US007510874B2

(12) **United States Patent**
Robertson et al.

(10) **Patent No.: US 7,510,874 B2**
(45) **Date of Patent: Mar. 31, 2009**

(54) STABLE CELL LINES EXPRESSING HERG1A AND HERG1B

(75) Inventors: **Gail A. Robertson**, Madison, WI (US); **Eugenia M. Jones**, Madison, WI (US); **Jinling Wang**, Pasadena, CA (US)

(73) Assignee: **Wisconsin Alumni Research Foundation**, Madison, WI (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 493 days.

(21) Appl. No.: **10/976,122**

(22) Filed: **Oct. 28, 2004**

(65) **Prior Publication Data**

US 2005/0164379 A1 Jul. 28, 2005

Related U.S. Application Data

(60) Provisional application No. 60/515,158, filed on Oct. 28, 2003.

(51) **Int. Cl.**

C12N 15/85	(2006.01)
C12N 5/08	(2006.01)
C07K 14/00	(2006.01)
C07K 16/00	(2006.01)
C07H 21/04	(2006.01)

(52) **U.S. Cl.** **435/325**; 435/366; 435/369; 530/350; 530/388.22; 536/23.1; 536/23.5

(58) **Field of Classification Search** .... None
See application file for complete search history.

(56) **References Cited**

OTHER PUBLICATIONS

Alomone Labs, "ERG K+ Channels," Modulator 13:1-2 (2001).

Crociani, O., et al., "Cell Cycle-Dependent Expression of HERG1 and HERG1B Isoforms in Tumor Cells," The Journal of Biological Chemistry 278:2947-2955 (2003).

Curran, M.E., et al., "A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome," Cell 80:795-803 (1995).

Delisle, B.P., et al., "Biology of Cardiac Arrhymias Ion Chanel Protein Trafficking," Circulation Research Jun. 11, 2004:1418-1428.

He, J.Q., et al., "Reduction in density of transverse tubules and L-type Ca(2+) channels in canine tachycardia-induced heart failure," Cardiovascular Research 49:298-307 (2001).

Jones, E. M., et al., "HERG-1b Protein is Experssed in Cardiac Ventricular Myocytes," Scientific Sessions 2003.

Kupershmidt, S., et al., "A K+ channel splice variant common in human heart lacks a C-terminal domain required for expression of rapidly activating delayed rectifier current," J. Biol. Chem. 273:27231-27235 (1998).

Lees-Miller, J.P., et al., "Electrophysiological characterization of an alternatively processed ERG K+ channel in mouse and human hearts," Circ. Res. 81:719-726 (1997).

London, B., et al., "N- and C-terminal isoforms of HERG in the human heart," Biophysical J. (1998).

O'Leary, M.E., "Inhibition of Human Ether-A-Go-Go Potassium Channels by Cocaine," Molecular Pharmacology 59:269-277 (2001).

Roti Roti, E.C., et al., "Interaction with GM130 during HERG Ion Channel Trafficking," The Journal of Biological Chemistry 277:47779-47785 (2002).

Sanguinetti, M.C., et al., "Two components of cardiac delayed rectifier K+ current. Differential sensitivity to block by class III antiarrhythmic agents," J. Gen. Physiol. 96:195-215 (1990).

Sanguinetti, M.C., et al., "A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel," Cell 81:299-307 (1995).

Sanguinetti, M.C., et al., "Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel," Nature 384:80-83 (1996).

Trudeau, M.C., et al., "HERG, a Human Inward Rectifier in the Voltage-Gated Potassium Channel Family," Science 269:92-95 (1995).

Wang, J., et al., "Dynamic Control of Deactivation Gating by a Soluble Amino-Terminal Domain in HERGK+ Channels," J. Gen Phyiol. 115:749-758 (2000).

Wang, J., et al., "Regulation of Deactivation by an Amino Terminal Domain in Human Ether-a-go-go-related Gene Potassium Channels," J. Gen. Phyiol. 112:637-647 (1998).

Zhou, A., et al., "HERG channel dysfunction in human long QT syndrom. Intracellular transport and functional defects," J. Biol. Chem. 273:21061-21066 (1998).

Zhou, A., et al., "Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature," Biophys. J. 74:230-241 (1998).

Jones E, et al., "Cardiac IKr channels minimally comprise hERG 1a and 1b subunits," J. Biol. Chem. 279:44690-44694 (2004).

London B, et al., "Two isoforms of the mouse ether-a-go-go-related gene coassemble to form channels with properties similar to the rapidly activating component of the cardiac delayed rectifier K+ current," Circ. Res. 81:870-878 (1997).

Ohya S, et al., "Functional and molecular identification of ERG channels in murine portal vein myocytes," Am. J. Physiol. Cell Physiol. 283:C866-877 (2002).

Pond L, et al., "Expression of distict ERG proteins in rat, mouse, and human heart. Relation to functional I(Kr) channels," J. Biol. Chem. 275:5997-6006 (2000).

Primary Examiner—Elizabeth C. Kemmerer
Assistant Examiner—Sandra Wegert
(74) *Attorney, Agent, or Firm*—Quarles & Brady LLP

(57) ABSTRACT

A line of cultured mammalian cells includes HERG1b subunits and optionally HERG1a subunits.

7 Claims, 2 Drawing Sheets

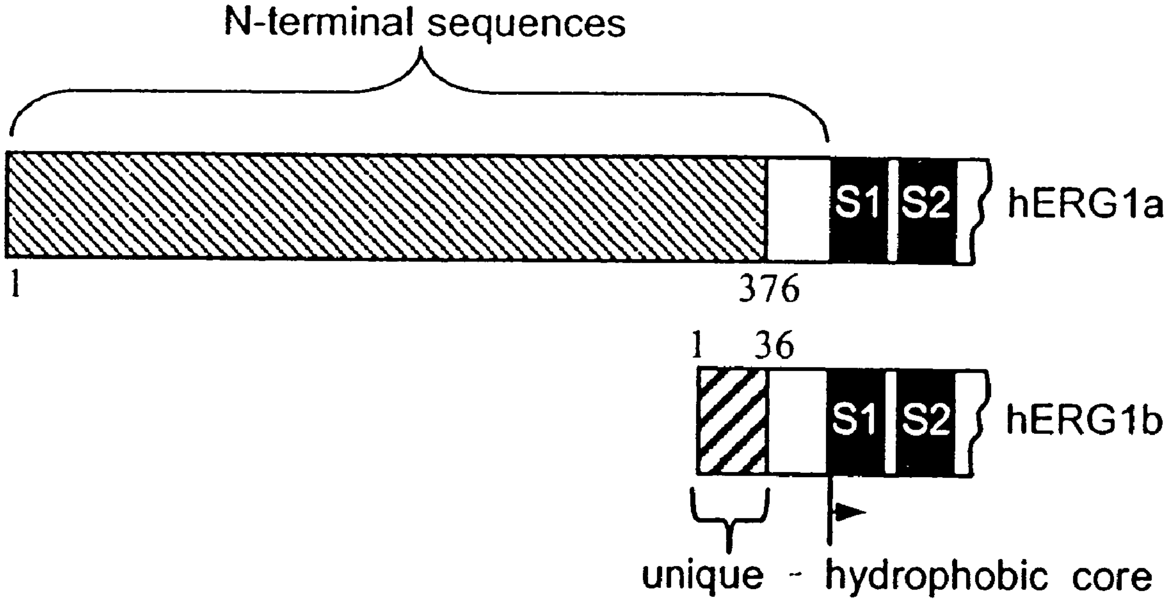
N-terminal sequences
S1
S2
hERG1a
1
376
1
36
S1
S2
hERG1b
unique
hydrophobic core

FIG 1

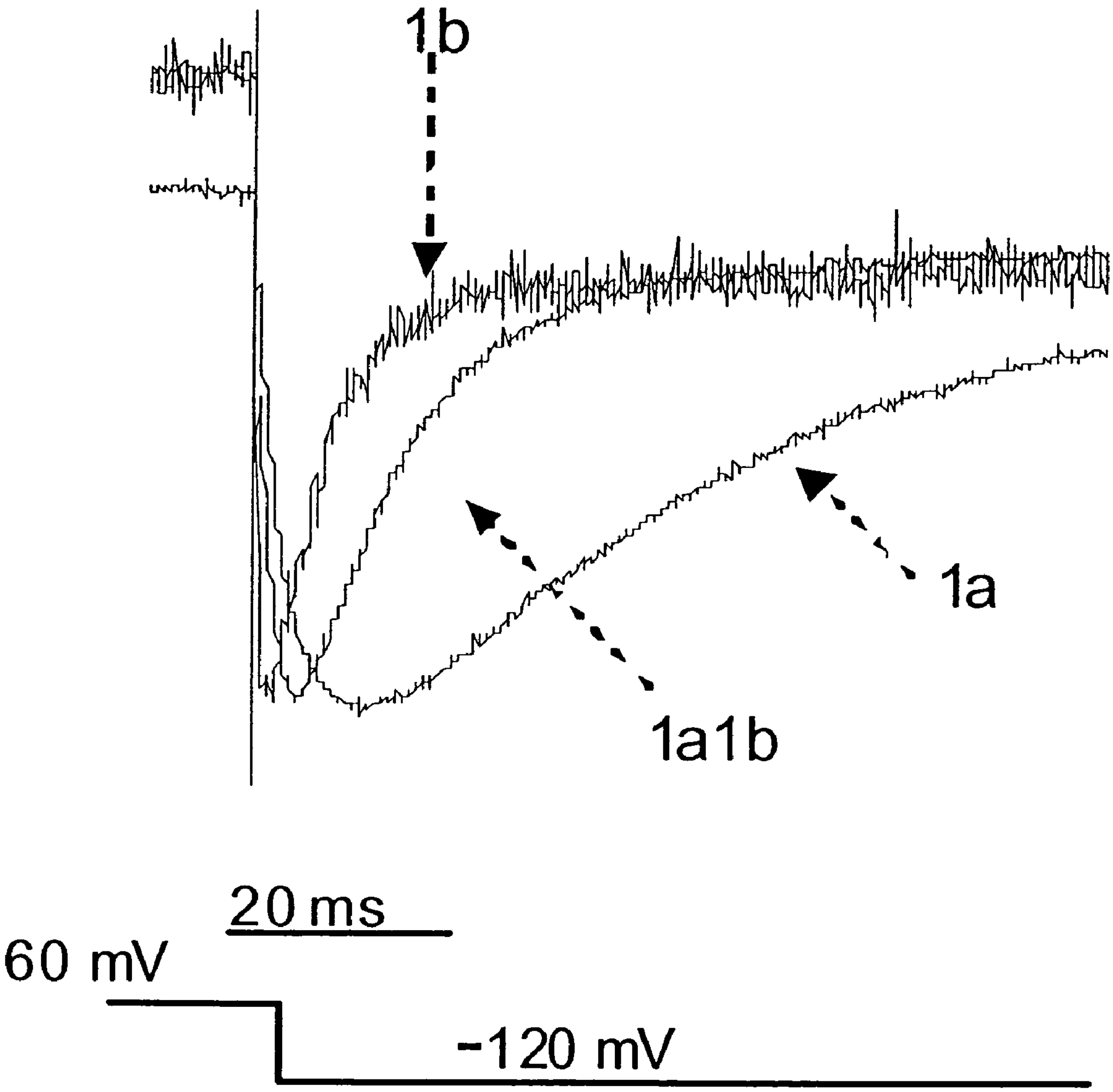

FIG 2

STABLE CELL LINES EXPRESSING HERG1A AND HERG1B

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/515,158, filed Oct. 28, 2003.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with United States government support awarded by the following agency: NIH Grant Number HL55973. The United States government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Ion channels expressed in the plasma membrane of excitable tissues (including heart) regulate the function of the tissues. Ion channels can comprise alpha, beta and auxiliary subunits. The alpha subunits are largely responsible for determining overall biophysical properties of the channels, such as ion selectivity, gating and drug sensitivity, whereas beta or auxiliary subunits modify these properties in important ways. Voltage-gated potassium channels comprise four alpha subunits that assemble into a pseudosymmetric array (MacKinnon, 1991), thereby providing the opportunity for heterogeneity by mixing of related subunits to form heterotetrameric channels (Christie et al., 1990; Isacoff et al., 1990). The potential for complexity and heterogeneity increases substantially when beta or auxiliary subunits are also present (England et al., 1995).

