudden cardiac death during sympathetic activation, associated predominantly with exercise activity or swimming. Accordingly, current guidelines recommend non-specific treatment with beta-blocker therapy in LQTS patients. Despite this, however, there are still a considerable number of LQT1 patients who experience life-threatening ventricular tachyarrhythmias during beta-blocker therapy. Furthermore, despite multiple attempts for risk stratification studies in long QT syndrome, currently there is limited ability to identify LQTS patients who will not respond to beta-blocker therapy.

The Applicants have recently discovered that mutations in the cytoplasmic interconnecting loops (C-loops) of the potassium channel result in severe impairment of sympathetic regulation of heart cells. The Applicants have determined from both expression studies and translational clinical studies the following important findings: 1) patients with missense mutations located in the C-loops exhibit the highest risk for life threatening cardiac events (including aborted cardiac arrest and sudden cardiac death), independently of clinical and electrocardiographic variables; 2) beta-blocker therapy is associated with a pronounced reduction in the risk of life-threatening cardiac events among carriers of missense C-loop mutations, whereas the benefit of this mode of medical therapy is significantly attenuated in LQT1 patients with other mutations; 3) mutations in the C-loops specifically increase the risk of arrhythmias during exercise activity; 4) C-loop mutations can be used to distinguish between LQT1 women with a high or low risk of sudden death. These findings, as well as other disclosed herein, have important implications for improved risk stratification, identification of response to therapies, and novel management strategies for the prevention of sudden cardiac death in LQT1 patients and possibly also patients of a more general population.

It is thus an object of the present invention to provide a method for risk stratification for sudden cardiac death in LQT1 patients based on mutation function. It is another object of the present invention to provide improved therapies by identifying responders and non-responders to beta-blocker therapy. It is another object of the present invention to provide targeted therapies for long-QT syndrome that are mutation specific. It is yet another object of the present

invention to provide improved guidance for LQT1 patients with regards to physical activity and competitive sports. It is another object of the present invention to provide improved risk assessment and risk management for women with long-QT syndrome. It is another object of the present invention to provide identification and management of at risk individuals in the general population who are at increased risk of arrhythmic events. It is yet another object of the present invention to provide a method for formulating a composition of a therapeutic agent for treatment of genetic long QT syndrome. It is another object of the present invention to provide a system for identifying and reducing the risk of life-threatening ventricular tachyarrhythmias and sudden cardiac death in patients with congenital type-1 long QT syndrome (LQT1). It is another object of the present invention to provide a method of targeted risk stratification and treatment for use in defining a therapeutic action to be taken. It is another object of the present invention to provide a method of quantifying optimal therapeutic efficacy of a drug for treatment of congenital long QT syndrome. It is another object of the present invention to provide a method of measuring a response to beta blocker therapy in patients with long QT syndrome. These and other objects of the present invention are not to be considered comprehensive or exhaustive, but rather, exemplary of objects that may be ascertained after reading this specification and claims with the accompanying drawings.

DISCLOSURE OF THE INVENTION

In accordance with the present invention, there is provided Systems And Methods for identifying and reducing the risk of life-threatening ventricular tachyarrhythmias and sudden cardiac death for patients with congenital type-1 long QT Syndrome (LQT1) comprising an instrument for genetic testing of LQT1 syndrome and a treatment construct using data from genetic testing for therapy of LQT1 syndrome.

The foregoing paragraph has been provided by way of introduction, and is not intended to limit the scope of the invention as described in this specification, claims and the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be described by reference to the following drawings, in which like numerals refer to like elements, and in which:

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Figure 1 is a diagram depicting frequency and location of mutations in the KCNQ1 Potassium Channel from a study of 860 subjects;

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Figure 2 is a graph of Kaplan-Meier estimates of cumulative probability of a life threatening cardiac event by mutation location and type;

Figure 3 is a bar graph depicting risk of life threatening cardiac events by mutation location and beta blocker treatment;

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Figure 4 depicts regulation of LQT1 mutant channels by PKA;

Figure 5 is a diagram depicting risk stratification for ACA or SCD in LQTS patients;

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Figure 6 is a diagram depicting risk stratification for ACA or SCD in LQT1 patients according to the present invention;

Figure 7 is a diagram depicting methods of targeted treatment of the present invention;

Figure 8 is a diagram depicting data sources used for treatment constructs of the present invention;

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Figure 9 is a diagram depicting a method of determining LQT1 patient responders and non-responders to beta blocker therapy;

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Figure 10 is a diagram depicting a method of determining LQT1 patients at increased or lower risk of exercise triggered events; and

Figure 11 is a diagram depicting a method of determining female LQT1 patients at increased risk of sudden cardiac death.

The present invention will be described in connection with a preferred embodiment, however, it will be understood that there is no intent to limit the invention to the embodiment described. On the contrary, the intent is to cover all alternatives, modifications, and equivalents
5 as may be included within the spirit and scope of the invention as defined by this specification, claims and the attached drawings.

BEST MODE FOR CARRYING OUT THE INVENTION

Congenital long QT syndrome is characterized by way of an electrocardiograph (ECG) where there is a prolonged QT interval manifested by ventricular arrhythmias that may result in life-threatening cardiac events and sudden cardiac death. Current treatment for congenital long QT syndrome includes beta-blockers, implantable cardioverter-defibrillators (ICDs), and surgical left cervicothoracic sympathetic denervation (LCSD). While beta-blocker therapy is considered a first line of therapy, and some practitioners administer beta-blockers to all long QT syndrome patients, there is a subset of individuals with long QT syndrome genotypes where beta-blocker therapy is not effective and they remain symptomatic. There remains a need for additional long QT syndrome therapies that may require a more targeted approach to treatment.

Genetic diagnostic testing for long QT syndrome has begun to provide identification of congenital long QT syndrome, and may be used to identify individuals with congenital long QT syndrome that are not diagnosed with traditional methods. Through extensive research with LQT1 genotyped individuals with varying genetic mutations, a Systems And Methods For Risk Stratification, Therapies, Targeted Treatment And Prevention of Life-Threatening Ventricular Tachyarrhythmias And Sudden Cardiac Death For Patients With Congenital Type-1 Long QT Syndrome (LQT1) has been developed. Long QT syndrome type-1 can be identified by mutations in the KCNQ1 gene encoding. Identification of mutations in the cytoplasmic loops of the KCNQ1 channel have been identified and used for the methods and systems described herein.

Long QT syndrome type-1 (LQT1) is the most common type of hereditary long QT syndrome, making up about 30 to 35 percent of genotyped patients (Goldenberg I, Moss AJ. Long QT syndrome. *Journal of the American College of Cardiology*. 2008;51:2291-2300). LQT1 arises from a decrease in repolarizing potassium current due to mutations in the *KCNQ1* gene encoding the α subunit of the slowly activating potassium channel I_{Ks} . Exercise is the main trigger for cardiac arrhythmic events in patients with LQT1 (Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT, Towbin JA, Beggs AH, Brink P, Wilde AA, Toivonen L, Zareba W, Robinson JL, Timothy KW, Corfield V, Wattanasirichaigoon D, Corbett C, Haverkamp W, Schulze-Bahr E, Lehmann MH, Schwartz K, Coumel P, Bloise R. Genotype-phenotype correlation in the long-QT syndrome: Gene-specific triggers for life-threatening arrhythmias. *Circulation*. 2001;103:89-95). Activation of β 1-adrenergic receptors (β 1-AR) is the major signaling pathway contributing

to increase in heart rate and cardiac output during exercise. β 1-AR activation leads to activation of protein kinase A (PKA), which directly phosphorylates the *KCNQ1* subunit, increasing I_{Ks} function (Walsh KB, Kass RS. Regulation of a heart potassium channel by protein kinase A and C. *Science*. 1988;242:67-69. Also, Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, Kass RS. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science*. 2002;295:496-499)

The increase in I_{Ks} is thought to suppress the premature beats and afterdepolarization induced by increased Ca^{2+} currents during β -adrenergic stimulation (Shimizu W, Antzelevitch C. Differential effects of beta-adrenergic agonists and antagonists in LQT1, LQT2 and LQT3 models of the long QT syndrome. *Journal of the American College of Cardiology*. 2000;35:778-786). Accordingly, β -blockers are the treatment of choice for cardiac events in LQT1 patients. Data from several prior long QT syndrome (LQTS) studies (Goldenberg I, Bradley J, Moss A, McNitt S, Polonsky S, Robinson JL, Andrews M, Zareba W. Beta-blocker efficacy in high-risk patients with the congenital long-QT syndrome types 1 and 2: Implications for patient management. *J Cardiovasc Electrophysiol*. 2010;21:893-901. Also, Goldenberg I, Moss AJ. Long QT syndrome. *J Am Coll Cardiol*. 2008;51:2291-2300) demonstrate that despite the significant reduction in the risk of cardiac events and sudden death with β -blocker therapy among LQT1 patients, there is still a residual event rate among high-risk patients who are being treated with this mode of medical therapy, suggesting that β -blockers may be less effective in certain subgroups of LQT1 patients.

The primary translated protein (isoform 1) of *KCNQ1* consists of 676 amino acid residues with an intracellular N-terminus region, 6 membrane-spanning segments with two connecting cytoplasmic loops (C-loops) and an intracellular C-terminus region (Jespersen T, Grunnet M, Olesen SP. The KCNQ1 potassium channel: From gene to physiological function. *Physiology (Bethesda)*. 2005;20:408-416). Prior genotype-phenotype studies have provided important information regarding the effect of location and coding type of the channel mutations on the phenotypic manifestations and clinical course of LQT1 patients. These studies have shown that missense mutations and mutations located at the transmembrane region (including the C-loops) were associated with greater risk for cardiac events (Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL, Qi M, Vincent GM, Ackerman MJ, Kaufman ES, Hofman N, Seth R, Kamakura S, Miyamoto Y, Goldenberg I, Andrews ML, McNitt S. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of

mutations involving the *kcnq1* gene. *Circulation*. 2007;115:2481-2489). However, the mechanism related to the increased risk associated with transmembrane mutations has not been studied. C-loops in the transmembrane region were suggested to affect adrenergic channel regulation by PKA (Matavel A, Medei E, Lopes CM. PKA and PKC partially rescue long QT type 1 phenotype by restoring channel-PIP2 interactions. *Channels (Austin)*. 2010;4:3-11). We therefore hypothesized that LQT1 patients with C-loop missense mutations would exhibit a differential clinical risk and response to β -blocker therapy compared with carriers of other mutations in the *KCNQ1* channel. Accordingly, we conducted a new study that was carried out in a large cohort of subjects having a spectrum of *KCNQ1* mutations from the International LQTS Registry, and was designed to: 1) investigate the clinical outcomes among *KCNQ1* mutation carriers by further dividing the transmembrane region into membrane spanning and C-loop domains; 2) determine a possible differential response to β -blocker therapy depending on mutation location and function related to PKA regulation; and 3) relate the clinical data to functional studies of changes in I_{Ks} function and β -AR regulation in mammalian cells.

Methods

Study Population.

The study comprised 860 patients with genetically confirmed *KCNQ1* mutations derived from 170 proband-identified families. The proband in each family had QTc prolongation not due to a known secondary cause. The subjects were drawn from the Rochester (n = 637), the Netherlands (n = 94), the Japanese (n = 82), the Danish (n = 43), and the Swedish (n=4) portions of the Multicenter Mutation Registry. All subjects or their guardians provided informed consent for the genetic and clinical studies. Patients with congenital deafness or patients with multiple LQTS associated mutations were excluded from the study.

Phenotype characterization

Routine clinical and electrocardiographic parameters were acquired at the time of enrollment in each of the registries. Follow-up was censored at age 41 years to minimize the influence of coronary disease on cardiac events. Measured parameters on the first recorded ECG included QT and R-R intervals in milliseconds, with QT corrected for heart rate by Bazett's formula. Clinical data were collected on prospectively designed forms with information on demographic characteristics, personal and family medical history, electrocardiographic findings, therapy, and end points during long-term follow-up.