Cardiac I_{Kr} is a rapidly-inactivating potassium current first identified by its sensitivity to the methanesulfonanilide drug E-4031 (Sanguinetti, M. C. and N. K. Jurkiewicz, 1990). Compared to all other known potassium currents, I_{Kr} has a unique functional profile characterized by the suppression of current during depolarization and large, rebounding tail currents produced upon repolarization. Currents are suppressed during depolarization because channels open only briefly and then rapidly inactivate. Upon repolarization, channels recover rapidly from inactivation and revisit the open state. Because deactivation is slow, the channels linger in this highly stable open state and produce the resurgent current that is a hallmark of I_{Kr}. Moreover, the sensitivity to E-4031 and other antiarrhythmic drugs is unique to I_{Kr}.

Currents with comparable biophysical and pharmacological properties are produced when HERG1, a gene encoding an inwardly rectifying potassium channel that was cloned from human hippocampus (Warmke and Ganetzky, 1994), is transiently expressed in *Xenopus* oocytes, suggesting that HERG1 is a central component of the channels that give rise to the I_{Kr} currents (Sanguinetti et al., 1995; Trudeau et al., 1995). Trudeau, M. C., et al., "HERG, a Human Inward Rectifier in the Voltage-Gated Potassium Channel Family," *Science* 269:92 (1995), incorporated by reference as if set forth herein in its entirety, described the HERG gene and also depicted the inwardly rectifying HERG currents and a gating model in the same paper.

Families with a form of inherited (familial) Long QT Syndrome (LQTS-2) have mutations the HERG1 gene (Curran et al., 1995). LQTS-2 is a life-threatening illness characterized by polymorphic ventricular arrhythmias known as torsades de pointes (Roden, 1993). Undiagnosed or untreated, LQTS often leads to sudden death by young adulthood. The expression studies of Trudeau et al. (1995), defining HERG as the primary component underlying I_{Kr}, thus explained the underlying cause of LQTS-2 as a loss of I_{Kr}.

More clinically prevalent than familial LQTS is an acquired form of the disease caused by block of I_{Kr} currents by a surprising variety of drugs, including antiarrhythmic drugs such as dofetilide (Tikosyn®) (Snyders and Chaudhary, 1996), the antihistamines terfenadine (Seldane®) (Roy et al., 1996; Suessbrich et al., 1996) and astemizole (Hismanal®) (Zhou et al., 1999b), the gastric motility drug cisapride (Propulsid®) (Mohammad et al., 1997; Rampe et al., 1997), and cocaine (Zhang S, 2001). An estimated 1-8% of the general public is susceptible to acquired LQTS. Despite their therapeutic value, several of these drugs have been withdrawn from the market because of an unacceptable risk of torsades. As a result, to avoid the risk of torsades and the lost investment associated with withdrawal of a drug from the market, standard pharmaceutical industry practice today dictates that all pharmaceutics in development are screened against cultured cells that express HERG1 in the cell membranes with monitoring for changes in potassium channel behavior. Commercially available HERG-expressing cell lines express only HERG1a channel subunits that assemble into HERG1 channels.

While it is accepted that I_{Kr} channels primarily contain HERG 1 subunits, the precise composition of these channels is unknown. The discovery of alternative HERG1a and HERG1b transcripts encoded by the HERG1 gene in human heart (Lees-Miller et al., 1997; London et al., 1997; Kupershmidt et al., 1998; London et al., 1998, each incorporated by reference as if set forth herein in its entirety), raised the possibility that alpha subunits other than HERG1a contribute to the I_{Kr} channels.

The proteins encoded by the HERG1a and HERG1b transcripts differ only at their amino termini, as shown in the attached Sequence Listing. The longer amino terminus of HERG1a confers slow deactivation; the shorter amino terminus of HERG1b confers rapid deactivation, relative to HERG1a. When transiently expressed together in a heterologous *Xenopus* oocyte system, the two subunits assemble to form heteromeric channels that produce currents with unique, intermediate deactivation properties that cannot be explained by the algebraic summation of two homomeric populations of channels. HERG1a DNA and amino acid sequences (SEQ ID NO:1 and 2, respectively) can be found at GenBank Accession No. NM_000238, and HERG1b DNA and amino acid sequences (SEQ ID NO:3 and 4, respectively) can be found at GenBank Accession No. NM_172057). The understanding of the art in this regard is presented in London, B. et al., "Two Isoforms of the Mouse Ether-a-go-go-Related Gene Co-assemble to Form Channels With Properties Similar to the Rapidly Activating Component of the Cardiac Delayed Rectifier K^+ Current," *Circ. Res.,* 81:870 (1997), which is incorporated by reference as if set forth herein in its entirety.

Although HERG1b transcripts have been observed in human heart tissue, until now there was no convincing evidence for the existence in the heart of HERG1b protein, nor was there a consensus as to whether HERG1a and HERG1b channel subunits co-assemble in the heart in vivo. It has heretofore been presumed that HERG channels in cardiac myocytes are uniformly formed of HERG1a subunits and a host of such HERG1a-containing cell lines are available for testing, as described. Even so, the potassium ion channel behavior of HERG1a-containing cell lines does not fully match the behavior of I_{Kr} currents observed in cardiac myocytes. Additionally, from the prior work in *Xenopus* oocytes one cannot predict co-assembly of HERG1a and HERG1b subunits, let alone production of an I_{Kr} current, in the membranes of mammalian cells, particularly upon heritable maintenance and expression of HERG1a and HERG1b in such cells. Understanding cardiac I_{Kr} physiology and the disease mechanisms of HERG-linked congenital and acquired LQTS necessitates approximating the native state in heterologous systems as closely as possible. It would be desirable to provide improved cell lines for pharmacologic testing, where the improved cell lines mirror the potassium ion channel behavior and subunit composition found in cardiac myocytes.

BRIEF SUMMARY OF THE INVENTION

ERG is understood by the skilled person to refer to the ether-a-go-go related gene, and ERG to the corresponding protein, identified in various mammalian, non-mammalian, and non-vertebrate species. HERG and HERG refer to the human ERG homolog, and corresponding protein, respectively. Reference herein to HERG refers to the human ether-a-go-go related gene while ERG indicates the homolog in lower mammals. When discussing an anti-ERG antibody, the applicants intend that the antibody reacts across species and interacts with the ERG protein (or ERG1a or ERG1b subunits) from both human and non-human animals. There is very close sequence similarity between the genes and the encoded proteins in higher and lower mammals, as well as interspecies cross-reactivity of isoform-specific antibodies.

The present invention relates to the direct demonstration by the inventors that both the HERG1a and HERG1b proteins are present in the cell membranes of heart tissue of human and non-human animals, and further that when both proteins are stably expressed in a mammalian cell line, a single antibody raised specifically against either the HERG1b or HERG1a subunits co-precipitates both the HERG1a and the HERG1b subunits. The inventors have demonstrated that ERG1a and ERG1b subunits of non-human animals are also expressed in non-human animal cardiac tissue.

Further, HERG1a and HERG1b expression in a heterologous mammalian cell system produced current with the characteristic hallmark pharmacological and biophysical properties of native I_{Kr} channels, namely sensitivity to a methanesulfonanilide drug, suppression of current during depolarization and large, rebounding tail currents produced upon repolarization. These hallmarks, and the ability to distinguish an I_{Kr} current from a non-I_{Kr} current, are understood by the skilled artisan familiar with the papers by Sanguinetti, M C and Jurkiewicz (1990), Sanguinetti, M C et al. (1995) and by Trudeau, M C, et al. (1995), each incorporated by reference herein as if set forth in its entirety.

A first aspect of the invention follows from these demonstrations. In accord with the first aspect, the invention is summarized in that cardiac I_{Kr} can be recapitulated in a line of cultured mammalian cells, notably human cells, having a cell membrane that comprises HERG potassium ion channels having HERG1a and HERG1b subunit components, wherein the HERG1a and HERG1b subunit components are heterologous to the line of cultured cells where the cultured cells do not contain the subunit components until polynucleotides encoding the components are provided in the cells. A cell line thus produced is useful for screening of a pharmacologic agent for an effect on potassium ion channel behavior, in the manner that cell lines comprising HERG1a channels alone are now used. A heterologous subunit is expressed in the line of cultured cells as a result of stable and heritable transfer of a subunit-encoding polynucleotide into cells used to produce the cell line. Relatedly, a line of cultured cells having a HERG1b subunit component without a HERG1a subunit component, wherein the HERG1b subunit component is heterologous to the cells, is also useful for evaluating the potassium ion channels formed in, and the membrane potential of, such cells, as a tool to evaluate ion channels in patients having a truncated HERG1a subunit or no HERG1a subunit and to screen and develop compounds that may be effective in enhancing HERG1b surface expression and thus rescuing the mutant phenotype. While it will be appreciated that mammalian cell lines comprising other ERG orthologs can be prepared and used as described herein, such cells are of less commercial interest than cells expressing HERG, as it is the behavior of the human ion channels in human cells that is of interest when screening prospective new drugs for use in humans. However, such cells could find utility in screening of veterinary pharmaceuticals for possible effects upon ERG ion channels in non-human animals. While the application is generally directed to HERG-expressing cells, it is understood that ERG-expressing cells are also within the scope of the disclosure.

A second aspect of the invention is summarized in that a method for screening a pharmacologic agent for an effect on potassium ion channel behavior includes the steps of (1) establishing a baseline potassium ion channel behavior of a line of cultured cells having a cell membrane that comprises HERG potassium ion channels having HERG1a and HERG1b subunit components, (2) exposing the line of cultured cells to a pharmacologic agent, (3) determining the potassium ion channel behavior of the exposed cells, (4) comparing the potassium ion channel behavior of the exposed cells to the baseline potassium ion channel behavior, and (5) determining whether the pharmacologic agent affects the potassium ion channel behavior of the cells. Ion channel behavior can be assessed using any conventional electrophysiological approach such as a square voltage clamp protocol or an action potential clamp protocol. The latter is advantageous as it approximates the physiological behavior of the ion channels in cardiac tissue, particularly with regard to the opening and closing of the channels, and thereby yields more meaningful information about the binding of a pharmacologic agent to an open channel and the associated risk to an individual.

In a related aspect, the invention is further summarized in that a method for establishing a line of cultured cells includes the steps of (1) introducing into an expression-component cell line one or more expression vectors containing polynucleotides that encode HERG1b (and, optionally, HERG1a) under the control of an upstream transcriptional promoter and an optional downstream polyA addition sequence under conditions favoring transcription of one or more transcripts from the vector and translation from the transcripts to yield HERG1a and HERG1b subunits for co-assembly into heterotetrameric HERG channels in the membranes of the cells, (2) selecting cells that express either the HERG1b subunit alone or the HERG1a and HERG1b subunits, and (3) expanding a single cell clone to establish the line of cultured cells. To confirm expression of the HERG1a and HERG1b in the cultured cell line, levels of HERG subunit expression can be evaluated in the line using biochemical or electrophysiological methods or both.

In still another related aspect, the invention is further summarized in that in the method for establishing a line of cultured cells, the polynucleotides that encode HERG1a and HERG1b are the published polynucleotides that encode conventional HERG1a and HERG1b, wherein such sequences are presented herein in SEQ ID NO:1 and SEQ ID NO:3. Optionally, additional related cell lines in keeping with the invention can be established by substituting for the conventional HERG1a- or HERG1b-encoding polynucleotides, or both, related polynucleotides carrying mutations known in the art to be associated with LQTS. The skilled artisan will appreciate that the evaluation of a pharmacologic agent can be customized for a particular individual if the HERG profile (protein sequences of the HERG1a and HERG1b subunits) in the tested line of cultured cells matches or substantially matches the HERG profile of the individual.