Genotype characterization

The KCNQ1 mutations were identified with the use of standard genetic tests performed in academic molecular-genetic laboratories. Genetic alterations of the amino acid sequence were characterized by location and by the specific type of mutation (missense, splice site, in-frame insertions/deletions, nonsense, stop codon, and frameshift).

We evaluated the risk associated with 4 main prespecified subgroups: 1) C- or N-terminus-missense; 2) membrane spanning-missense; 3) C-loops-missense; and 4) non-missense (i.e. splice sites, in-frame insertions, in-frame deletions, stop codons, and frameshift). The membrane spanning region of the KCNQ1-encoded channel was defined as the coding sequence involving amino acid residues between 120-170 (S1-S2), 196-241 (S3-S4), and 263-355 (S5-S6), with the C-loops region between residues 171-195 (S2-S3) and 242-262 (S4-S5), as depicted in Figure 1. The N-terminus region was defined before residue 120 and the C-terminus region after residue 355. Figure 1 is a diagram depicting frequency and location of mutations in the KCNQ1 Potassium Channel from a study of 860 subjects. In Figure 1, the α subunit involves the N-terminus (N), 6 membrane-spanning segments, 2 intracytoplasmic loops (S2-S3 and S4-S5) and the C-terminus portion (C). The size of the circles in Figure 1 reflect the number of subjects with mutations at the respective locations.

Cellular Expression Studies

In order to study the mechanism underlying the risk for cardiac events in patients with missense C-loop mutations as compared with other missense mutation located in different domains of the channel, we measured channel function and regulation for channels formed with two mutant subunits present in C-loops (V254M and R243C) and two mutant subunits present in the membrane spanning domain (G168R and S225L). The mutations chosen included the most common mutations in each domain in the LQT1 registry. Wild type-and mutant KCNQ1 subunits, together with KCNE1 subunits cDNA were transfected into HEK293T cells. Mutant KCNQ1 cDNA was transfected in combination with WT-KCNQ1 to mimic the heterozygous nature of the disease (WT-KCNQ1:mutant KCNQ1:KCNE1=0.5:0.5:1). We measured ion channel currents, in the presence and absence of Forskolin, a protein kinase A activator. Details of the molecular biology and electrophysiological methods are described further later in this specification.

End point

The primary end point of the study was the occurrence of a first life-threatening cardiac event, comprising aborted cardiac arrest (ACA) requiring external defibrillation as part of the resuscitation) or LQTS-related sudden cardiac death (SCD) abrupt in onset without evident cause, if witnessed, or death that was not explained by any other cause if it occurred in a non-witnessed setting). The consistency of the results among patients who received an implantable cardioverter defibrillator (ICD) during follow-up was evaluated in a secondary analysis that included the occurrence of a first appropriate ICD shock in the composite ACA or SCD end point.

Statistical analysis

Characteristics of the 4 subgroup of patients categorized by mutation location and type were compared with the one way ANOVA test or Chi square and Fisher exact tests, as appropriate. The probability of a first life-threatening cardiac event by the mutation-location and type subgroups was graphically displayed according to the method of Kaplan and Meier, with comparison of cumulative probability of events by the log-rank test. The Cox proportional-hazards survivorship model was used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of a life-threatening cardiac event from birth through age 40 years. The Cox regression models, stratified by decade of birth year and allowing for time-dependent covariates, were fitted to estimate the adjusted hazard ratio function of age (Hobbs JB, Peterson DR, Moss AJ, McNitt S, Zareba W, Goldenberg I, Qi M, Robinson JL, Sauer AJ, Ackerman MJ, Benhorin J, Kaufman ES, Locati EH, Napolitano C, Priori SG, Towbin JA, Vincent GM, Zhang L. Risk of aborted cardiac arrest or sudden cardiac death during adolescence in the long-QT syndrome. *Journal of the American Medical Association*. 2006;296:1249-1254). Therefore, to fulfill the assumption of proportional hazards for sex over the entire age range, a time-dependent covariate for sex (via an interaction with time) was incorporated, allowing for different hazard ratios by sex before and after age 13 years. Patients who did not have an ECG for QTc measurement (n=127) were identified in the Cox models as "QTc missing", and all Cox models were adjusted for this QTc missing parameter. The influence of time-dependent β -blocker therapy (the age at which β -blocker therapy was initiated) on outcome in the subgroups of patients with and without C-loop-missense mutations was determined by adding "time-dependent β -blocker "-by-"mutation category" interaction

term to the multivariate Cox model. Since almost all subjects were first- and second-degree relatives of probands, we adjusted for the effect of potential lack of independence between subjects using the robust sandwich estimator for family membership. We have carried out the following additional secondary analyses: 1) Including the biophysical function of the mutations (categorized as dominant-negative, haploinsufficiency, and unknown) (Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL, Qi M, Vincent GM, Ackerman MJ, Kaufman ES, Hofman N, Seth R, Kamakura S, Miyamoto Y, Goldenberg I, Andrews ML, McNitt S. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the *kcna1* gene. *Circulation*. 2007;115:2481-2489) as a covariate in the model, 2) excluding the large subgroup of patients with V254M mutations, and 3) including appropriate ICD shocks in the composite end point. The statistical software used for the analyses was SAS version 9.20 (SAS Institute Inc, Cary, NC). A 2-sided 0.05 significance level was used for hypothesis testing. Additional statistical methods regarding molecular biology and expression studies are provided herein.

Results

Study population

The spectrum of mutations as categorized by location and type and their respective number of carriers are presented in Table 1s below. The location and frequency of missense mutations is presented diagrammatically in Figure 1. Of the total 100 different *KCNQ1* mutations identified, 79 were missense mutations and 21 non-missense mutations. Missense mutations were further categorized according to their location: there were 29 different mutations in C-terminus or N-terminus regions (27 in C-terminus), 34 mutations in membrane spanning regions, and 16 mutations in the C-loop regions (8 in S4-S5 loop and 8 in S2-S3 loop).

Material and Methods

Molecular Biology

Human *KCNQ1* and *KCNE1* in pCDNA3.1(+) vector were gifts from Dr. Robert S. Kass (Columbia University, New York, NY). PCR based site direct mutagenesis was performed using PFU ultra DNA polymerase (Stratagene, La Jolla CA). Construct sequences were confirmed by DNA sequencing (Cornell University, Ithaca, NY and University of Rochester, Rochester, NY).

Electrophysiology

KCNQ1:KCNE1 DNA was expressed either at a ratio of 1:1 or 0.5:1 in order to mimic the haploinsufficient phenotype in HEK293T cells. Each mutant *KCNQ1* was expressed in combination with WT-*KCNQ1* to mimic heterozygous mutation (WT-*KCNQ1*:mutant *KCNQ1:KCNE1*=0.5:0.5:1). Each mutant current was compared to the current observed from haploinsufficient control channel (0.5 ng WT-*KCNQ1*: 1ng *KCNE1*) from the same passage cells. Cells were also co-transfected with 0.2 ng pEGFP-N1 (Clontech, La Jolla, CA) for positive identification of transfected cells by fluorescence. All the transfection was done by using Eugene HD transfection kit (Roche, Mannheim, Germany). Cells were re-plated on small glass cover slide (VWR, West Chester, PA) coated with 0.02 % gelatin 24 hours after transfection by using Accutase (Innovative Cell technologies, Inc., San Diego, CA) and use for experiment 48 hours after transfection.

Currents were activated by 2s depolarizing pulse to +20 mV every 10sec from a -80mV holding potential. These were followed by a step to -20mV. One-way ANOVA followed by Dunnett's Post Hoc test was applied for the assessment of statistical significance. All experiments were performed at room temperature.

Table 1s. Distribution of Mutation Location and Type in LQT1 patients

Missense						Nonmissense	
C/N terminal		Transmembrane		Cytoplasmic loops			
Codon	n, %	Codon	n, %	Codon	n, %	Type	n, %
All	173 (20)	All	376 (44)	All	125 (15)	All	186 (21)
R591H	19 (11)	G168R	67 (18)	V254M	62 (50)	A344A/sp	41 (22)
H363N	17 (10)	G269S	41 (11)	Y184S	18 (14)	Q530X	25 (13)
R366W	15 (9)	G269D	24 (6)	R243C	13 (10)	R518X	19 (10)
R594Q	14 (8)	L266P	24 (6)	A178P	5 (4)	K598K	17 (9)
D611Y	10 (6)	A341V	22 (6)	R259L	5 (4)	448 insG	11 (6)
K393N	10 (6)	G314S	19 (5)	G189R	4 (3)	delF340	9 (5)
S373P	8 (5)	A344V	18 (5)	R190Q	4 (3)	IVS +5 G>A	8 (4)
G568R	7 (4)	T312I	17 (5)	D242N	3 (2)	IVS7 +5 G>A	8 (4)
I567S	7 (4)	W305S	16 (4)	G189E	2 (2)	L191fs/90	8 (4)
R555C	7 (4)	S225L	15 (4)	R174H	2 (2)	P400fs/62	7 (4)
V524G	6 (3)	S349W	15 (4)	R259C	2 (2)	Y171X	7 (4)
R397W	5 (3)	E160K	12 (3)	A178T	1 (1)	S349X	6 (3)
R591C	5 (3)	A341E	10 (3)	D242Y	1 (1)	intron2,-2 G/A	4 (2)
S546L	5 (3)	L273F	10 (3)	R190W	1 (1)	S571fs/20	3 (2)
R562S	4 (2)	Y315C	10 (3)	R243S	1 (1)	639+5G>A	2 (1)
A590T	3 (2)	G325R	8 (2)	R258C	1 (1)	A150fs/133	2 (1)
D455Y	3 (2)	S277L	8 (2)			A636fs/28	2 (1)
I517T	3 (2)	D317G	5 (1)			IVS2 +1 G>A	2 (1)
K557E	3 (2)	L353P	4 (1)			IVS4 +5 G>A	2 (1)
M520R	3 (2)	Y281C	4 (1)			R195fs/40	2 (1)
Q357H	3 (2)	A226V	3 (1)			K422fsX10	1 (1)

R360G	3 (2)	A302V	3 (1)				
R583H	3 (2)	G292D	3 (1)				
S566F	3 (2)	W305C	3 (1)				
R562M	2 (1)	E284K	2 (1)				
W120C	2 (1)	F296S	2 (1)				
A525T	1 (1)	G306R	2 (1)				
G57V	1 (1)	T322M	2 (1)				
M1V	1 (1)	V310I	2 (1)				
		I274V	1 (0)				
		P320H	1 (0)				
		P343S	1 (0)				
		R237P	1 (0)				
		Y315S	1 (0)				

The clinical characteristics of patients in the 4 mutation location/type subgroups are presented in Table 1 below. Of the 860 study subjects, 20 percent had C/N terminal-missense mutations, 44 percent had membrane spanning-missense mutations, 15 percent had C-loop-missense mutations, and 22 percent had non-missense mutations. Patients with C-loop-missense mutations exhibited the longest QTc interval at enrollment, were treated with beta-blockers more frequently during follow-up, and had a significantly higher frequency of cardiac events of any type, including, syncope, ACA, and LQTS death, as compared with the other mutation subgroups.