These findings have significant implications for N-terminal mutations that are causally associated with LQTS. Approximately 20% of LQTS-2 mutations reside in the N terminus of HERG1a, where they can truncate the protein, alter gating properties, and/or cause trafficking deficiencies. Since HERG1a and HERG1b are alternate transcripts produced by the HERG1 gene, mutations in exons encoding the HERG1a N-terminus are not likely to affect the production of wild-type HERG1b from this gene. These findings suggest the importance of screening LQTS patients for mutations in the HERG1b-specific exon and for assessing the disease mechanism of all mutations in heterologous expression systems in which HERG1a and HERG1b are co-expressed.

In another aspect, the invention relates to a polyclonal or monoclonal antibody specific for the ERG1b isoform. In this regard, the inventors have produced a polyclonal antibody specific for the ERG1b isoform. It is the only such antibody known to be in existence, and it is useful in many applications, especially for establishing and characterizing the cell lines of the invention and for localizing the ERG1b isoform in vivo. The anti-ERG1b antibody recognizes HERG1b (from humans) and ERG1b (from non-human species).

In a related aspect, the invention further relates to a polyclonal or monoclonal antibody specific for the ERG1a isoform, the antibody being raised against an epitope in the ERG1a subunit, where the epitope shares amino acid similarity with the ERG1b epitope used to produce the ERG1b antibody and has a Jameson-Wolf antigenic index greater than 1. A portion of ERG1a between amino acids 264 and 286, inclusive, has these attributes. Interestingly, the characteristics of this epitope are also found in the 1a/1b C-terminal epitope and the HERG1b N-terminal epitope, used to raise the ERG-KA antibody and the ERG1b antibody, respectively, but in no other contiguous ~20 amino acid long portion of the HERG1a or HERG1b protein. In particular, these regions are characterized by a first pair of basic amino acid residues (such as conservatively related arginine or lysine residues) spaced apart by a single residue and a second pair of adjacent basic residues separated from the aforementioned pair by three to six amino acid residues.

In another related aspect, the cultured cells of the invention can also be employed in a screen for anti-cancer HERG blocker drugs, since it has been reported that HERG channels are upregulated in tumor cells and that proliferation of tumor cells is blocked by HERG blockers (Crociani et al., 2003).

It is an object of the invention to provide a line of cultured cells for screening of pharmacologic agents for an effect on potassium ion channel behavior where the line of cells recapitulates native cardiac I_{Kr} or the I_{Kr} observed in cardiac cells having mutant HERG channels.

It is a feature of the invention that the cultured cells have a cell membrane that comprises HERG potassium ion channels having native or mutant HERG1a and HERG1b subunit components.

Other objects, advantages and features of the invention will become apparent upon consideration of the following description taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. **1** illustrates the schematic topology of N-terminal regions of HERG1a and HERG1b proteins showing divergent N termini (hatched) and identical regions (white and black). Predicted molecular mass for ERG1a is 127 kD, and 90 kD for ERG1b.

FIG. **2** illustrates an overlay of normalized currents recorded from HEK-293 cells expressing HERG1a, HERG1b and both HERG1a/1b.

DETAILED DESCRIPTION OF THE INVENTION

Conventional or native HERG1a and HERG1b channel subunits are encoded by the HERG1 gene and arise as the result of differential splicing. The polynucleotide that encodes native HERG1a is published by NCBI and is presented herein at SEQ ID NO:1. The polynucleotide that encodes native HERG1b is published at http://www.ncbi.nlm.nih.gov/ and is presented herein at SEQ ID NO:3. Additionally, several hundred separate mutations in the HERG1a polynucleotides (often in the portion shared by HERG1a and HERG1b) known to cause various changes in cardiac I_{Kr} are also known, and additional mutations in HERG1 will undoubtedly be reported. The skilled artisan is in possession of various databases of such mutations (as well as polymorphisms). For example, the Working Group on Arrhythmias of the European Society of Cardiology maintains an online database. Also, the Statens Serum Institute maintains a very similar online database. The current data are provided herein at Table 1, but it will be understood that the skilled artisan can be readily apprised of further cataloged mutations in HERG by consulting the literature or database of such mutations. Additionally, one or a plurality of mutations in either HERG1a, HERG1b, or both, can be incorporated using standard tools of the skilled molecular biologist into one or more of the HERG1 subunits.

It will be understood by the skilled artisan that a line of cultured cells produced according to the invention can comprise in the cell membrane a conventional HERG1b subunit or a mutant HERG1b subunit, optionally in combination with a conventional or mutant HERG1a subunit. It will be appreciated that if the cell line is used in a screening method as described herein, then it is desirable for the cell line to express both the HERG1b and the HERG1a subunits. It is of particular interest to evaluate the potassium ion channel behavior of cell lines carrying conventional HERG1a and HERG1b. Also of significant interest is the potassium ion channel behavior of cell lines carrying conventional HERG1b in combination with HERG1a mutants, even more particularly those HERG1a mutants that differ from conventional HERG1a in the N-terminal portion of the protein not shared with HERG1b. Further, when screening ion channels characteristic of HERG1a-defective mutants, a cell line that comprises HERG1b channels alone is indicated. A HERG1a-defective mutant subunit can be expressed in such cells to simulate channel formation and behavior in native tissue. The skilled artisan will appreciate that mutations in HERG1a- or HERG1b (which can be deletions, insertions, or substitutions) can alter, or can have no effect upon, the activity of the subunits or the channels formed therefrom. The attached list of mutations give a flavor for the types of mutations contemplated, but is not to be considered an exhaustive list.

Any of the aforementioned polynucleotides can be cloned into an expression vector (or plurality of expression vectors) engineered to support expression from the polynucleotides.

Suitable expression vectors comprise a transcriptional promoter active in a recipient cell upstream of the HERG1 polynucleotide and can optionally comprise a polyA-addition sequence downstream of the polynucleotide.

Suitable commercially available expression vectors are pcDNA3.1 and pcDNA3.1zeo (Invitrogen), which differ from one another in that pcDNA3.1 includes sequences conferring resistance to neomycin while pcDNA3.1zeo includes sequences conferring resistance to zeocin. The polyA-addition sequences, not required for expression, can be excised from these vectors by digesting both with ApaI (nuc. 1002) and BbsI (nuc. 1217), respectively, filling in, and self-ligating. The vectors can be prepared to receive the HERG1a or HERG1b polynucleotides, by cleavage with EcoRI and BamHI. For convenience during the subsequent selection step, HERG1a can then be cloned into the cleaved pcDNA3.1 vector; HERG1b can be cloned into the cleaved pcDNA3.1zeo vector. In addition, HERG1a and 1b polynucleotides can be ligated into the two multiple cloning sites of a vector with an internal ribosomal entry site, such as pIRES (Novagen), which allows for production of two separate proteins from a single transcript. Use of this vector to produce clonal cell lines allows for selection with a single antibiotic.

The vector(s) can be introduced (or co-introduced) by, for example, transfection or lipofection, into cells competent to receive and express the HERG1 subunits in their cell membrane. A commercially lipofection kit, such as a kit available for this purpose from Mirus Corporation, Madison, Wis., can be employed. Preferably, the recipient cells do not natively contain HERG1 subunits in their cell membranes, so that the presence of HERG1 channels in the membrane is completely attributable to expression from the introduced expression vector. Suitable recipient cells are human embryonic kidney cells such as cells of the HEK-293 cell line, commercially available from the American Type Culture Collection (Accession Number CRL-1573).

Later, preferably about 24 hours later, cells can be harvested, distributed into wells and grown in selective media. In the exemplified embodiment, a selective medium suitable for selecting cells carrying the HERG1a-vector contains neomycin at 500 micrograms/ml, a medium suited for carrying the HERG1b-vector contains zeocin at 100 micrograms/ml, and a medium suited for growing cells carrying both vectors contains both antibiotics. Cells can be grown under selection for 2-3 weeks until the wells are confluent. Resulting clonal lines (24-48 for each type) can be examined biochemically or electrophysiologically to confirm the presence of the HERG1 channel subunit(s) and the level(s) of HERG produced.

EXAMPLES

Materials and Methods

Cell lines and Antibodies. Human embryonic kidney 293 (HEK-293) cell lines stably expressing conventional HERG1a have been previously described by Zhou, Z., et al., *Biophys J.* 74, 230-241 (1998), and by Furutani, M., et al., *Circulation* 99:2290-2294 (1999), each incorporated by reference as if set forth in its entirety. Cell lines stably expressing HERG1a and HERG1b were prepared by transfecting HEK-293/HERG1a stable cells with HERG1b containing a Kozak consensus sequence cloned into the Bam HI/Eco RI sites of pcDNA3.1zeo (Invitrogen, Carlsbad, Calif.). Separate cell colonies were selected after plating cells at low density and grown in media containing 100 μg/ml Zeocin, 500 μg/ml neomycin for selection. All HEK-293 cells were cultured in Dulbecco's modified Eagle's medium at 37° C.

A polyclonal antibody (termed "ERG-KA") raised against a peptide having an amino acid sequence of CRQRKRKLSFRRRTDKDTEQ (corresponding to amino acids 883 through 901 of SEQ ID NO:1 plus a non-essential N-terminal cysteine residue provided to permit coupling of the peptide to an immunogenic carrier) can co-precipitate both HERG1a and HERG1b subunits from cardiac myocytes of the HERG channel protein and is diagnostic for the presence of both HERG1a and HERG1b in HERG channels in vivo or in cultured cell lines in vitro. See Roti Roti, E. et al., "Interaction with GM130 during HERG Ion Channel Trafficking," *J. B. C.,* 277:47779 (2002), incorporated herein by reference as if set forth in its entirety. ERG1 isoform-specific antibodies were produced by Bethyl Laboratories (Montgomery, Tex., USA) in rabbits. Antisera were affinity purified using the same peptides employed in immunization. An immunogenic ERG1b peptide was amino acids 12-25 (GALRPRAQKGRVRR) of SEQ ID NO:4 (HERG1b). The ERG1b antibody was raised against CGALRPRAQKGRVRR, corresponding to the aforementioned amino acids 12-25 plus a non-essential N-terminal cysteine residue provided to permit coupling of the peptide to an immunogenic carrier. An immunogenic ERG1a peptide was amino acids 140-153 (SPAHDTNHRGPPTS) (Neoclone, Madison, Wis.) of SEQ ID NO:2 (HERG1a). A HERG1a-specific antibody raised in goat (HERG N-20) was purchased from Santa Cruz Biotechnologies (Santa Cruz, Calif.). Horseradish Peroxidase—(HRP-) coupled secondary antibodies were purchased from Pierce (Rockford, Ill.) and Santa Cruz Biotechnology (Santa Cruz, Calif.). Fluorophore-coupled secondary antibodies were purchased from Molecular Probes (Lake Oswego, Oreg.).

Cardiac tissue preparation. Human male ventricular lysate was purchased from ProSci Inc. (Poway, Calif.). Canine ventricular myocytes were isolated from mongrel males and enzymatically treated as described by He, J. Q., et al. (2001), incorporated herein by reference as if set forth in its entirety. Sprague-Dawley rat ventricles were excised from anesthetized adult males after injection of sodium Pentobarbital (100 mg/kg body weight intraperitoneal) as described by He, J. Q, et al. Rat ventricular myocytes were prepared using the same procedure as described for the canine tissue. All procedures were approved by the Research Animal Resources Center (RARC) at UW-Madison.

Cell membrane protein preparations. Membranes were prepared from myocytes or ventricular tissue after suspension in homogenization buffer (in mM: 25 Tris-HCl, pH 7.4; 10 NaEGTA; 20 NaEDTA). All buffers used in this procedure contained the following protease inhibitor cocktail: 5 μg/ml aprotinin, 50 μg/ml 1,10 phenanthroline, 0.7 μg/ml pepstatin A, 1.56 μg/ml benzamidine, and 1× Complete minitab (Roche, Indianapolis, Ind.). Suspensions were homogenized using a Polytron homogenizer at setting 6 for two bursts of 15 seconds each, followed by sonication on ice twice at an amplitude of 20 for 20 seconds each. Suspensions were spun at 2,000×g at 4° C. for 10 minutes to remove cellular debris. The supernatants were subjected to further centrifugation at 40,000×g for 30 minutes at 4° C. The resultant pellet was solubilized on a rotary shaker at 4° C. for 2 hours, in either Triton buffer (in mM: 150 NaCl; 25 Tris-HCl, pH 7.4; 20 NaEDTA; 10 NaEGTA; 5 glucose, and 1% v/v Triton X-100), or RIPA buffer (in mM: 150 NaCl; 50 Tris-HCl, pH 7.4, 1 NaEDTA, and 1% v/v Triton X-100, 1% v/v sodium deoxycholate, 0.1% v/v sodium dodecylsulfate). Samples were then spun at 10,000×g to remove insoluble material. Cell line membrane pellets were prepared by washing plates gently with PBS, aspirating, and adding either Triton buffer or RIPA buffer. Cells were then scraped, collected in a microfuge tube, and sonicated on ice twice at an amplitude of 20 for 20 seconds each. The suspension was rotated at 4° C. for 2 hours and then centrifuged at 10,000×g for 10 minutes to remove insoluble material. Protein concentrations of all samples were determined using a modified Bradford assay (DC Protein Assay, Bio-Rad, Hercules, Calif.).

Biochemical Analysis. Membrane proteins were deglycosylated using PNGase F and Endoglycosidase H (Roche Applied Science, Indianapolis, Ind.) as described by Zhou, Z., et al. (1998), supra, and by Zhou, Z., et al., J Biol Chem 273, 21061-21066 (1998), incorporated herein by reference as if set forth in its entirety. Proteins were denatured at 60° C. to avoid thermal aggregation at higher temperatures. To determine which proteins were expressed on the surface membrane, proteins were surface biotinylated using sulfo-NHS-LC-Biotin reagent. Briefly, 100 mm tissue culture dishes with growth at 70-80% confluency were rinsed three times with cold PBS, and incubated with freshly prepared Biotin reagent (5 mg/ml) in PBS for 45 minutes at 4° C. Cells were then rinsed once with 25 mM Tris-HCl (pH 7.5) to quench the reaction, followed by three washes with cold PBS. Membrane proteins were prepared as indicated above.

Western Blot analysis. Membrane proteins (cell lines 2-10 μg/lane; heart lysates 30-50 μg/lane) were separated on 7.5% SDS-polyacrylamide electrophoresis gels along with prestained molecular weight markers (Bio-Rad, Hercules, Calif.), and were then transferred to PVDF membranes (Immobilon-P, Bedford, Mass.) for 1 hour at 100 mV. Western blots were blocked, probed, and analyzed as described. For peptide block experiments, 5 μl antibody was incubated with 10 μg peptide in 100 μl TBS for 6 hours at 4° C., then centrifuged at 10,000×g for 20 min. The supernatant was carefully removed and used to probe Western blots. Western blot controls include probing blots with secondary antibody alone, and peptide block of primary antibody. In the case of heart lysates, a lane containing HERG1a/1b cell membrane preparation was included as a positive control.

Co-immunoprecipitation. Membrane lysates (cell lines: 100-200 μg/reaction; heart lysates: 500-1000 μg/reaction) in 1 ml TBS (150 mM NaCl, 25 mM Tris-HCl, pH 7.4) were cleared with 50 μl Protein A or G sepharose beads (Amersham, Palatine, Ill.) on immunoprecipitating (IP) antibody; Protein A was used for rabbit and Protein G for goat IP antibodies. Cleared lysates were incubated with antibody (anti-ERG1b at 1/100 or N-20 at 1/20) on a rotating platform for 3-16 hours at 4° C. 50 μl Protein A or G coupled beads were added and samples were incubated at 4° C. for an additional 1-3 h. Beads were collected by centrifugation at 10,000×g, and washed three times with 150 mM NaCl, 25 mM Tris-HCl, pH 7.4, 5 mM NaEDTA, 1% (v/v) Trition X-100, followed by one wash with 150 mM NaCl, 25 mM Tris-HCl, pH 7.4. Proteins were eluted with 200 ng/ml antibody-specific peptide for 1 hour at 4° C. Samples were centrifuged at 10,000×g and the supernatant was collected. 100 μl LSB (25 mM Tris-HCl, pH 6.8, 2% v/v sodium dodecylsulfate, 10% glycerol) was added to the beads to elute any proteins that remained bound. Additional controls included lysates processed without antibody. Eluted proteins were Western blotted as described above.

Immunohistochemistry. Isolated canine myocytes were fixed in 2% paraformaldehyde-PBS, pH 7.4 for 10 minutes at room temperature, and were washed 3× in PBS (pH 7.4). Myocytes were then either stored at 4° C. (for up to 8 weeks) or processed immediately. Myocytes were washed once in PBS (pH 7.4)+1% Triton X-100, and permneabilized in PBS (pH 7.4)+0.5% Triton X-100 for 10 minutes at room temperature followed by incubation in 0.75% glycine-PBS (pH 7.4) for 10 minutes at room temperature to quench any free aldehydes, and incubation in blocking buffer (PBS, pH 7.4,+0.1% Tween-20+10% donkey serum+2% BSA) for 2 hours at 4° C., with rotation. Cells were washed 3× with PBS (pH 7.4)+0.1% Tween-20, and divided into 0.5 ml aliquots. Each myocyte aliquot was incubated overnight at 4° C. in diluted primary antibody. ERG1b antibodies were diluted 1:1000, ERG1a antibodies (N-20), 1:10, and myosin binding protein C antibodies, 1:500. Myocytes were washed 3×1 hour in PBS (pH 7.4)+0.1% Tween-20. Secondary antibodies were diluted in PBS (pH 7.4)+0.1% Tween-20+5% BSA, and spun to remove any aggregates. Myocytes were suspended in 0.5 ml of diluted secondary antibody and incubated in the dark 2 hours at room temperature with rotation. Donkey anti-rabbit Alexa 488 and donkey anti-goat Alexa 568 antibodies were diluted 1:1000. Myocytes were washed briefly 3× with PBS (pH 7.4)+0.1% Tween-20 followed by two 1 hour washes with PBS, pH 7.4 and were stored at 4° C. until viewed on a Zeiss Axiovert 200 with a 63× objective. Optical sectioning was accomplished using the Apotome, and 3D rendering done within Axiovision software. Fluorescent excitation-emission filter set for Alexa 488 (excitation 450-490 nm: emission 515-565 nm) and Alexa 568 (excitation 500-639 nm, emission 560-700 nm) do not overlap. Species specificity of secondary antibodies was confirmed by incubating cells probed with one primary with secondary antibody raised against the other species. No signal was detected demonstrating each secondary is species specific. Secondary alone controls were also used to ensure signal was specific.

Results

On a Western blot of rat heart tissue, the ERG-KA antibody identified three bands at 160, 120, and 95 kD. The two higher molecular mass bands are consistent in size with maturely glycosylated and unglycosylated rat ERG1a, respectively. The 95 kD band is consistent in size with ERG1b protein produced in heterologous expression systems (see below) but had not been previously observed in native tissue. The 95 kD band cannot represent ERG-USO, another HERG1 transcript that produces a protein of approximately the same size, since ERG-USO does not contain the C terminal sequence against which the ERG-KA antibody was raised.

To test the hypothesis that the 95 kD band represents ERG1b, Western blots of membrane proteins prepared from HEK-293 cells stably expressing HERG1a and HERG1b were evaluated with the ERG1a- and ERG1b N termini-specific antibodies. The ERG-KA antibody recognized bands at 155 and 135 kD, consistent with previously published results identifying these bands as mature and immature HERG1 glycoforms, respectively. As expected, blots probed with the ERG1a-specific antibody recognized the 155 and 135 kD bands but not the three lower-mass bands. The 155 and 135 kD bands were eliminated upon incubation of the ERG1a antisera with the antigenic HERG1a peptide prior to probing the blots. Notably, cells expressing HERG1a alone produced only the 155 and 135 kD bands, representing the mature and immature HERG1a species, respectively.

ERG-KA also recognized three lower molecular mass bands at 95, 85 and 80 kD. The ERG1b-specific antibody recognized the 95, 85, and 80 kD bands but not the two higher-mass HERG1a bands. These bands were similarly eliminated by preincubation of the antisera with the antigenic HERG1b peptide. These data show that ERG-KA antisera recognize both HERG1a and HERG1b isoforms, and that ERG1a and HERG1b antisera are specific for their corresponding isoforms.

Membrane proteins from stable HEK-293 HERG1a/1b cell lines were incubated with glycosidases to determine if the multiple HERG1b bands on Western blots correspond to different glycoforms, as shown previously for the HERG1a 155 and 135 kD bands. Removing all glycans from the HERG1b proteins by incubating membrane preparations with PNGase F reduced the higher molecular mass HERG1b species to a single 80 kD band. Digestion with Endoglycosidase H, which removes only glycans that are attached in the ER but not yet processed in the Golgi, reduced the 85 kD band to 80 kD but left the 95 kD band unaltered. Thus, the 95 kD band represents the maturely glycosylated (Golgi-processed) HERG1b isoform, the 85 kD band the core glycosylated, ER-retained form, and the 80 kD band the unglycosylated form. To determine if the mature HERG1b glycoform is expressed on the cell surface, where it could contribute to HERG1 currents, surface proteins were biotinylated prior to cell lysis. Biotinylated proteins were affinity purified with streptavidin beads, Western blotted, and probed with ERG-KA antisera. Like HERG1a, only the maturely glycosylated HERG1b (95 kD) protein band was biotinylated, showing that it is expressed on the cell surface in HEK-293 cells.

In Western blots from two separate human ventricular membrane preparations, the ERG-KA antibody revealed bands at 140, 120, 94 and 83 kD. The 140 and 120 kD bands are consistent with previous reports from human tissue and represent the maturely glycosylated and unglycosylated HERG1a, respectively. The HERG1b-specific antibody recognized the 94 and 83 kD bands, demonstrating that ERG1b protein is expressed in human ventricle.

ERG1b was also observed in Western blots of canine ventricular tissue. There the ERG-KA antibody consistently recognized proteins at 160-165 and 90-95 kD and less consistently at 140-145, 115-125 and 80-85 kD. High molecular weight bands at 165 and 140 kD were recognized by both ERG-KA and ERG1a antibodies and thus represent ERG1a isoforms. The ERG1b antibody recognized the bands at 95 and 83 kD, which were also recognized by ERG-KA, demonstrating that these bands represent ERG1b isoforms. These data show conclusively both ERG1a and ERG1b proteins are expressed within the ventricle across a range of mammalian species.

The antibodies were also characterized by immunocytochemistry using confocal microscopy. The HERG-KA antibody detected HERG1a and HERG1b with a very high sensitivity and little background. The HERG1b-specific antibody stained cells expressing HERG1b but not those expressing HERG1a. The ERG1a antibody gave a high background in immunocytochemistry.

Co-immunoprecipitation Evidence for of HERG1a and HERG1b Heteromerization in Animal Tissue and in HEK-293 Cells

Immunoprecipitation studies confirmed that HERG1a and HERG1b co-assemble in heterologous expression systems and in animal tissue. Bidirectional co-immunoprecipitations were carried out in four separate canine cardiac membrane preparations to confirm in vivo assembly. Extracts of fresh, solubilized canine heart tissue were incubated with the HERG1b-specific antibody conjugated to sepharose beads. The beads were concentrated by centrifugation and washed, and the bound proteins were eluted, size-fractionated by SDS-PAGE and transferred to PVDF membrane. At least two membranes were prepared for each eluate. One was probed with the HERG1b-specific antibody to confirm HERG1b immunoprecipitation. The second blot probed with the ERG1a-specific antibody showed HERG1a mature bands, demonstrating HERG1a associates with HERG1b in vivo. The immunoprecipitated proteins were visualized with the HERG-KA antibody, identifying both the precipitating and the associating subunits. The ERG1a-specific antibody immunoprecipitates both mature and immature HERG1a, and co-immunoprecipitates HERG1b. Interestingly, the ERG1a antibody enriched for the mature HERG1b species, relative to its abundance in lysate. Signals were visualized on blots using ECL (Amersham) chemiluminescent detection. The converse IP experiment using bead-bound ERG1a-specific antibody was run to confirm the first IP results. A no-antibody, bead-only control was included in each immunoprecipitation experiment to control for nonspecific precipitation.