Table 1. Demographic and clinical characteristics

Parameter	Missense			Non-missense	p-value
	C/N-Term	membrane spanning	C-loops		
Patients (n, %)	173 (20.1)	376 (43.7)	125 (14.5)	186 (21.6)	-
Female (n, %)	94 (54.3)	221 (58.8)	70 (56.0)	119 (64.0)	0.28
QTc at enrollment, msec	467±63	480±51	503±58	470±41	<0.001

(mean±SD)*					
QTc at enrollment ≥ 500 msec (n, %)	39/154 (25.3)	94/312 (30.1)	47/102 (46.1)	41/165 (24.8)	0.001
Therapy during follow-up					
β-blocker s (n, %)	64 (37.0)	167 (44.4)	63 (50.4)	74 (39.8)	0.09
Pacemaker (n, %)	2 (1.2)	7 (1.9)	1 (0.8)	4 (2.2)	0.75
Defibrillator (n, %)	6 (3.5)	25 (6.6)	10 (8.0)	13 (7.0)	0.36
Sympathectomy (n, %)	0 (0.0)	1 (0.3)	1 (0.8)	1 (0.5)	-
Cardiac event during follow-up					
Syncope (n, %)	52 (30.1)	122 (32.4)	69 (55.2)	46 (24.7)	<0.001
Aborted cardiac arrest (n, %)	6 (3.5)	10 (2.7)	8 (6.4)	3 (1.6)	0.11
Sudden cardiac death (n, %)	18 (10.4)	29 (7.7)	24 (19.2)	13 (7.0)	0.001
Any cardiac event (n, %)	63 (36.4)	144 (38.3)	84 (67.2)	55 (29.6)	<0.001

*127 patients did not have an ECG for QTc measurement.

5

Clinical outcome of patients according to mutation location and type

Figure 2 is a graph of Kaplan-Meier estimates of cumulative probability of a life threatening cardiac event by mutation location and type in 4 subgroups. There was a significantly higher event rate in the C-loop-missense subgroup as compared with the other 3 subgroups (p log rank < 0.001). Thus, at age 40 years the rate of life threatening cardiac events was 33 percent in patients with C-loop-missense mutations as compared with ≤ 16 percent in patients with other mutations, as illustrated in Figure 2. In Figure 2, ACA= aborted cardiac arrest. LQTS= long QT syndrome. The numbers in parentheses in Figure 2 reflect the cumulative event rate at that point in time.

15 The findings from the multivariate analysis for the end point of a first life threatening cardiac event are shown in Table 2 below. Notably, the adjusted hazard ratio for C-loop-missense vs. non-missense mutation was 2.75 (P=0.009), and there was no statistically significant difference in the risk among the other mutation location/type subgroups.

Table 2. Multivariate analysis: Risk factors for aborted cardiac arrest or sudden cardiac death

	Hazard ratio	95% CI	P Value
Gender/age			
Males age < 13 yrs	1.93	1.08-3.45	0.03
Females age 13 to 40 yrs	0.89	0.52-1.50	0.65
QTc $\geq$ 500 msec (vs. QTc<500)	3.55	1.83-6.89	<0.001
Mutation type and location			
Cytoplasmatic loops (missense) vs. non-missense	2.75	1.29-5.86	0.009
C/N Terminus (missense) vs. non-missense	1.47	0.64-3.39	0.37
Membrane spanning (missense) vs. non-missense	0.85	0.41-1.78	0.67

Secondary confirmatory analyses showed that patients with C-loop-missense mutations had an adjusted hazard ratio of 2.74 (95 percent confidence interval 1.68 to 4.46 [P<0.001]) for life threatening events as compared with patients with other mutations. The results were consistent when the biophysical function of the mutations was added as a covariate to the multivariate model or after excluding the large subgroup of patients with V254M mutations. The results were also consistent after inclusion of appropriate ICD shocks in the composite end point (adjusted hazard ratios for C-loop-missense mutations vs. non-missense mutations of 2.64 [95 percent confidence interval 1.64 to 4.23; P<0.001]).

β -blocker therapy

Multivariate analysis showed a significant differential effect of β -blocker therapy on the outcome of patients with C-loop-missense mutations as compared with those who had other mutations, as summarized in Table 3 below. β -blocker therapy was associated with a significant 88% reduction (P=0.02) in the risk of life threatening events among patients with C-loop-missense mutations, whereas the benefit of β -blocker therapy was significantly attenuated

among patients with other mutations in the *KCNQ1* channel (adjusted hazard ratio of 0.82 [P=0.68]; P-value for treatment-by-mutation-location/type interaction = 0.04). Figure 3 is a bar graph depicting risk of life threatening cardiac events by mutation location and beta blocker treatment. Consistent with those findings, the rate of Aborted Cardiac Arrest or Sudden Cardiac Death, as depicted in Figure 3, was lowest among patients with C-loop-missense mutations who were treated with Beta-blockers and highest among patients with C-loop-missense mutations who were not treated with beta-blockers (0.17 vs. 1.11 per 100 patient-years, respectively), whereas patients with other mutations in the *KCNQ1* channel exhibited intermediate and similar rates of life-threatening events with- and without beta-blocker therapy (0.36 and 0.38 per 100 patient-years, respectively). Event rates per 100 person-years were calculated by dividing the number of events during the period of β -blocker therapy or the absence of β -blocker therapy by person-years, and multiplying the results by 100. ACA= aborted cardiac death. LQTS= long QT syndrome.

Table 3. Multivariate analysis: response to β -blocker therapy

β -blocker vs. no β -blocker therapy	Hazard ratio	95% CI	P Value
All LQT1 patients	0.49	0.19-1.23	0.13
LQT1 patients with C-loop-missense mutations*	0.12	0.02-0.73	0.02
LQT1 patients with other mutations (non-[C-loop-missense] mutations*)	0.82	0.31-2.13	0.68

The models are adjusted for sex X age, corrected QT category (including missing QT), mutation type and location category, and time-dependent β -blocker treatment.

* p for interaction for mutation location-by- β blocker treatment =0.04

Cellular Expression Studies

In order to understand the mechanism underlying the increase in risk associated with C-loop mutations we measured channel basal function and regulation in four mutant channels associated with LQT1, two in the membrane spanning domains (G168R and S225L) and two located in C-loops (V254M and R243C). The mutations chosen included the most common mutation in each domain in the LQT1 registry (See Table 1s). Figure 4 depicts regulation of LQT1 mutant channels by PKA. Channel current was strongly decreased for all four mutations studied when compared to wild type subunits (Figure 4A and B). In addition, because activation by PKA is thought to be particularly important for I_{Ks} function and to underlie arrhythmogenesis in LQT1 (Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, Kass RS. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science*. 2002;295:496-499. Also, Terrenoire C, Clancy CE, Cormier JW, Sampson KJ, Kass RS. Autonomic control of cardiac action potentials: Role of potassium channel kinetics in response to sympathetic stimulation. *Circ Res*. 2005;96:e25-34. Also, Potet F, Scott JD, Mohammad-Panah R, Escande D, Baro I. AKAP proteins anchor cAMP-dependent protein kinase to KvLQT1/IsK channel complex. *Am J Physiol Heart Circ Physiol*. 2001;280:H2038-2045), we measured the effect of the PKA activator Forskolin (Figure 4 A, C and D). Both C-loop mutations showed dramatically impaired response to Forskolin, whereas the mutations located in the membrane spanning domain showed a strong activation by Forskolin, as did the wild type KCNQ1 channel.

In Figure 4, G168R and S225L are missense mutations located in the membrane spanning region; R243C and V254M are missense mutations located in the cytoplasmic loops region. **A:** Typical ion channel current measured before and after 10 min application of the PKA activator Forskolin (10 μ M) for left: wild-type (WT) and right: WT and mutant subunits co-expressed at a 1:1 ratio. Currents were activated by 2sec depolarizing steps to +20 mV from a -80mV holding potential. These were followed by a step to -20mV. **B:** Average current measured for cells expressing WT and mutant subunits measured at +40 mV after 2sec depolarization. **C:** Typical time course of current regulation measured at +20 mV after 2sec depolarization for channels formed by either WT or mutant co-expressed with WT subunits, as indicated. Current was normalized to current in the absence of Forskolin application. **D:** Summary data for experiments done as in C. KCNE1 was co-expressed with the KCNQ1 subunits at a molar ratio 1:1.

The present analysis among 860 LQT1 patients with a wide range of mutations in the *KCNQ1* channel provides several important implications regarding risk assessment and management in this population: (1) patients with missense mutations located in the C-loops exhibit the highest risk for life threatening cardiac events, independently of clinical and electrocardiographic variables; (2) Beta-blocker therapy is associated with a pronounced reduction in the risk of ACA or SCD among carriers of missense mutations in the C-loop, whereas the benefit of this mode of medical therapy is significantly attenuated in LQT1 patients with other mutations; (3) expression studies of C-loop mutations suggest that an impaired regulation by PKA in combination with basal decrease in channel function is the mechanism underlying the increased risk for cardiac events, and may explain the pronounced response to medical therapy with Beta-blockers among patients with C-loop mutation carriers.

We have recently shown that patients with mutations located in the transmembrane region of the channel have a significantly higher rate of cardiac events than those with mutations located in the C-terminus (Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL, Qi M, Vincent GM, Ackerman MJ, Kaufman ES, Hofman N, Seth R, Kamakura S, Miyamoto Y, Goldenberg I, Andrews ML, McNitt S. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the *kenq1* gene. *Circulation*. 2007;115:2481-2489). In addition, mutations in the transmembrane domain were suggested to be associated with greater prolongation in the QTc during exercise (Shimizu W, Horie M, Ohno S, Takenaka K, Yamaguchi M, Shimizu M, Washizuka T, Aizawa Y, Nakamura K, Ohe T, Aiba T, Miyamoto Y, Yoshimasa Y, Towbin JA, Priori SG, Kamakura S. Mutation site-specific differences in arrhythmic risk and sensitivity to sympathetic stimulation in the LQT1 form of congenital long QT syndrome: Multicenter study in Japan. *J Am Coll Cardiol*. 2004;44:117-125). However, the mechanisms related to those effects have not been studied. Our study extends these observations and shows that mutations in part of the transmembrane domain, the C-loops, are associated with a longer QTc on the ECG, and with a significant 2.7-fold increased risk of life threatening events as compared with other mutations in the *KCNQ1* channel (including the membrane-spanning [non-C-loop] mutations).

The S2-S3 and S4-S5 C-loops have been previously suggested to have an important functional role in modifying the function of voltage gated potassium channels (Isacoff EY, Jan YN, Jan LY. Putative receptor for the cytoplasmic inactivation gate in the shaker K⁺ channel. *Nature*. 1991;353:86-90). In particular for I_{Ks}, the S4-S5 loop has been suggested to mediate a functional interaction with the auxiliary *KCNE1* subunits (Franqueza L, Lin M, Shen J,

Splawski I, Keating MT, Sanguinetti MC. Long QT syndrome-associated mutations in the S4-S5 linker of KvLQT1 potassium channels modify gating and interaction with mink subunits. *J Biol Chem.* 1999;274:21063-21070). Most recently, LQT1 mutations in C-loops, when expressed in the absence of wild-type subunits, were suggested to affect adrenergic channel regulation (Matavel A, Medei E, Lopes CM. PKA and PKC partially rescue long QT type 1 phenotype by restoring channel-PIP2 interactions. *Channels (Austin).* 2010;4:3-11). Our results showed that even when expressed in the presence of wild-type subunits, C-loop mutations can dramatically affect channel regulation. It is conceivable that a decrease in channel regulation, as observed for the C-loop mutations, will lead to an increase in the burden of the mutation during adrenergic stimulus. The increase in cardiac risk associated with C-loop mutations is independent of traditional clinical variables; this can be explained by a blunted PKA-mediated activation because QTc is generally measured at rest. Thus, our results suggest that exercise may exacerbate the QTc prolongation for C-loop domain mutants.