Similar results were obtained from one human myocyte preparation immunoprecipitated with the ERG1a specific antibody. These data show that HERG1a and HERG1b proteins associate in mammalian ventricular myocytes in vivo.

To confirm that ERG1a and HERG1b isoforms co-assemble to form heteromeric channels in native tissues, we demonstrated that both ERG1a and ERG1b isoforms are present in rat and canine heart lysates, and that they can be visualized in confocal immunofluorescence microscopy images as puncta along the myocyte sarcolemma, characteristic of a T-tubular distribution. Localization of ERG1a and ERG1b to T-tubular structures in canine ventricular myocytes is consistent with electron microscopy studies in rat myocytes showing ERG1 protein predominantly localized to the T-tubules, where it could regulate action potential duration at the site of excitation-contraction coupling. The signal is similar for both fixed and live cells. No signal was observed when probed only with the secondary antibody. These data suggests that both subunits express at the surface membrane in a similar pattern.

To characterize ERG1 localization more precisely, we stained myocytes concurrently with ERG1a and Myosin binding protein C (MyBP-C) antisera. Three-dimensional images were rendered from a stack of deconvolved two-dimensional immunofluorescent images. MyBP-C signal, in green, appeared as a repeating pattern of doublets separated by regions devoid of fluorescence that span the cell's width. MyBP-C signal localized to the myosin-containing sarcomere A-band; the unstained areas, between doublets, represent M-lines. ERG1a fluorescent signal, shown in red, was seen in I-bands, adjacent to A-bands. Both Z-lines and T-tubules were located in the I-band. The punctate red ERG1a signal extended in columns from the cell surface to the interior, as expected of a T-tubular-restricted protein, where it borders the green MyBP-C signal. These data indicate ERG1 signal in canine myocytes is consistent with a T-tubular distribution.

Co-assembly of HERG1a and HERG1b subunits in stably transfected cell lines.

HERG1a and HERG1b subunits can be co-immunoprecipitated by antibodies specific to either ERG1a or ERG1b subunits from HEK-293 cells. Membrane currents resulting from the co-assembly of these two subunits display characteristic I_{Kr} current profile and sensitivity to E-4031 and anti-arrhythmic drug quinidine.

FIG. **2** illustrates an overlay of normalized currents recorded from HEK-293 cells expressing HERG1a, HERG1b and both HERG1a/1b. The current of the cells that express HERG1a and HERG1b has the characteristic electrophysiological properties of an I_{Kr} current.

The present invention is not intended to be limited to the foregoing embodiments, but rather to encompass all such modifications and variations as come within the scope of the appended claims.

LITERATURE CITED AND INCORPORATED BY REFERENCE AS IF SET FORTH HEREIN IN ITS ENTIRETY

Abbott, G W, Sesti F, Splawski I, Buck M E, Lehmann M H, Timothy K W, Keating M T, Goldstein S A (1999) MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell 97:175-187.

Brette, F., and Orchard, C. (2003) *Circ Res* 92, 1182-1192

Chen, J., Zou, A., Splawski, I., Keating, M. T., and Sanguinetti, M. C. (1999) *J Biol Chem* 274, 10113-10118

Christie, M J, North R A, Osborne P B, Douglass J, Adelman J P (1990) Heteropolymeric potassium channels expressed in *Xenopus* oocytes from cloned subunits. Neuron 4:405-411.

Craig, R., and Offer, G. (1976) *Proc R Soc Lond B Biol Sci* 192, 451-461

Crociani, O, Guasti L, Balzi M, Becchetti A, Wanke E, Olivotto M, Wymore R S, Arcangeli A (2003) Cell cycle-dependent expression of HERG1and HERG1B isoforms in tumor cells. J. Biol. Chem. 278: 2947-2955.

Cui, J, Melman Y, Palma E, Fishman G I, McDonald T V (2000) Cyclic AMP regulates the HERG K(+) channel by dual pathways. Curr Biol 10:671-674.

Curran, M E, Splawski I, Timothy K W, Vincent G M, Green E D, Keating M T (1995) A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell 80:795-803.

Delisle, B. P., Anderson, C. L., Balijepalli, R. C., Anson, B. D., Kamp, T. J., and January, C. T. (2003) *J Biol Chem* 278, 35749-35754

Deutsch, C (2002) Potassium channel ontogeny. Annu Rev Physiol 64:19-46.

Ellgaard, L, Helenius A (2003) Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol 4:181-191.

England, S K, Uebele V N, Shear H, Kodali J, Bennett P B, Tamkun M M (1995) Characterization of a voltage-gated K+ channel beta subunit expressed in human heart. Proc Natl Acad Sci U S A 92:6309-6313.

Ficker, E K, Thomas D, Viswanathan P, Rudy Y, Brown A M (2000) Rescue of a misprocessed mutant HERG channel linked to hereditary long QT syndrome. Biophysical Journal 78:342A.

Finley, M. R., Li, Y., Hua, F., Lillich, J., Mitchell, K. E., Ganta, S., Gilmour, R. F., and Freeman, L. C. (2002) *Am J Physiol* 283, H126-H138

Gossen, M, Freundlieb S, Bender G, Muller G, Hillen W, Bujard H (1995) Transcriptional activation by tetracyclines in mammalian cells. Science 268:1766-1769.

Gulbis, J M, Zhou M, Mann S, MacKinnon R (2000) Structure of the cytoplasmic beta subunit-T1 assembly of voltage-dependent K+ channels. Science 289:123-127.

Guo, W, Li H, Aimond F, Johns D C, Rhodes K J, Trimmer J S, Nerbonne J M (2002) Role of heteromultimers in the generation of myocardial transient outward K+ currents. Circ Res 90:586-593.

Harris, S. P., Bartley, C. R., Hacker, T. A., McDonald, K. S., Douglas, P. S., Greaser, M. L., Powers, P. A., and Moss, R. L. (2002) *Circ Res* 90, 594-601

He, J. Q., Conklin, M. W., Foell, J. D., Wolff, M. R., Haworth, R. A., Coronado, R., and Kamp, T. J. (2001) *Cardiovascular Research* 49, 298-307

Huxley, A. F., and Niedergerke, R. (1954) *Nature* 173, 971-973

Isacoff, E Y, Jan Y N, Jan L Y (1990) Evidence for the formation of heteromultimeric potassium channels in *Xenopus* oocytes. Nature 345:530-534.

Ishii, M, Kurachi Y (2002) The 14-3-3 protein as a novel regulator of ion channel localisation. J Physiol 545:2.

Kagan, A, Melman Y F, Krumerman A, McDonald T V (2002) 14-3-3 amplifies and prolongs adrenergic stimulation of HERG K+ channel activity. EMBO J 21:1889-1898.

Kosolapov, A, Deutsch C (2003) Folding of the voltage-gated K+ channel T1 recognition domain. J Biol Chem 278: 4305-4313.

Kupershmidt, S, Snyders D J, Raes A, Roden D M (1998) A K+ channel splice variant common in human heart lacks a C-terminal domain required for expression of rapidly activating delayed rectifier current. J Biol Chem 273:27231-27235.

Lees-Miller, J P, Kondo C, Wang L, Duff H J (1997) Electrophysiological characterization of an alternatively processed ERG K+ channel in mouse and human hearts. Circ Res 81:719-726.

Lees-Miller, J P, Guo J, Somers J R, Roach D E, Sheldon R S, Rancourt D E, Duff H J (2003) Selective knockout of mouse ERG1 B potassium channel eliminates I(Kr) in adult ventricular myocytes and elicits episodes of abrupt sinus bradycardia. Mol Cell Biol 23:1856-1862.

Li, M, Jan Y N, Jan L Y (1992) Specification of subunit assembly by the hydrophilic amino-terminal domain of the Shaker potassium channel. Science 257:1225-1230.

Li, X, Xu J, Li M (1997) The human delta1261 mutation of the HERG potassium channel results in a truncated protein that contains a subunit interaction domain and decreases the channel expression. J Biol Chem 272:705-708.

London, B, Aydar E, Lewarchik C, Seibel J S, January C T, Robertson G A (1998) N and C-terminal isoforms of HERG in the human heart. Biophysical J.

London, B, Trudeau M C, Newton K P, Beyer A K, Copeland N G, Gilbert D J, Jenkins N A, Satler C A, Robertson G A (1997) Two isoforms of the mouse ether-a-go-go-related gene co-assemble to form channels with properties similar to the rapidly activating component of the cardiac delayed rectifier K+ current. Circ Res 81:870-878.

Lu, J, Robinson J M, Edwards D, Deutsch C (2001) T1-T1 interactions occur in ER membranes while nascent Kv peptides are still attached to ribosomes. Biochemistry 40:10934-10946.

Larsen, L. A., Andersen, P. S., Kanters, J., Svendsen, I. H., Jacobsen, J. R., Vuust, J., Wettrell, G., Tranebjaerg, L., Bathen, J., and Christiansen, M. (2001) *Clinical Chemistry* 47, 1390-1395

MacKinnon, R (1991) Determination of the subunit stoichiometry of a voltage-activated potassium channel. Nature 350:232-235.

Mohammad, S, Zhou Z, Gong Q, January C T (1997) Blockage of the HERG human cardiac K+ channel by the gastrointestinal prokinetic agent cisapride. Am J Physiol 273: H2534-2538.

Morais, Cabral J H, Lee A, Cohen S L, Chait B T, Li M, Mackinnon R (1998) Crystal structure and functional analysis of the HERG potassium channel N terminus: a eukaryotic PAS domain. Cell 95:649-655.

Newitt, R A, Houamed K M, Rehm H, Tempel B L (1991) Potassium channels and epilepsy: evidence that the epileptogenic toxin, dendrotoxin, binds to potassium channel proteins. Epilepsy Res Suppl 4:263-273.

O'Kelly, I, Butler M H, Zilberberg N, Goldstein S A (2002) Forward transport. 14-3-3 binding overcomes retention in endoplasmic reticulum by dibasic signals. Cell 111:577-588.

Papazian, D M, Shao X M, Seoh S A, Mock A F, Huang Y, Wainstock D H (1995) Electrostatic interactions of S4 voltage sensor in Shaker K+ channel. Neuron 14:1293-1301.

Paulussen, A., Raes, A., Matthijs, G., Snyders, D. J., Cohen, N., and Aerssens, J. (2002) *J Biol Chem* 277, 48610-48616

Petcherski, A G, Kimble J (2000) LAG-3 is a putative transcriptional activator in the C. elegans Notch pathway. Nature 405:364-368.

Pond, A L, Scheve B K, Benedict A T, Petrecca K, Van Wagoner D R, Shrier A, Nerbonne J M (2000) Expression of distinct ERG proteins in rat, mouse, and human heart. Relation to functional I(Kr) channels. J Biol Chem 275: 5997-6006.

Rampe, D, Roy M L, Dennis A, Brown A M (1997) A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. FEBS Lett 417:28-32.

Rasmussen, H. B., Moller, M., Knaus, H. G., Jensen, B. S., Olesen, S. P., and Jorgensen, N. K. (2004) *Am J Physiol* 286, H1300-H1309

Robertson, G A (2000) LQT2: amplitude reduction and loss of selectivity in the tail that wags the HERG channel [editorial; comment]. Circ Res 86:492-493.

Roden, D M (1993) Torsade de pointes. Clin Cardiol 16:683-686.