Current guidelines recommend empiric therapy with β -blockers in all LQTS patients (Zipes DP, Camm AJ, Borggrefe M, Buxton AE, Chaitman B, Fromer M, Gregoratos G, Klein G, Moss AJ, Myerburg RJ, Priori SG, Quinones MA, Roden DM, Silka MJ, Tracy C, Smith SC, Jr., Jacobs AK, Adams CD, Antman EM, Anderson JL, Hunt SA, Halperin JL, Nishimura R, Ornato JP, Page RL, Riegel B, Blanc JJ, Budaj A, Dean V, Deckers JW, Despres C, Dickstein K, Lekakis J, McGregor K, Metra M, Morais J, Osterspey A, Tamargo JL, Zamorano JL. ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: A report of the american college of cardiology/american heart association task force and the european society of cardiology committee for practice guidelines (writing committee to develop guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death): Developed in collaboration with the european heart rhythm association and the heart rhythm society. *Circulation.* 2006;114:e385-484). The present study shows for the first time, a mutation-specific response to beta-blocker therapy in type-1 long QT syndrome, demonstrating that beta-blockers were associated with a significant greater reduction in the risk of life-threatening cardiac events among patients with mutations located in the C-loops as compared with all other mutations. It is conceivable that during β -adrenergic stimulation patients with mutations located in the C-loops have an unopposed increase in inward Ca^{2+} currents and prolongation of repolarization due to blunted PKA-mediated activation of I_{Kr} .⁵ β -blockers may decrease these unopposed inward Ca^{2+} currents, shorten repolarization and reduce the risk for ventricular

arrhythmias (Huffaker R, Lamp ST, Weiss JN, Kogan B. Intracellular calcium cycling, early afterdepolarizations, and reentry in simulated long QT syndrome. *Heart Rhythm*. 2004;1:441-448), whereas patients with other mutations do not exhibit such an effect.

5 Study limitations

The International LQTS Registry records therapies that are prescribed at the discretion of the treating physicians to enrolled subjects, and therefore β -blocker administration was not randomized. We have therefore carried out multivariate analyses that used beta-blocker therapy as a time-dependent covariate, and have further evaluated the benefit of medical therapy with
10 beta-blockers within higher- and lower- risk subsets of LQT1 patients. Our results from the time-dependent adjusted models suggest that the benefit of medical therapy is attenuated among lower-risk patients with non-C-loop-missense mutations; however, these lower-risk patients should still be treated with beta-blocker therapy according to guidelines (Zipes DP, Camm AJ, Borggrefe M, Buxton AE, Chaitman B, Fromer M, Gregoratos G, Klein G, Moss AJ, Myerburg
15 RJ, Priori SG, Quinones MA, Roden DM, Silka MJ, Tracy C, Smith SC, Jr., Jacobs AK, Adams CD, Antman EM, Anderson JL, Hunt SA, Halperin JL, Nishimura R, Ornato JP, Page RL, Riegel B, Blanc JJ, Budaj A, Dean V, Deckers JW, Despres C, Dickstein K, Lekakis J, McGregor K, Metra M, Morais J, Osterspey A, Tamargo JL, Zamorano JL. ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: A report of the american college of cardiology/american heart association task force and the european society of cardiology committee for practice guidelines (writing committee to develop guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death): Developed in collaboration with the european heart rhythm association and the heart rhythm society. *Circulation*. 2006;114:e385-484), since the
20 cumulative probability of ACA or SCD from birth through age 40 years among patients with non-C-loop-missense mutations was still considerable (between 11 and 16 percent).

We used a combination of clinical analysis and cellular electrophysiology experiments to investigate the molecular determinants and mechanism underlying the clinical outcomes of a large cohort of subjects having a spectrum of *KCNQ1* mutations categorized by their code type
30 and location. Patients with *KCNQ1* missense mutations located in the C-loop domains had a significantly greater risk for life threatening cardiac events and gained greater benefit when treated with β -blockers as compared with patients having other *KCNQ1* missense or non-missense mutations independently of clinical risk factors. We suggest that a combination of

decrease in basal function and altered adrenergic regulation of the I_{Ks} channel underlies the increased cardiac risk in this subgroup of patients. Mutation-specific information should be assessed and incorporated into the risk assessment and the management of LQT1 patients. Our results highlight the importance of understanding the molecular determinants and mechanisms
5 underlying arrhythmogenesis to identify cardiac risk factors for LQT1 patients, and have implications for risk stratification and treatment in LQT1 patients.

Genetic testing, as described herein, includes all forms of testing used to test for and detect genetic disorders. Many forms of genetic testing involve examination of the DNA molecule itself, or may involve biochemical tests for enzymes, chromosomes, proteins, or other
10 substances. Genetic testing includes techniques to examine genes or markers near the genes. Genetic testing is often times performed on a sample collected from a patient, such as a sample of blood, hair, skin, or other tissue. One example of such collection is that of a buccal smear, where a sample of cells is collected from the inside surface of a patient's cheek by way of a swab or brush.

The ability to identify specific mutations that are not only indicative of patients that have LQT1 syndrome, but also contain additional, heretofore unknown information related to LQT1 syndrome provides the scientific basis for new and useful methods of treatment, devices for improved diagnosis and treatment, devices and methods for improved risk stratification, methods and devices for targeted therapies, and the use and development of therapeutic drugs
20 and drug delivery systems. Various novel uses of the scientific information produced by the processes, techniques and equipment described herein has profound utility in targeted treatment, therapies, risk stratification, and prevention of life-threatening ventricular tachyarrhythmias and sudden cardiac death for patients with congenital type-1 long QT syndrome (LQT1).

For example, Figure 5 is a diagram depicting risk stratification for Aborted Cardiac Arrest or Sudden Cardiac Death in LQTS patients without use of the present invention and its various embodiments. Various risk factors are considered in the risk stratification diagram of Figure 5. For example, age, gender, prior syncope, frequency of prior syncope, number of syncope events in a specified time period, QTc variables, and the like. With the use of the
30 present invention and its various embodiments described and envisioned herein, risk stratification is genotype-specific. Figure 6 is a diagram depicting risk stratification for Aborted Cardiac Arrest or Sudden Cardiac Death in LQT1 patients according to the present invention. Mutation specific risk factors are considered in the risk stratification diagram of

Figure 6. Current risk stratification in LQTS patients relies mostly on non-specific clinical parameters, including the duration of the corrected QT interval (QTc) and a history of symptoms, whereas genetic/functional information is usually not incorporated into risk assessment. A prior study in 2007 showed that mutations located in the transmembrane region of the potassium channel are associated with increased risk of cardiac events in LQT1 patients. This study, however, did not assess specifically the risk of sudden cardiac death and did not identify functional mechanisms related to mutation-specific risk. Our new data show that only missense mutations within the transmembrane region increase the risk of sudden death while other transmembrane mutations are associated with a significantly lower risk. Thus, while the rate of aborted cardiac arrest or sudden cardiac death from birth through age 40 years among LQT1 patients with missense C-loops mutations is very high -- 33%, those who have other mutations in the transmembrane region have a significantly lower rate of only 13%. Therefore, various methods of targeted risk stratification and treatment for use in defining a therapeutic action to be taken can be envisioned. The methods may be manual, or they may, in some embodiments of the present invention, be performed on a computer or a computing device or instrument. Examples of steps to be taken may include, for example, performing genetic testing on patients suspected of having long QT syndrome to identify genetic mutations indicative of long QT syndrome and processing the results of the genetic testing on a computer, identifying by way of genetic testing mutations in KCNQ1 channel C-loops and processing the results of the genetic testing on a computer, evaluating electrocardiogram patient information on a computer, evaluating patient clinical history on a computer; and defining a therapeutic action to be taken. Therapeutic actions may include, but are not limited to, use of a beta blocker, use of an implantable cardioverter-defibrillator, alteration of the operation of an implantable cardioverter-defibrillator (such as, for example, by programming), use of surgical left cervicothoracic sympathetic denervation, or use of pharmacological agents, for example, IKs openers, PKC inhibitors, peptides based on the sequence of the c-loop region, c-loop interacting partners that may restore normal channel regulation, drugs that mimic c-loop peptide action, drugs that bind to c-loop peptides, and drugs that bind to c-loop peptide binding partners.

Therefore, disclosed is a system for identifying and reducing the risk of life-threatening ventricular tachyarrhythmias and sudden cardiac death in patients with congenital type-1 long QT Syndrome (LQT1) comprising an instrument for genetic testing of LQT1 syndrome and for identifying KCNQ1 channel mutations; an instrument for identifying C-loop missense mutations in the KCNQ1 channel mutations; and a computer for associating a therapeutic action

with identified C-loop missense mutations in the KCNQ1 channel. The computer may contain listings of therapeutic actions to be taken, as will be further disclosed herein. Therapeutic actions may include, but are not limited to, formulating a pharmacological agent containing a beta blocker, formulating or directing mixing or adding a type of beta blocker to a pharmacological agent, formulation of a pharmacological agent that includes, for example, IKs openers, PKC inhibitors, peptides based on the sequence of the c-loop region, c-loop interacting partners that may restore normal channel regulation, drugs that mimic c-loop peptide action, drugs that bind to c-loop peptides, or drugs that bind to c-loop peptide binding partners, use of an implantable cardioverter-defibrillator, programming or modification of an implantable cardioverter-defibrillator, the use of surgical left cervicothoracic sympathetic denervation, or the like. Such a system that correlates the heretofore described genetic testing with a therapeutic action may operate on a computer such as a laptop computer, a tablet computer, a smart phone, an instrument containing a processor, a wearable device such as a wristwatch, heart rate monitor, blood pressure monitor, or the like. Therapeutic actions may also comprise alerts from the computer that are activated upon reaching a specified bodily indicator such as a specified heart rate, a specified heart rhythm, a specified percent oxygen saturation in blood, a specified blood pressure, a specified chemical marker in blood, or the like. The term specified is defined to be any value, numerical or otherwise, that is entered or otherwise programmed into the computer, either by a human or through another source, such as another computer, a database, a calculation or calculations, and the like.

Turning now to Figure 7, there is shown a diagram depicting methods of targeted risk stratification and treatment using the present invention. Instruments such as processes, techniques and equipment heretofore described for genetic testing and subsequent identification of mutations in C-loops of the KCNQ1 channel as shown in 701 of Figure 7. ECG findings are also evaluated in step 703. Clinical history is also used in step 705, with the useful output being the definition and subsequent taking of therapeutic actions in step 707 through a treatment construct. Treatment constructs and related therapeutic actions may include, but are not limited to, the use of beta-blockers, the use of an implantable cardioverter-defibrillator (ICD), the use of surgical left cervicothoracic sympathetic denervation (LCSD). Additional possible novel therapeutic modalities (which are currently not given in LQTS patients) that may be developed and/or evaluated as a result of the present invention include the use of various pharmacological agents such as an IKs opener (for example, L-364373 from Merck, Retigabine or ezogabine from Valeant Pharmaceuticals and GlaxoSmithKline), a PKC inhibitor (for example,

Ruboxistaurin, a calcium dependent PKC inhibitor), peptides based on the sequence of the c-loop region or c-loop interacting partners (such as KCNQ1(S6)/KCNQ1(Cterm)/KCNE1(C-term) that may restore normal channel regulation, drugs that mimic c-loop peptide action such as drugs that can either bind to c-loop peptides or to c-loop peptide binding partners, or the like.

5 Figure 8 is a diagram depicting data sources used for treatment constructs of the present invention. The data sources may, in one embodiment of the present invention, be contained in a data storage device such as a hard disk, solid state memory, an optical device, a magnetic device, a semiconductor device, or the like. The data sources contained on physical media may also be organized or patterned by way of a database, relational database, software program, spreadsheet, file partition, or the like. The data sources may also be connected within or
10 between one another by way of a network, electrical, or optical connection. To provide treatment constructs such as a treatment regimen 801, various data inputs are used. Clinical data 803 that may include, for example, further medical observations of the patient, along with Electrocardiogram data 805, historical data 807 such as prior syncope data, and genetic testing
15 data 809, are provided. Treatment constructs may be developed or specified by way of a computer or microprocessor based system.

Figure 9 is a diagram depicting a method of determining LQT1 patient responders and non-responders to beta blocker therapy. A predominant medical treatment available for the management of LQT1 patients is beta-blocker therapy. Beta blockers are beta-adrenergic
20 blocking agents, beta-adrenergic antagonists, beta-adrenoreceptor antagonists, or beta antagonists that are considered a class of drugs. Examples of beta blockers include, but are not limited to, Propranolol, Sotalol, Timolol, Pindolol, Penbutolol, Pxprenolol, Nadolol, Carteolol, Bucindolol, Alprenolol, Eucommia, Carvedilol, Labetalol, and the like. Other beta blockers, such as B1 selective agents, may also be used. Examples include, but are not limited to,
25 Acebutolol, Atenolol, Betaxolol, Bisoprolol, Celiprolol, Esmolol, Metoprolol, Nebivolol, and the like. However, through our research with large population studies we show that some LQT1 patients experience life-threatening events despite being treated with beta-blockers. Our new information shows for the first time a significant difference in response to beta-blockers between LQT1 patients who have C-loop mutations (who experience a pronounced 88%
30 reduction in the risk of aborted cardiac arrest and sudden cardiac death with beta-blocker therapy) and those who have other LQT1-associated mutations (who do not derive any significant benefit from beta-blockers). These findings have important implications for recommendations for therapy in LQT1 patients that take into account mutation-specific

response to therapy. Figure 9 further describes our method where an LQT1 patient 901 undergoes genetic testing to generate genetic testing data 903 related to long QT syndrome. The presence of C-loop mutations 905 are indicative of greater response to beta-blocker therapy 907; whereas LQT1 patients with no C-loop mutations show lesser or no response to beta-blocker therapy 909. Therefore, various methods of formulating the composition of a therapeutic agent for treatment of genetic long QT syndrome are provided by way of this disclosure. For example, a method of formulating the composition of a therapeutic agent for treatment of genetic long QT syndrome may include the steps of performing genetic testing on patients suspected of having long QT syndrome to identify genetic mutations indicative of long QT syndrome, identifying with genetic testing the specific types of genetic mutations that are indicative of long QT syndrome, separating KCNQ1 channel C-loop missense mutations from other mutations in the KCNQ1 channel, and formulating therapeutic agent compositions that include Beta Blockers for reduction of life threatening events in patients with KCNQ1 channel C-loop missense mutations. Another example of a method of formulating the composition of a therapeutic agent for treatment of genetic long QT syndrome may include the steps of performing genetic testing on patients suspected of having long QT syndrome to identify genetic mutations indicative of long QT syndrome, identifying with genetic testing the specific types of genetic mutations that are indicative of long QT syndrome, separating KCNQ1 channel C-loop missense mutations from other mutations in the KCNQ1 channel, and formulating therapeutic agent compositions that do not include Beta Blockers for reduction of life threatening events in patients with genetic mutations that do not include KCNQ1 channel C-loop missense mutations. Other methods are evident after reading this disclosure, and are included in the spirit and broad scope of the present invention as described and depicted herein. For example, a method of quantifying optimal therapeutic efficacy of a drug for treatment of congenital long QT syndrome may include performing genetic testing on patients suspected of having long QT syndrome to identify genetic mutations indicative of long QT syndrome, identifying by way of genetic testing the specific types of genetic mutations indicative of long QT syndrome, separating KCNQ1 channel C-loop missense mutations from other mutations in the KCNQ1 channel, and assigning a greater probability of therapeutic efficacy of drugs containing beta blockers to patients with KCNQ1 channel C-loop missense mutations than to those patients without KCNQ1 channel C-loop missense mutations.

In addition, a method of measuring a response to beta blocker therapy in patients with long QT syndrome may include the steps of performing genetic testing on patients suspected of having long QT syndrome to identify genetic mutations indicative of long QT syndrome, identifying by way of genetic testing KCNQ1 channel C-loop missense mutations in genetic testing results, and correlating KCNQ1 channel C-loop missense mutations with an improved response to beta blocker therapy over other mutations responsible for genetic long QT syndrome.

Figure 10 is a diagram depicting a method of determining LQT1 patients at increased or lower risk of exercise triggered events. Currently, there is a nonspecific recommendation for all LQT1 patients to avoid participation in competitive sports and intense physical activity. Our findings show that patients with C-loop mutations have increased risk for arrhythmic events during exercise, whereas other LQT1 mutations are indicative of a significantly lower risk for exercise-triggered events. This data may change current recommendations for sports participation in patients with inherited arrhythmic disorders. Figure 10 further describes our method where an LQT1 patient 1001 undergoes genetic testing to generate genetic testing data 1003 related to long QT syndrome. The presence of C-loop mutations 1005 are indicative of increased risk of arrhythmic events during exercise; whereas LQT1 patients with no C-loop mutations show lower risk of exercise triggered events 1009.

Figure 11 is a diagram depicting a method of determining female LQT1 patients at increased risk of sudden cardiac death. Prior studies suggest that LQT1 women have a lower risk of cardiac events than men. Our new data shows that LQT1 women with C-loops mutations have a very high-risk of sudden cardiac death that is similar to that of men. This new finding has important implications for the management and risk assessment of an important subgroup of LQTS for whom current information regarding arrhythmic risk is very limited. Figure 11 further describes our method where a female LQT1 patient 1101 undergoes genetic testing to generate genetic testing data 1103 related to long QT syndrome. The presence of C-loop mutations 1105 are indicative of a similar risk of cardiac events as compared to a male LQT1 patient 1107; whereas female LQT1 patients with no C-loop mutations show lower risk of cardiac events than comparable male LQT1 patients 1109.

Our findings show a very high risk of arrhythmic events in patients with mutations in the C-loop region of the potassium channel. Therefore, it is possible that single nucleotide polymorphisms (that are more common in the general population) are responsible for unexplained syncopal and/or arrhythmic events that are currently left undiagnosed and

untreated. Thus, our methods, systems, and devices can be used in related ways, for example, as a possible new test to identify risk of arrhythmic events in non-LQTS patients, and further to develop therapies that can be used for the reduction in the risk of sudden death in the general population.

5 It is, therefore, apparent that there has been provided, in accordance with the various objects of the present invention, Systems And Methods For Risk Stratification, Therapies, Targeted Treatment And Prevention of Life-Threatening Ventricular Tachyarrhythmias And Sudden Cardiac Death For Patients With Congenital Type-1 Long QT Syndrome (LQT1).

10 . While the various objects of this invention have been described in conjunction with preferred embodiments thereof, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of this specification, claims and the attached drawings.

What is claimed is:

1. A system for identifying and reducing the risk of life-threatening ventricular
5 tachyarrhythmias and sudden cardiac death in patients with congenital type-1 long QT
Syndrome (LQT1) comprising:

an instrument for genetic testing of LQT1 syndrome and for identifying KCNQ1
channel mutations;

10 an instrument for identifying C-loop missense mutations in the KCNQ1 channel
mutations; and

a computer for associating a therapeutic action with identified C-loop missense
mutations in the KCNQ1 channel.

2. The system for identifying and reducing the risk of life-threatening ventricular
15 tachyarrhythmias and sudden cardiac death in patients with congenital type-1 long QT
Syndrome (LQT1) as recited in claim 1, wherein the therapeutic action is the
formulation of a pharmacological agent containing a beta blocker.

3. The system for identifying and reducing the risk of life-threatening ventricular
20 tachyarrhythmias and sudden cardiac death in patients with congenital type-1 long QT
Syndrome (LQT1) as recited in claim 1, wherein the therapeutic action is the
formulation of a pharmacological agent selected from the group consisting of IKs
openers, PKC inhibitors, peptides based on the sequence of the c-loop region, c-loop
interacting partners that may restore normal channel regulation, drugs that mimic c-loop
25 peptide action, drugs that bind to c-loop peptides, and drugs that bind to c-loop peptide
binding partners.

4. The system for identifying and reducing the risk of life-threatening ventricular
tachyarrhythmias and sudden cardiac death in patients with congenital type-1 long QT
30 Syndrome (LQT1) as recited in claim 1, wherein the therapeutic action is the use of an
implantable cardioverter-defibrillator.

5. The system for identifying and reducing the risk of life-threatening ventricular tachyarrhythmias and sudden cardiac death in patients with congenital type-1 long QT Syndrome (LQT1) as recited in claim 1, wherein the therapeutic action is the programming of an implantable cardioverter-defibrillator.

5

6. The system for identifying and reducing the risk of life-threatening ventricular tachyarrhythmias and sudden cardiac death in patients with congenital type-1 long QT Syndrome (LQT1) as recited in claim 1, wherein the therapeutic action is the modification of an implantable cardioverter-defibrillator.

10

7. The system for identifying and reducing the risk of life-threatening ventricular tachyarrhythmias and sudden cardiac death in patients with congenital type-1 long QT Syndrome (LQT1) as recited in claim 1, wherein the therapeutic action is the use of surgical left cervicothoracic sympathetic denervation.

15

8. The system for identifying and reducing the risk of life-threatening ventricular tachyarrhythmias and sudden cardiac death in patients with congenital type-1 long QT Syndrome (LQT1) as recited in claim 1, wherein the therapeutic action is the activation of an alert by the computer upon reaching a specified metabolic function.

20

9. A method of formulating the composition of a therapeutic agent for treatment of genetic long QT syndrome comprising the steps of:

performing genetic testing on patients suspected of having long QT syndrome to identify genetic mutations indicative of long QT syndrome;

25

identifying with genetic testing the specific types of genetic mutations that are indicative of long QT syndrome;

separating KCNQ1 channel C-loop missense mutations from other mutations in the KCNQ1 channel; and

formulating therapeutic agent compositions that include Beta Blockers for reduction of life threatening events in patients with KCNQ1 channel C-loop missense mutations.

30

10. A method of formulating the composition of a therapeutic agent for treatment of genetic long QT syndrome comprising the steps of:

performing genetic testing on patients suspected of having long QT syndrome to identify genetic mutations indicative of long QT syndrome;
identifying with genetic testing the specific types of genetic mutations that are indicative of long QT syndrome;
5 separating KCNQ1 channel C-loop missense mutations from other mutations in the KCNQ1 channel; and
formulating therapeutic agent compositions that do not include Beta Blockers for reduction of life threatening events in patients with genetic mutations that do not include KCNQ1 channel C-loop missense mutations.

10
11. A method of targeted risk stratification and treatment for use in defining a therapeutic action to be taken comprising the steps of:
performing genetic testing on patients suspected of having long QT syndrome to identify genetic mutations indicative of long QT syndrome and processing the results of
15 the genetic testing on a computer;
identifying by way of genetic testing mutations in KCNQ1 channel C-loops and processing the results of the genetic testing on a computer;
evaluating electrocardiogram patient information on a computer;
evaluating patient clinical history on a computer; and
20 defining a therapeutic action to be taken.

12. The method of claim 11, wherein the therapeutic action to be taken is defined to be the use of a beta blocker.

25 13. The method of claim 11, wherein the therapeutic action to be taken is defined to be the use of an implantable cardioverter-defibrillator.

14. The method of claim 11, wherein the therapeutic action to be taken is defined to be alteration of the operation of an implantable cardioverter-defibrillator

30 15. The method of claim 11, wherein the therapeutic action to be taken is defined to be the use of surgical left cervicothoracic sympathetic denervation.

16. The method of claim 11, wherein the therapeutic action to be taken is defined to be the use of a pharmacological agent selected from the group consisting of IKs openers, PKC inhibitors, peptides based on the sequence of the c-loop region, c-loop interacting partners that may restore normal channel regulation, drugs that mimic c-loop peptide action, drugs that bind to c-loop peptides, and drugs that bind to c-loop peptide binding partners.