Roti Roti, E C, Myers C D, Ayers R A, Boatman D E, Delfosse S A, Chan E K, Ackerman M J, January C T, Robertson G A (2002) Interaction with GM130 during HERG ion channel trafficking. Disruption by type 2 congenital long QT syndrome mutations. Human Ether-a-go-go-Related Gene. J Biol Chem 277:47779-47785.

Roy, M, Dumaine R, Brown A M (1996) HERG, a primary human ventricular target of the nonsedating antihistamine terfenadine [see comments]. Circulation 94:817-823.

Sanguinetti, M C, Jurkiewicz N K (1990) Two components of cardiac delayed rectifier K+ current. Differential sensitivity to block by class III antiarrhythmic agents. J Gen Physiol 96:195-215.

Sanguinetti, M C, Jiang C, Curran M E, Keating M T (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell 81:299-307.

Sanguinetti, M C, Curran M E, Zou A, Shen J, Spector P S, Atkinson D L, Keating M T (1996), Co-assembly of K(V) LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. Nature 384:80-83.

Shen, N V, Pfaffinger P J (1995) Molecular recognition and assembly sequences involved in the subfamily-specific assembly of voltage-gated K+ channel subunit proteins. Neuron 14:625-633.

Shi, W, Wymore R S, Wang H S, Pan Z, Cohen I S, McKinnon D, Dixon J E (1997) Identification of two nervous system-specific members of the erg potassium channel gene family. J Neurosci 17:9423-9432.

Snyders, D J, Chaudhary A (1996) High affinity open channel block by dofetilide of HERG expressed in a human cell line. Mol Pharmacol 49:949-955.

Splawski, I, Tristani-Firouzi M, Lehmann M H, Sanguinetti M C, Keating M T (1997) Mutations in the hminK gene cause long QT syndrome and suppress IKs function. Nat Genet 17:338-340.

Suessbrich, H, Waldegger S, Lang F, Busch A E (1996) Blockade of HERG channels expressed in *Xenopus* oocytes by the histamine receptor antagonists terfenadine and astemizole. FEBS Lett 385:77-80.

Thomas, D, Zhang W, Karle C A, Kathofer S, Schols W, Kubler W, Kiehn J (1999) Deletion of protein kinase A phosphorylation sites in the HERG potassium channel inhibits activation shift by protein kinase A. J Biol Chem 274:27457-27462.

Trudeau, M C, Warmke J W, Ganetzky B, Robertson G A (1995) HERG, a human inward rectifier in the voltage-gated potassium channel family. Science 269:92-95.

Tu, L, Deutsch C (1999) Evidence for dimerization of dimers in K+ channel assembly. Biophys J 76:2004-2017.

Wang, J, Myers C D, Robertson G A (2000) Dynamic control of deactivation gating by a soluble amino-terminal domain in HERG K(+) channels. J Gen Physiol 115:749-758.

Warmke, J W, Ganetzky B (1994) A family of potassium channel genes related to eag in Drosophila and mammals. Proc Natl Acad Sci U S A 91:3438-3442.

Xu, J, Yu W, Jan Y N, Jan L Y, Li M (1995) Assembly of voltage-gated potassium channels. Conserved hydrophilic motifs determine subfamily-specific interactions between the alpha-subunits. J Biol Chem 270:24761-24768.

Yang, T., Wathen, M. S., Felipe, A., Tamkun, M. M., Snyders, D. J., and Roden, D. M. (1994) *Circ Res* 75, 870-878

Yuan, H, Michelsen K, Schwappach B (2003) 14-3-3 dimers probe the assembly status of multimeric membrane proteins. Curr Biol 13:638-646.

Zerangue, N, Schwappach B, Jan Y N, Jan L Y (1999) A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. Neuron 22:537-548.

Zhou, Z, Gong Q, January C T (1999a) Correction of defective protein trafficking of a mutant HERG potassium channel in human long QT syndrome. Pharmacological and temperature effects. J Biol Chem 274:31123-31126.

Zhou, Z, Gong Q, Epstein M L, January C T (1998a) HERG channel dysfunction in human long QT syndrome. Intracellular transport and functional defects. J Biol Chem 273: 21061-21066.

Zhou, Z, Vorperian V R, Gong Q, Zhang S, January C T (1999b) Block of HERG potassium channels by the antihistamine astemizole and its metabolites desmethylastemizole and norastemizole. J Cardiovasc Electrophysiol 10:836-843.

Zhou, Z, Gong Q, Ye B, Fan Z, Makielski J C, Robertson G A, January C T (1998b) Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. Biophys J 74:230-241.

TABLE 1

KCNH2 (HERG) mutations

Nucleotide change	Mutation	Coding effect	Region	Ethnic background	Reference	More data
—	S26I	Missense	PAS domain	USA	Moss et al, 2002	
87C > A	F29L	Missense	PAS domain	USA	Chen et al, 1999, Splawski et al, 2000	

TABLE 1-continued

KCNH2 (HERG) mutations

Nucleotide change	Mutation	Coding effect	Region	Ethnic background	Reference	More data
98A > C	N33T	Missense	PAS domain	USA	Chen et al, 1999, Splawski et al, 2000	
132C > A	C44X	Missense	PAS domain	USA	Splawski et al, 2000	
140G > T	G47V	Missense	PAS domain	USA	Splawski et al, 2000	
157G > C	G53R	Missense	PAS domain	USA	Chen et al, 1999, Splawski et al, 2000	
167G > A	R56Q	Missense	PAS domain	USA	Chen et al, 1999, Splawski et al, 2000	
196T > G	C66G	Missense	PAS domain	USA	Chen et al, 1999, Splawski et al, 2000	
209A > G	H70R	Missense	PAS domain	USA	Chen et al, 1999, Splawski et al, 2000	
215C > A	P72Q	Missense	PAS domain	USA	Splawski et al, 2000	
221–251del	31 bp deletion	Frameshift	PAS domain	USA	Splawski et al, 2000	
234–250dup	16 bp duplication	Frameshift	PAS domain	USA	Splawski et al, 2000	
—	A78P	Missense	PAS domain	USA	Moss et al, 2002	
241C > T	Q81X	Nonsense	Pas domain	USA	Splawski et al, 2000	
244–252 ins9	82–84insIAQ	Duplication	PAS-domain	Da	Larsen et al, 2001	
—	fs83/37aa	Frameshift	PAS domain	USA	Moss et al, 2002	
257G > T	L86R	Missense	PAS domain	USA	Chen et al, 1999, Splawski et al, 2000	
260T > C	L87P	Missense	PAS domain	Du	Jongbloed et al, 2002	
287T > C	I96T	Missense	PAS-domain	Da	Larsen et al, 2001	
296A > C	Y99S	Missense	PAS domain	Du	Jongbloed et al, 2002	
391A > G	K101E	Missense	PAS-domain	Da	Larsen et al, 2001	
—	M124R	Missense	—	USA	Moss et al, 2002	
422insC	1 bp insertion	Frameshift	PAS-S1	USA	Splawski et al, 2000	
453insC	1 bp insertion	Frameshift	PAS-S1	USA	Splawski et al, 2000	
453delC	1 bp deletion	Frameshift	PAS-S1	Fi	Swan et al, 1999, Lätinen et al, 2000	
526C > T	R176W	Missense	PAS-S1	Fi	Swan et al, 1999, Lätinen et al, 2000	
558–600dup	42 bp duplication	Frameshift	PAS-S1	Mediteranean	Hoorntje et al, 1999	
582–587 delCCGTG	G192fs328	Deletion	PAS-S1	Du	Jongbloed et al, 2002	
724insC	1 bp insertion	Frameshift	PAS-S1	USA	Splawski et al, 2000	
885delC	1 bp insertion	Frameshift	PAS-S1	USA	Splawski et al, 2000	
—	FsV295/63aa	Frameshift	PAS-S1	USA	Moss et al, 2002	
934C > T	R312C	Missense	PAS-S1	USA	Splawski et al, 2000	
1039G > A	P347S	Missense	PAS-S1	USA	Splawski et al, 2000	
1039C > T	P347S	Missense	PAS-S1	Du	Jongbloed et al, 2002	
1096C > T	R366X	Nonsense	PAS-S1	Da	Larsen et al, 2001	
—	Q376-	Splice site	PAS-S1	USa	Moss et al, 2002	
1128G > A	—	Splice site	PAS-S1	USA	Splawski et al, 2000	
1129-2 G > A	—	Splice site	PAS-S1	USA	Splawski et al, 2000	
1199T > A	I100N	Missense	S1	Da	Larsen et al, 2001	
1261delA	1 bp deletion	Frameshift	S1	It	Curran et al, 1995	
1283C > A	S428X	Nonsense	S1-S2	It	Priori et al, 1999	
—	S428L	Missense	S1-S2	USA	Moss et al, 2002	
1307C > T	T436M	Missense	S1-S2	It	Priori et al, 1999	
1341C > A	Y447X	Nonsense	S1-S2	Du	Jongbloed et al, 2002	
1352C > T	P451L	Missense	S2	Fi	Lä¨tinen et al, 2000	
1408A > G	N470D	Missense	S2	Me, Ge, Eng, Da	Curran et al, 1995	
1421C > T	T474I	Missense	S2-S3	Ja	Tanaka et al, 1997	
1479C > G	Y493X	Nonsense	S2-S3	Ja	Itoh et al, 1998	
1496–1523dle	I500-F508del	Deletion	S3	Me, Ge, Eng, Da	Curran et al, 1995	
1501A > G	D501N	Missense	S3	Du	Jongbloed et al, 2002	