17. A method of quantifying optimal therapeutic efficacy of a drug for treatment of congenital long QT syndrome, the method comprising the steps of:

performing genetic testing on patients suspected of having long QT syndrome to identify genetic mutations indicative of long QT syndrome;

identifying by way of genetic testing the specific types of genetic mutations indicative of long QT syndrome;

separating KCNQ1 channel C-loop missense mutations from other mutations in the KCNQ1 channel; and

assigning a greater probability of therapeutic efficacy of drugs containing beta blockers to patients with KCNQ1 channel C-loop missense mutations than to those patients without KCNQ1 channel C-loop missense mutations.

18. A method of measuring a response to beta blocker therapy in patients with long QT syndrome comprising the steps of:

performing genetic testing on patients suspected of having long QT syndrome to identify genetic mutations indicative of long QT syndrome;

identifying by way of genetic testing KCNQ1 channel C-loop missense mutations in genetic testing results; and

correlating KCNQ1 channel C-loop missense mutations with an improved response to beta blocker therapy over other mutations responsible for genetic long QT syndrome.

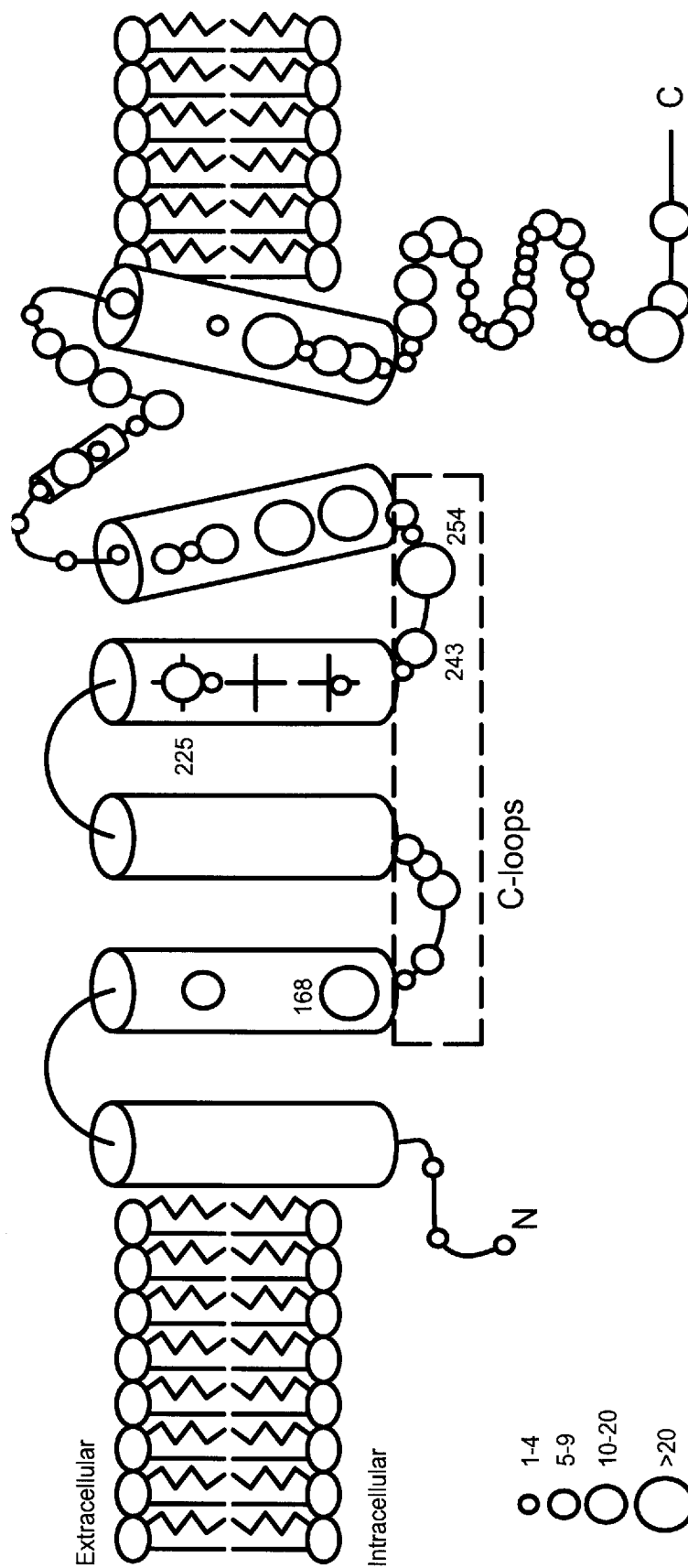


Fig. 1

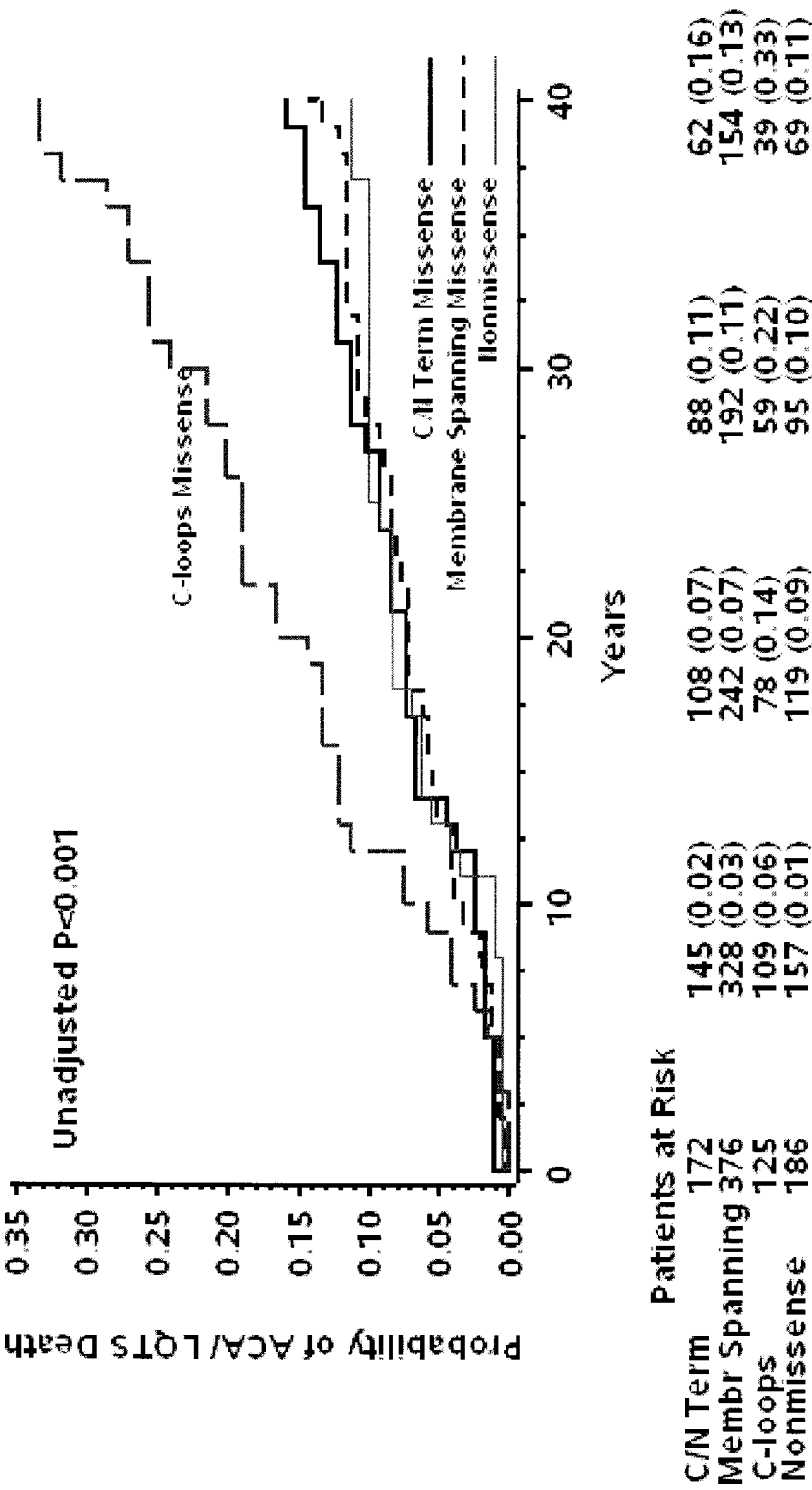


Fig. 2

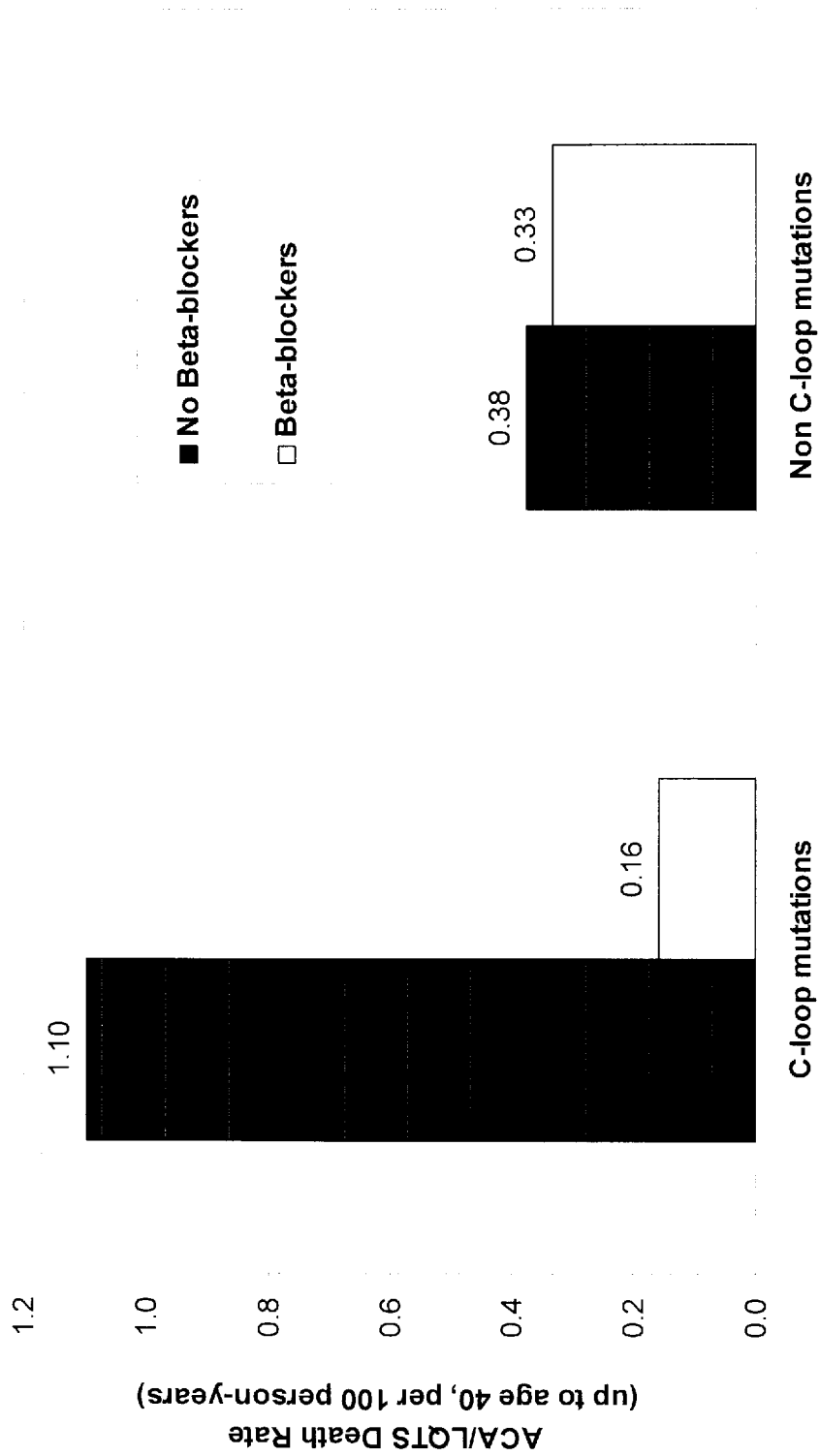


Fig. 3

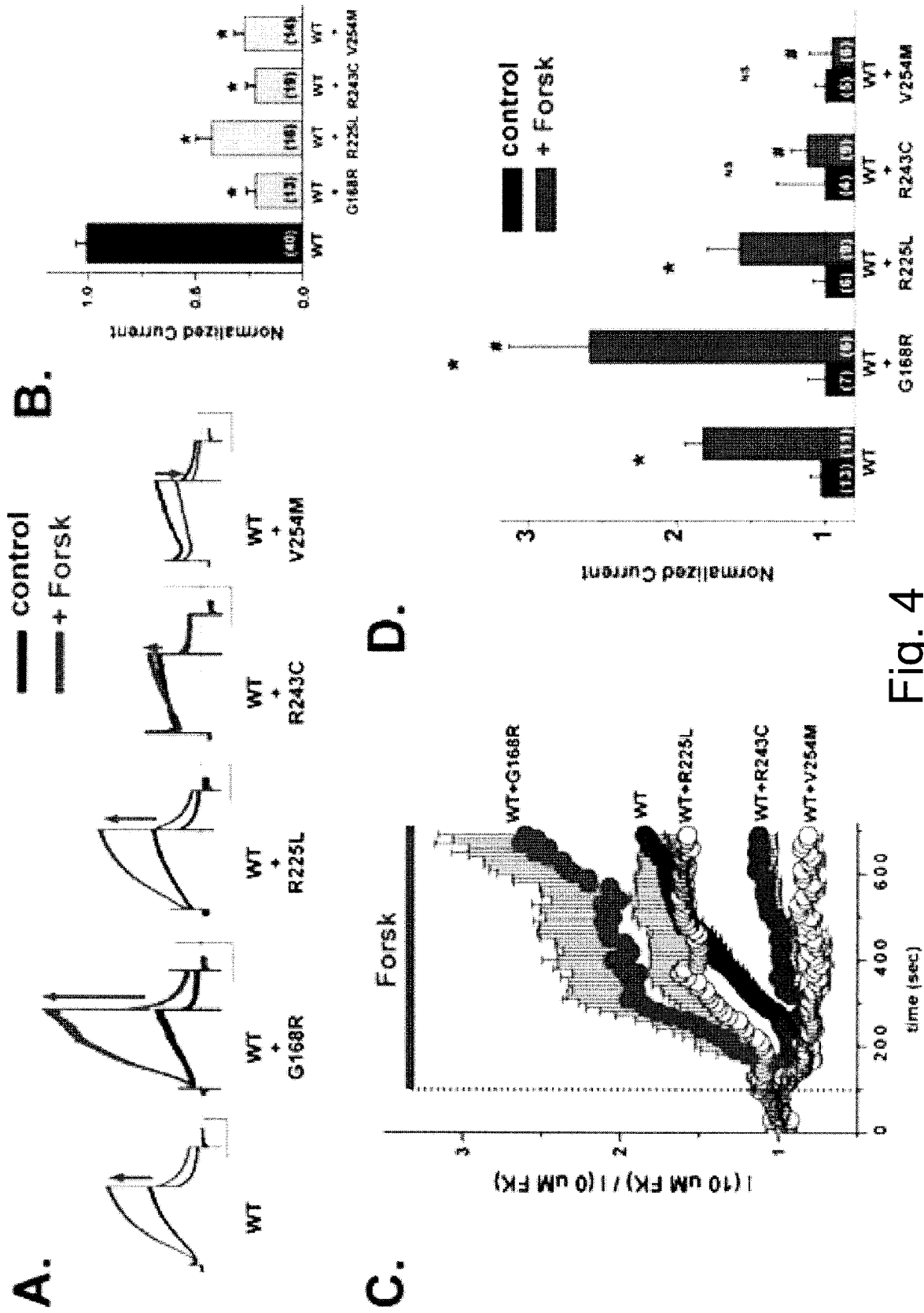


Fig. 4

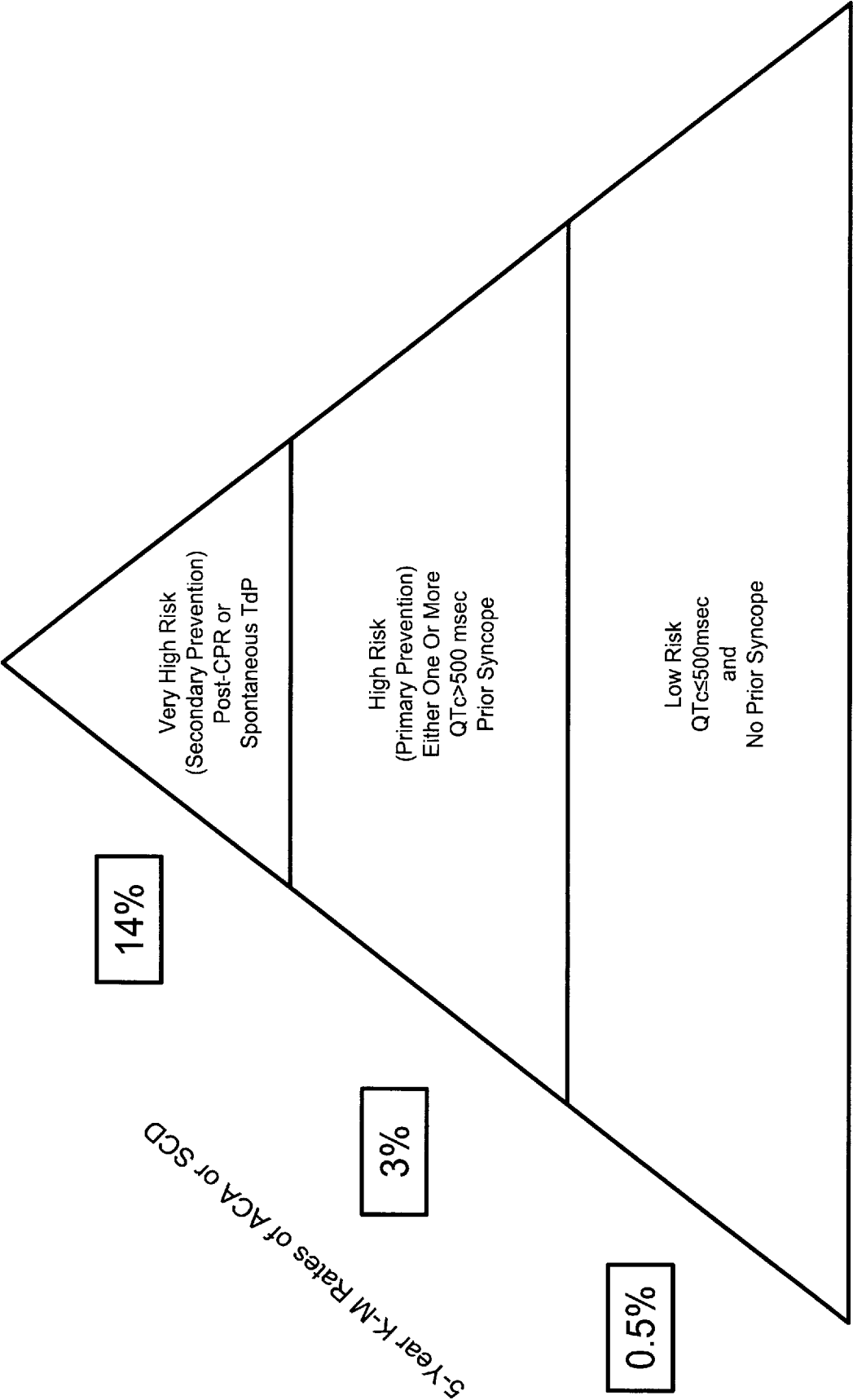


Fig. 5

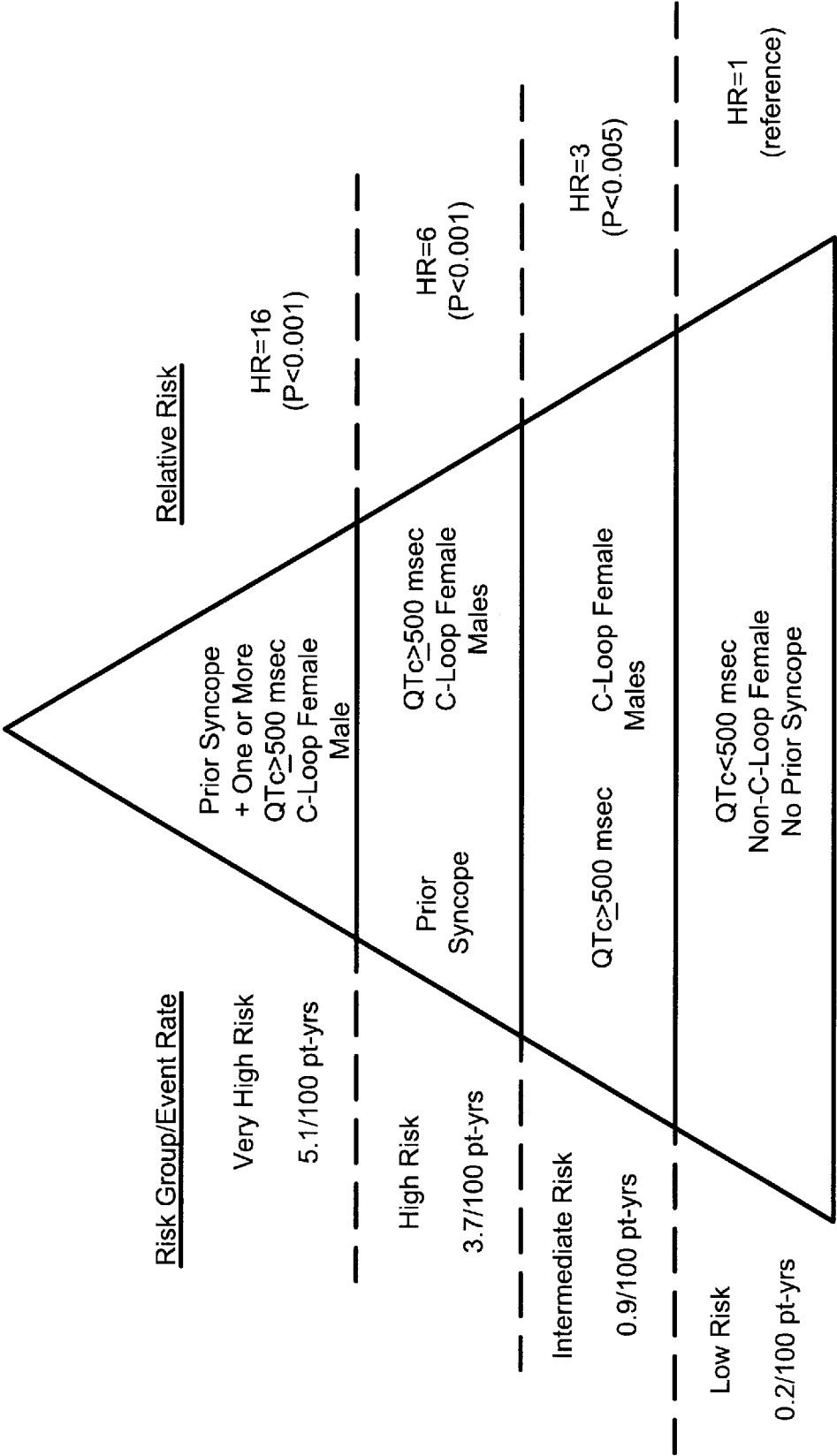


Fig. 6

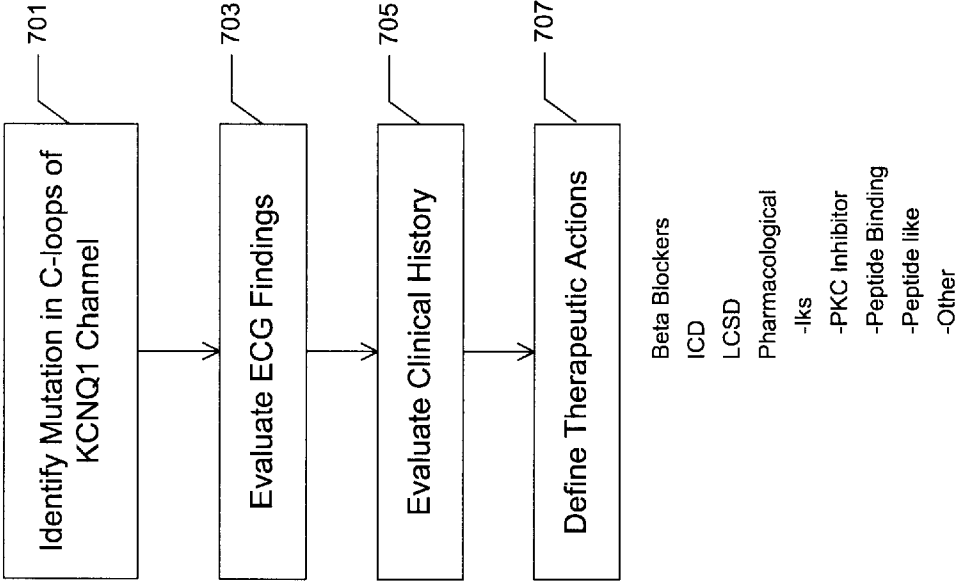


Fig. 7

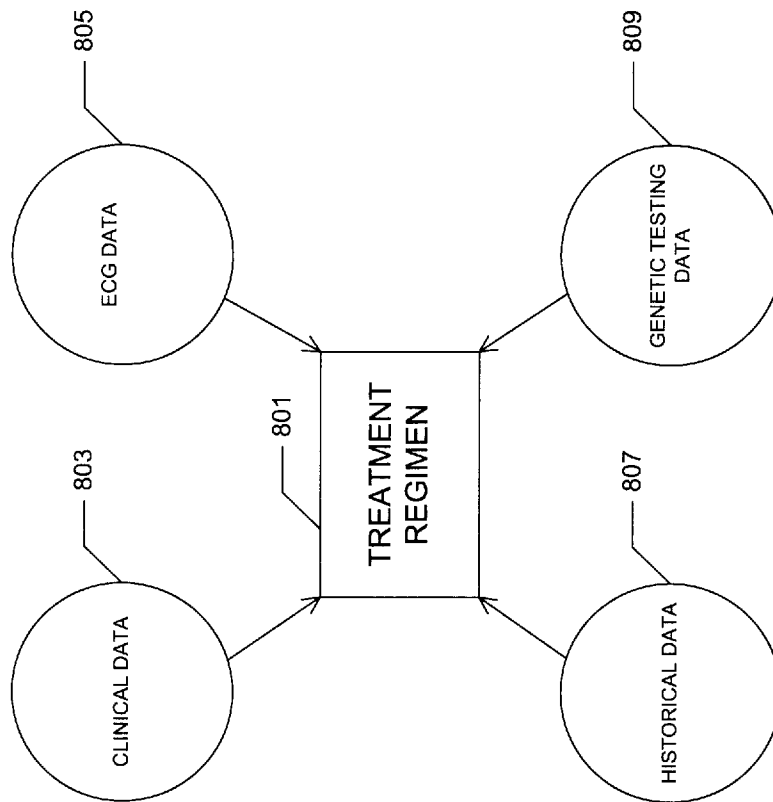


Fig. 8

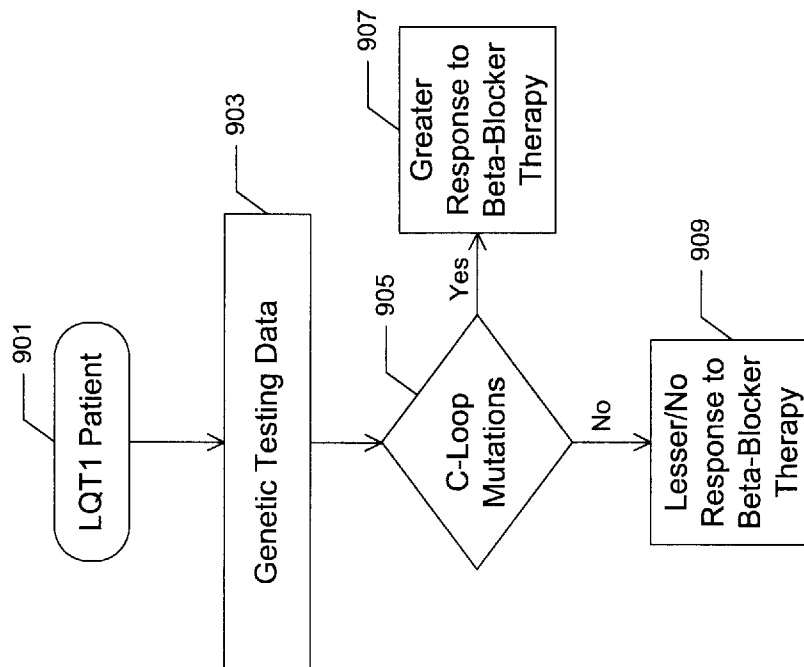


Fig. 9

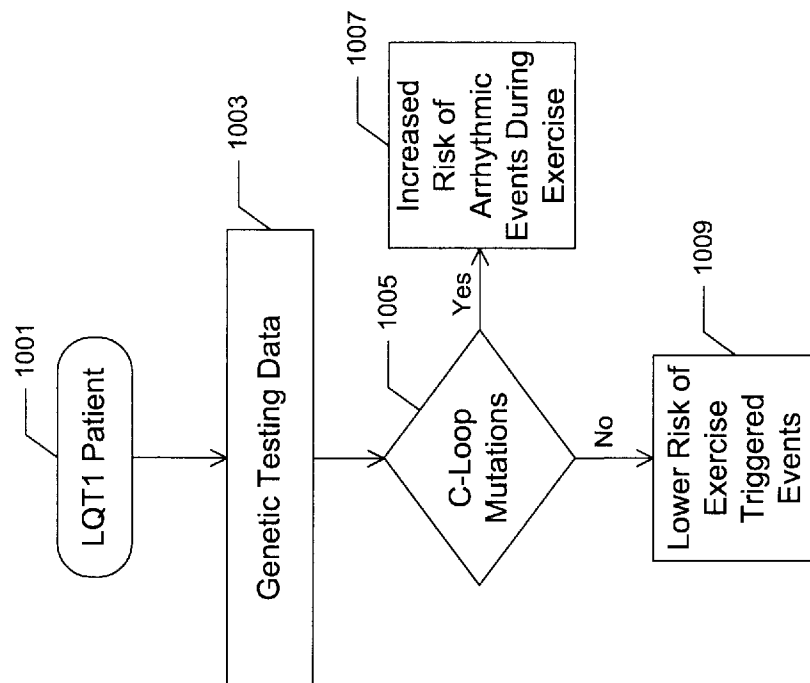


Fig. 10

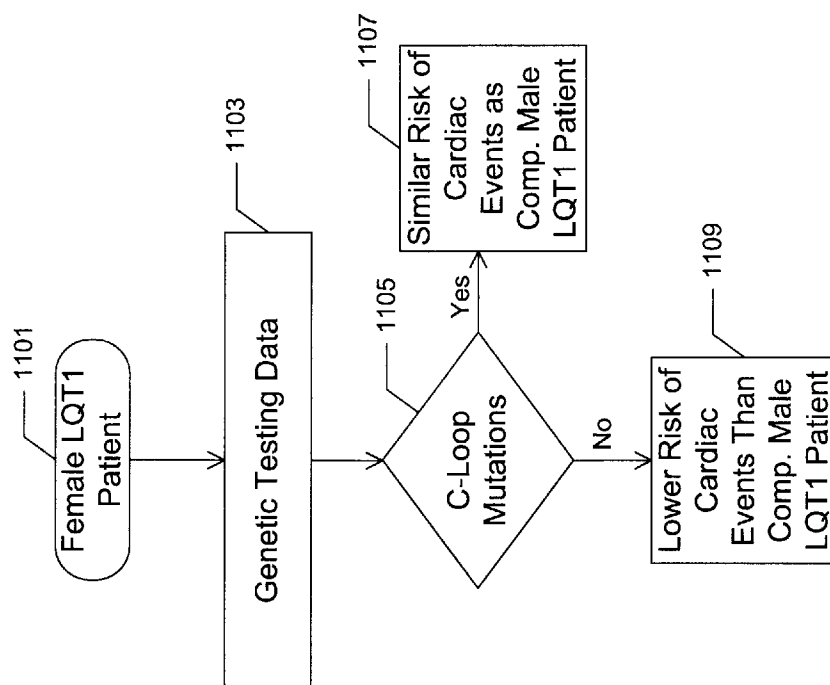


Fig. 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/36004

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (2012.01)

USPC - 435/6.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C12Q 1/68 (2012.01)

USPC - 435/6.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/4, 6.16, 283.1, 287.1; 600/300, 481, 508, 509, 515, 516; 607/1, 2, 5; 702/1, 19, 20Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Dialog Classic Files -- 2,6,35,64,144,155,315,371,344,347,340,348,349,654,652,345,351; Google Scholar; Search terms -- tachyarrhythmias; ventricular; SUDDEN CARDIAC death; CONGENITAL; LONG QT SYNDROME; LQT1 CHANNEL; MUTATION; channelopathy; GENETIC PROFILE; GENETIC TEST; ALLELE; MARKER; GENE; MISSENSE; SNP; COMPUTER; THERAPEUTIC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2005/0142591 A1 (Ackerman) 30 June 2005 (06.03.2005); para [0004], [0005], [0023], [0026]-[0028], [0031], [0038], [0043], [0066]; Fig. 1; Table 3	1-18