TABLE 1-continued

KCNH2 (HERG) mutations

Nucleotide change	Mutation	Coding effect	Region	Ethnic background	Reference	More data
1592G > A	R531Q	Missense	S3	USA	Splawski et al, 2000	
—	L552S	Missense	S3	USA	Moss et al, 2002	
1600C > T	R635C	Missense	S4	Ja	Itoh et al, 1998, Nakajima et al, 1999	
1631delAG	2 bp deletion	frameshift	S4-S5	Fi	Wsan et al, 1999, Lätinen et al, 2000	
1655T > C	L552S	Missense	S5	Fi, USA	Swan et al, 1999, Splawski et al, 2000	
1672G > C	A558P	Missense	S5	Du	Jongbloed et al, 1999	
1681G > A	A561T	Misense	S5	Fr, USA	Dausse et al, 1996, Splawski et al, 2000	
1682C > T	A561V	Missense	S5	Me, Ge, Emg, Da, Ja, USA	Curran et al, 1995, Tanaka et al, 1997, Priori et al, 1999, Larsen et al, unpublished, Splawski et al, 2000	
1691T > C	L564P	Missense	S5	Fr, Ca	St-Pierre et al, 2000	
1705T > C	Y569H	Missense	S5	Fi	Swan et al, 1999, Lätinen et al, 2000	
1714G > C	G572R	Missense	S5-pore	Da	Larsen et al, 2000	
1714G > T	G572C	Missense	S5-pore	Du	Jongbloed et al, 1999	
1750G > A	W585C	Missense	S5-pore	Fi, USA	swan et al, 1999, Splawski et al, 2000, Lätinen et al, 2000	
1755G > T	W585C	Missense	S5-pore	USA	Splawski et al, 2000	
1762A > G	N588D	Missense	S5-pore	USA	Splawski et al, 2000	
1778T > C	I593B	Missense	S5-pore	Eng?, USA	Benson et al, 1996, Splawski et al, 2000	
1778T > G	I593G	Missense	S5-pore	USA	Benson et al, 1996, Splawski et al, 2000	
—	I593X	Nonsense	S5-pore	USA	Moss et al, 2002	
—	P596L	Missense	S5-pore	USA	Moss et al, 2002	
1801G > A	G601S	Missense	S5-pore	Ja, Fi	Akimoto et al, 1997, Swan et al, 1999, Lätinen et al, 2000	
1810G > A	G604S	Missense	S5-pore	Du, USA	Jongbloed et al, 1999, Splawski et al, 2000	
1825G > A	D609N	Missense	S5-pore	USA	Splawski et al, 2000	
1831T > C	Y611H	Missense	S5-pore	Ja	Tanaka et al, 1997	
1833T > A/G	Y611X	Nonsense	S5-pore	Ge	Schultze-Bahr et al, 1995	
1834G > T	V612L	Missense	S5-pore	Ir, It, Du	Satler et al, 1998	
1838C > T	T613M	Missense	S5-Pore	Du, Fi	Jongbloed et al, 1999, Lätinen et al, 2000	
1842C > T	A614V	Missense	Pore	Ja, Ir, Cz, Eng, Fr, Fi, It, Tw	Tanaka et al, 1997, Satler et al, 1998, Splawski er al, 1998, Priori et al, 1999, Lee-Chen et al, 1999	
1843G >	L615V	Missense	Pore	USA	Splawski et al, 2000	
1862G > A	S621N	Missense	Pore	Da	Larsen et al, 2001	
1876G > A	G626S	Missense	Pore	USA	Splawski et al, 2000	
1877G > T	G626V	Missense	pore	Ge	Jahr et al, 2000	
1881G > C	F627L	Missense	Pore	USA	splawski et al, 2000	
1882G > A	G628S	Missense	Pore	Me, Ge, Eng, Da	Curran et al, 1995, Splawski et al, 2000	
1885A > G	N629D	Missense	Pore	Ge, Ir, Na	Satler et al, 1998, Lees-Miller et al, 2000	
1886A > G	N629S	Missense	Pore	Unknown, Da	Satler et al, 1998, Larsen et al, unpublished	
—	N629K	Missense	Pore	Ja	Yoshida et al, 1999	
1888G > C	V630L	Missense	Pore	Ja	Tanaka et al, 1997	
1889T > C	V630A	Missense	Pore	USA	Splawski et al, 1998	
1894C > T	P632S	Missense	Pore	USA	Splawski et al, 2000	
1898A > G	N633S	Missense	Pore	Eng	Satler et al, 1998	
1912A > G	K638E	Missense	S6	USA	Splawski et al, 2000	
1913-15del	K638del	Deletion	S6	USA	Splawski et al, 2000	
1920C > A	F640L	Missense	S6	Du	Jongbloed et al, 1999	
1933A > T	M645L	Missense	S6	USA	Splawski et al, 2000	
1951–1952 delAT	2 bp deletion	Frameshift	S6	Ja	Itoh et al, 1998	
2001C > A	Y667X	Nonsense	S6	Be	Paulussen et al, 2000	
2044G > T	E682X	Nonsense	S6	USA	Splawski et al, 2000	
2092G > T	E698X	Nonsense	S6	Du	Jongbloed et al, 2002	
2173C > T	Q725X	Nonsense	S6	Ja	Itoh et al, 1998	
2218insT	1 bp insertion	Frameshift	S6--	USA	Splawski et al, 2000	
2232C > T	R744X	Nonsense	S6--	Tw	Ko et al, 2001	
2254C > T	R752W	Nonsense	S6--	USA	Splawski et al, 2000, Ficher e al, 2000	
2350C > T	R784W	Missense	S6--	USA	Yang et al, 2002	

TABLE 1-continued

KCNH2 (HERG) mutations

Nucleotide change	Mutation	Coding effect	Region	Ethnic background	Reference	More data
2356–2376 dup	31 bp duplication	Frameshift	CNB domain	Ja	Itoh et al, 1998	
2395delC	1 bp deletion	Frameshift	CNB domain	USA	Splawski et al, 2000	
2398 + 1G > C	IVS9 DS + 1	Splice mutation	CNB domain	USA	Curran et al, 1995	
2414T > C	F805S	Missense	CNB domain	USA	Splawski et al, 2000	
2414T > G	F805C	Missense	CNB domain	USA	Splawski et al, 2000	
2453C > T	S818L	Missense	CNB domain	Fr	Berthet et al, 1999	
2464G > A	V822M	Missense	CNB domain	Ir, Fr	Satler etal, 1996, Berthet et al,	
2467C > T	R823W	Missense	CNB domain	USA	Spalwski et al, 2000	
2471insG	R823fs828	Deletion	CNB domain	Du	Jongbloed et al, 2000	
2582A > T	N861I	Missense	C-terminal	USA	Splawski et al, 2000	
2592 + 1G > A	IVS1 + DS + 1	Frameshift	C-terminal	Be, USA	Berthet et al, 1999 Splawski et al, 2000	
2616delC	P872fs877	Deletion	C-terminal	Du	Jongbloed et al, 2002	
2660delG	1 bp deletion	Frameshift	C-termnal	USA	Splawski et al, 2000	
2750C > T	R917L	Missense	C-terminal	USA	Splawski et al, 2000	
2762delA	1bP deletion	Frameshift	C-terminal	USA	Splawski et al, 2000	
2764C > T	R922W	Missense	C-terminal	USA	Splawski et al, 2000	
2775insG	1 bp insertion	Frameshift	C-terminal	USA	Splawski et al, 2000	
2906delG	1 bp deletion	Frameshift	C-terminal	USA	Splawski et al, 2000	
—	P968/4aa	Frameshift	C-terminal	USA	Moss et al, 2002	
2959delCT	2 bp deletion	Frameshift	C-terminal	USA	Splawski et al, 2000	
—	W1001X	Nonsense	C-terminal	USA	Moss et al, 2002	
3040C > T	R1014X	Nonsense	C-terminal	USA	Splawski et al, 2000	
3094delC	1 bp deletion	Frameshift	C-terminal	USA	Splawski et al, 2000	
3108insG	—	Frameshift	C-terminal	Sp	Berthet et al, 1999	
3303insC	1 bp insertion	Frameshift	C-terminal	USA	Splawski et al, 2000	
			Aminoacid polymorphisms			
2690A > C	K897T	—	—	—	Iwasa et al, 2000, Lätinen et al, 2000, Yang et al, 2002	
—	R1047L	—	—	—	Larsen et al, 2001	
			Single nucleotide polymorphisms (SNPs)			
IVS2 + 27G > C	Intron variant	SNP	—	—	Jongbloed et al, 2002	
IVS8-61 A/G	Intron variant	SNP	—	—	Larsen et al, 2001	
IVS13 + 12 C/A	Intron variant	SNP	—	—	Larsen et al, 2001	
IVS13 + 22 A/G	Intron variant	SNP	—	—	Larsen et al, 2001	
1467C > T	I4891	SNP	—	—	Akimoto et al, 1997	
1539C > T	F513F	SNP	—	—	Akimoto et al, 1997	
1692A > G	L564L	SNP	—	—	Akimoto et al, 1997	
1956T > C	Y652Y	SNP	—	—	Larsen et al, 1999	
2965 + 22 A > G	Intronic variant	—	—	—	Iwasa et al, 2000	

[1] Ja: Japanese, Ir: Irish, Cz: Czech, Eng: English., Ge: German, Fr: French, Fil: Filipino, Fi: Finnish, Na: Native american, Me. Mexican, Da: Danish, It: Italian, Du: Dutch, Sp: Spanish. Be: Belgian.Tw: Taiwanese.

[2] Region distal to S6.

SEQUENCE LISTING

```
<160> NUMBER OF SEQ ID NOS: 4

<210> SEQ ID NO 1
<211> LENGTH: 3900
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (14)..(3493)

<400> SEQUENCE: 1

ccatgggctc agg atg ccg gtg cgg agg ggc cac gtc gcg ccg cag aac        49
               Met Pro Val Arg Arg Gly His Val Ala Pro Gln Asn
               1               5                   10

acc ttc ctg gac acc atc atc cgc aag ttt gag ggc cag agc cgt aag       97
Thr Phe Leu Asp Thr Ile Ile Arg Lys Phe Glu Gly Gln Ser Arg Lys
        15                  20                  25

ttc atc atc gcc aac gct cgg gtg gag aac tgc gcc gtc atc tac tgc      145
Phe Ile Ile Ala Asn Ala Arg Val Glu Asn Cys Ala Val Ile Tyr Cys
    30                  35                  40

aac gac ggc ttc tgc gag ctg tgc ggc tac tcg cgg gcc gag gtg atg      193
Asn Asp Gly Phe Cys Glu Leu Cys Gly Tyr Ser Arg Ala Glu Val Met
45                  50                  55                  60

cag cga ccc tgc acc tgc gac ttc ctg cac ggg ccg cgc acg cag cgc      241
Gln Arg Pro Cys Thr Cys Asp Phe Leu His Gly Pro Arg Thr Gln Arg
                65                  70                  75

cgc gct gcc gcg cag atc gcg cag gca ctg ctg ggc gcc gag gag cgc      289
Arg Ala Ala Ala Gln Ile Ala Gln Ala Leu Leu Gly Ala Glu Glu Arg
            80                  85                  90

aaa gtg gaa atc gcc ttc tac cgg aaa gat ggg agc tgc ttc cta tgt      337
Lys Val Glu Ile Ala Phe Tyr Arg Lys Asp Gly Ser Cys Phe Leu Cys
        95                  100                 105

ctg gtg gat gtg gtg ccc gtg aag aac gag gat ggg gct gtc atc atg      385
Leu Val Asp Val Val Pro Val Lys Asn Glu Asp Gly Ala Val Ile Met
    110                 115                 120

ttc atc ctc aat ttc gag gtg gtg atg gag aag gac atg gtg ggg tcc      433
Phe Ile Leu Asn Phe Glu Val Val Met Glu Lys Asp Met Val Gly Ser
125                 130                 135                 140

ccg gct cat gac acc aac cac cgg ggc ccc ccc acc agc tgg ctg gcc      481
Pro Ala His Asp Thr Asn His Arg Gly Pro Pro Thr Ser Trp Leu Ala
                145                 150                 155

cca ggc cgc gcc aag acc ttc cgc ctg aag ctg ccc gcg ctg ctg gcg      529
Pro Gly Arg Ala Lys Thr Phe Arg Leu Lys Leu Pro Ala Leu Leu Ala
            160                 165                 170

ctg acg gcc cgg gag tcg tcg gtg cgg tcg ggc ggc gcg ggc ggc gcg      577
Leu Thr Ala Arg Glu Ser Ser Val Arg Ser Gly Gly Ala Gly Gly Ala
        175                 180                 185

ggc gcc ccg ggg gcc gtg gtg gtg gac gtg gac ctg acg ccc gcg gca      625
Gly Ala Pro Gly Ala Val Val Val Asp Val Asp Leu Thr Pro Ala Ala
    190                 195                 200

ccc agc agc gag tcg ctg gcc ctg gac gaa gtg aca gcc atg gac aac      673
Pro Ser Ser Glu Ser Leu Ala Leu Asp Glu Val Thr Ala Met Asp Asn
205                 210                 215                 220

cac gtg gca ggg ctc ggg ccc gcg gag gag cgg cgt gcg ctg gtg ggt      721
His Val Ala Gly Leu Gly Pro Ala Glu Glu Arg Arg Ala Leu Val Gly
                225                 230                 235

ccc ggc tct ccg ccc cgc agc gcg ccc ggc cag ctc cca tcg ccc cgg      769
Pro Gly Ser Pro Pro Arg Ser Ala Pro Gly Gln Leu Pro Ser Pro Arg
            240                 245                 250
```