Y	US 6,497,655 B1 (Linberg et al.) 24 December 2002 (24.12.2002); col 8, ln 22-63; claim 11	5, 6, 8, 14
Y	US 7,123,961 B1 (Kroll et al.) 17 October 2006 (17.10.2006); col 8, ln 56-67; col 9, ln 1-2	7, 15
Y	US 2009/0299645 A1 (Colby et al.) 3 December 2009 (03.12.2009); para [0009], [0055], [0077], [0569], [0635], [0638], [0911], [0916], [1011], [1035]-[1042], [1251]; Fig. 1, 6C, 11A, 11B, 146, 152C	1-8, 11-16
Y	Matavel et al. "PKA and PKC partially rescue long QT type 1 phenotype by restoring channel-PIP2 interactions" Channels (February 2010) 1: 3-11; pg 8, Fig 3 caption; pg 9, Fig 5	3, 16
Y	Kobori et al. "Additional Gene Variants Reduce Effectiveness of Beta-Blockers in the LQT1 Form of Long QT Syndrome" Journal of Cardiovascular Electrophysiology (February 2004) 15:190-199; abstract; pg 197 right col, pg 198 left col	9, 17, 18
Y	Wilson et al. "Abnormal KCNQ1 trafficking influences disease pathogenesis in hereditary long QT syndromes (LQT1)" Cardiovascular Research (June 2005) 67:476-486; abstract; pg 482, section 3.6; pg 483, Fig 7 and legend	10

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 July 2012 (30.07.2012)

Date of mailing of the international search report

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Name and mailing address of the ISA/US

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