-continued

```
gcg cac agc ctc aac ccc gac gcc tcg ggc tcc agc tgc agc ctg gcc       817
Ala His Ser Leu Asn Pro Asp Ala Ser Gly Ser Ser Cys Ser Leu Ala
        255                 260                 265

cgg acg cgc tcc cga gaa agc tgc gcc agc gtg cgc cgc gcc tcg tcg       865
Arg Thr Arg Ser Arg Glu Ser Cys Ala Ser Val Arg Arg Ala Ser Ser
    270                 275                 280

gcc gac gac atc gag gcc atg cgc gcc ggg gtg ctg ccc ccg cca ccg       913
Ala Asp Asp Ile Glu Ala Met Arg Ala Gly Val Leu Pro Pro Pro Pro
285                 290                 295                 300

cgc cac gcc agc acc ggg gcc atg cac cca ctg cgc agc ggc ttg ctc       961
Arg His Ala Ser Thr Gly Ala Met His Pro Leu Arg Ser Gly Leu Leu
                305                 310                 315

aac tcc acc tcg gac tcc gac ctc gtg cgc tac cgc acc att agc aag      1009
Asn Ser Thr Ser Asp Ser Asp Leu Val Arg Tyr Arg Thr Ile Ser Lys
            320                 325                 330

att ccc caa atc acc ctc aac ttt gtg gac ctc aag ggc gac ccc ttc      1057
Ile Pro Gln Ile Thr Leu Asn Phe Val Asp Leu Lys Gly Asp Pro Phe
        335                 340                 345

ttg gct tcg ccc acc agt gac cgt gag atc ata gca cct aag ata aag      1105
Leu Ala Ser Pro Thr Ser Asp Arg Glu Ile Ile Ala Pro Lys Ile Lys
    350                 355                 360

gag cga acc cac aat gtc act gag aag gtc acc cag gtc ctg tcc ctg      1153
Glu Arg Thr His Asn Val Thr Glu Lys Val Thr Gln Val Leu Ser Leu
365                 370                 375                 380

ggc gcc gac gtg ctg cct gag tac aag ctg cag gca ccg cgc atc cac      1201
Gly Ala Asp Val Leu Pro Glu Tyr Lys Leu Gln Ala Pro Arg Ile His
                385                 390                 395

cgc tgg acc atc ctg cat tac agc ccc ttc aag gcc gtg tgg gac tgg      1249
Arg Trp Thr Ile Leu His Tyr Ser Pro Phe Lys Ala Val Trp Asp Trp
            400                 405                 410

ctc atc ctg ctg ctg gtc atc tac acg gct gtc ttc aca ccc tac tcg      1297
Leu Ile Leu Leu Leu Val Ile Tyr Thr Ala Val Phe Thr Pro Tyr Ser
        415                 420                 425

gct gcc ttc ctg ctg aag gag acg gaa gaa ggc ccg cct gct acc gag      1345
Ala Ala Phe Leu Leu Lys Glu Thr Glu Glu Gly Pro Pro Ala Thr Glu
    430                 435                 440

tgt ggc tac gcc tgc cag ccg ctg gct gtg gtg gac ctc atc gtg gac      1393
Cys Gly Tyr Ala Cys Gln Pro Leu Ala Val Val Asp Leu Ile Val Asp
445                 450                 455                 460

atc atg ttc att gtg gac atc ctc atc aac ttc cgc acc acc tac gtc      1441
Ile Met Phe Ile Val Asp Ile Leu Ile Asn Phe Arg Thr Thr Tyr Val
                465                 470                 475

aat gcc aac gag gag gtg gtc agc cac ccc ggc cgc atc gcc gtc cac      1489
Asn Ala Asn Glu Glu Val Val Ser His Pro Gly Arg Ile Ala Val His
            480                 485                 490

tac ttc aag ggc tgg ttc ctc atc gac atg gtg gcc gcc atc ccc ttc      1537
Tyr Phe Lys Gly Trp Phe Leu Ile Asp Met Val Ala Ala Ile Pro Phe
        495                 500                 505

gac ctg ctc atc ttc ggc tct ggc tct gag gag ctg atc ggg ctg ctg      1585
Asp Leu Leu Ile Phe Gly Ser Gly Ser Glu Glu Leu Ile Gly Leu Leu
    510                 515                 520

aag act gcg cgg ctg ctg cgg ctg gtg cgc gtg gcg cgg aag ctg gat      1633
Lys Thr Ala Arg Leu Leu Arg Leu Val Arg Val Ala Arg Lys Leu Asp
525                 530                 535                 540

cgc tac tca gag tac ggc gcg gcc gtg ctg ttc ttg ctc atg tgc acc      1681
Arg Tyr Ser Glu Tyr Gly Ala Ala Val Leu Phe Leu Leu Met Cys Thr
                545                 550                 555

ttt gcg ctc atc gcg cac tgg cta gcc tgc atc tgg tac gcc atc ggc      1729
Phe Ala Leu Ile Ala His Trp Leu Ala Cys Ile Trp Tyr Ala Ile Gly
            560                 565                 570
```

-continued

```
aac atg gag cag cca cac atg gac tca cgc atc ggc tgg ctg cac aac     1777
Asn Met Glu Gln Pro His Met Asp Ser Arg Ile Gly Trp Leu His Asn
        575                 580                 585

ctg ggc gac cag ata ggc aaa ccc tac aac agc agc ggc ctg ggc ggc     1825
Leu Gly Asp Gln Ile Gly Lys Pro Tyr Asn Ser Ser Gly Leu Gly Gly
    590                 595                 600

ccc tcc atc aag gac aag tat gtg acg gcg ctc tac ttc acc ttc agc     1873
Pro Ser Ile Lys Asp Lys Tyr Val Thr Ala Leu Tyr Phe Thr Phe Ser
605                 610                 615                 620

agc ctc acc agt gtg ggc ttc ggc aac gtc tct ccc aac acc aac tca     1921
Ser Leu Thr Ser Val Gly Phe Gly Asn Val Ser Pro Asn Thr Asn Ser
                625                 630                 635

gag aag atc ttc tcc atc tgc gtc atg ctc att ggc tcc ctc atg tat     1969
Glu Lys Ile Phe Ser Ile Cys Val Met Leu Ile Gly Ser Leu Met Tyr
            640                 645                 650

gct agc atc ttc ggc aac gtg tcg gcc atc atc cag cgg ctg tac tcg     2017
Ala Ser Ile Phe Gly Asn Val Ser Ala Ile Ile Gln Arg Leu Tyr Ser
        655                 660                 665

ggc aca gcc cgc tac cac aca cag atg ctg cgg gtg cgg gag ttc atc     2065
Gly Thr Ala Arg Tyr His Thr Gln Met Leu Arg Val Arg Glu Phe Ile
    670                 675                 680

cgc ttc cac cag atc ccc aat ccc ctg cgc cag cgc ctc gag gag tac     2113
Arg Phe His Gln Ile Pro Asn Pro Leu Arg Gln Arg Leu Glu Glu Tyr
685                 690                 695                 700

ttc cag cac gcc tgg tcc tac acc aac ggc atc gac atg aac gcg gtg     2161
Phe Gln His Ala Trp Ser Tyr Thr Asn Gly Ile Asp Met Asn Ala Val
                705                 710                 715

ctg aag ggc ttc cct gag tgc ctg cag gct gac atc tgc ctg cac ctg     2209
Leu Lys Gly Phe Pro Glu Cys Leu Gln Ala Asp Ile Cys Leu His Leu
            720                 725                 730

aac cgc tca ctg ctg cag cac tgc aaa ccc ttc cga ggg gcc acc aag     2257
Asn Arg Ser Leu Leu Gln His Cys Lys Pro Phe Arg Gly Ala Thr Lys
        735                 740                 745

ggc tgc ctt cgg gcc ctg gcc atg aag ttc aag acc aca cat gca ccg     2305
Gly Cys Leu Arg Ala Leu Ala Met Lys Phe Lys Thr Thr His Ala Pro
    750                 755                 760

cca ggg gac aca ctg gtg cat gct ggg gac ctg ctc acc gcc ctg tac     2353
Pro Gly Asp Thr Leu Val His Ala Gly Asp Leu Leu Thr Ala Leu Tyr
765                 770                 775                 780

ttc atc tcc cgg ggc tcc atc gag atc ctg cgg ggc gac gtc gtc gtg     2401
Phe Ile Ser Arg Gly Ser Ile Glu Ile Leu Arg Gly Asp Val Val Val
                785                 790                 795

gcc atc ctg ggg aag aat gac atc ttt ggg gag cct ctg aac ctg tat     2449
Ala Ile Leu Gly Lys Asn Asp Ile Phe Gly Glu Pro Leu Asn Leu Tyr
            800                 805                 810

gca agg cct ggc aag tcg aac ggg gat gtg cgg gcc ctc acc tac tgt     2497
Ala Arg Pro Gly Lys Ser Asn Gly Asp Val Arg Ala Leu Thr Tyr Cys
        815                 820                 825

gac cta cac aag atc cat cgg gac gac ctg ctg gag gtg ctg gac atg     2545
Asp Leu His Lys Ile His Arg Asp Asp Leu Leu Glu Val Leu Asp Met
    830                 835                 840

tac cct gag ttc tcc gac cac ttc tgg tcc agc ctg gag atc acc ttc     2593
Tyr Pro Glu Phe Ser Asp His Phe Trp Ser Ser Leu Glu Ile Thr Phe
845                 850                 855                 860

aac ctg cga gat acc aac atg atc ccg ggc tcc ccc ggc agt acg gag     2641
Asn Leu Arg Asp Thr Asn Met Ile Pro Gly Ser Pro Gly Ser Thr Glu
                865                 870                 875

tta gag ggt ggc ttc agt cgg caa cgc aag cgc aag ttg tcc ttc cgc     2689
Leu Glu Gly Gly Phe Ser Arg Gln Arg Lys Arg Lys Leu Ser Phe Arg
            880                 885                 890
```

-continued

```
agg cgc acg gac aag gac acg gag cag cca ggg gag gtg tcg gcc ttg      2737
Arg Arg Thr Asp Lys Asp Thr Glu Gln Pro Gly Glu Val Ser Ala Leu
        895                 900                 905

ggg ccg ggc cgg gcg ggg gca ggg ccg agt agc cgg ggc cgg ccg ggg      2785
Gly Pro Gly Arg Ala Gly Ala Gly Pro Ser Ser Arg Gly Arg Pro Gly
    910                 915                 920

ggg ccg tgg ggg gag agc ccg tcc agt ggc ccc tcc agc cct gag agc      2833
Gly Pro Trp Gly Glu Ser Pro Ser Ser Gly Pro Ser Ser Pro Glu Ser
925                 930                 935                 940

agt gag gat gag ggc cca ggc cgc agc tcc agc ccc ctc cgc ctg gtg      2881
Ser Glu Asp Glu Gly Pro Gly Arg Ser Ser Ser Pro Leu Arg Leu Val
                945                 950                 955

ccc ttc tcc agc ccc agg ccc ccc gga gag ccg ccg ggt ggg gag ccc      2929
Pro Phe Ser Ser Pro Arg Pro Pro Gly Glu Pro Pro Gly Gly Glu Pro
            960                 965                 970

ctg atg gag gac tgc gag aag agc agc gac act tgc aac ccc ctg tca      2977
Leu Met Glu Asp Cys Glu Lys Ser Ser Asp Thr Cys Asn Pro Leu Ser
        975                 980                 985

ggc gcc ttc tca gga gtg tcc aac att ttc agc ttc  tgg ggg gac agt     3025
Gly Ala Phe Ser Gly Val Ser Asn Ile Phe Ser Phe  Trp Gly Asp Ser
    990                 995                 1000

cgg  ggc cgc cag tac cag  gag ctc cct cga tgc  ccc gcc ccc acc       3070
Arg  Gly Arg Gln Tyr Gln  Glu Leu Pro Arg Cys  Pro Ala Pro Thr
1005                 1010                 1015

ccc  agc ctc ctc aac atc  ccc ctc tcc agc ccg  ggt cgg cgg ccc       3115
Pro  Ser Leu Leu Asn Ile  Pro Leu Ser Ser Pro  Gly Arg Arg Pro
1020                 1025                 1030

cgg  ggc gac gtg gag agc  agg ctg gat gcc ctc  cag cgc cag ctc       3160
Arg  Gly Asp Val Glu Ser  Arg Leu Asp Ala Leu  Gln Arg Gln Leu
1035                 1040                 1045

aac  agg ctg gag acc cgg  ctg agt gca gac atg  gcc act gtc ctg       3205
Asn  Arg Leu Glu Thr Arg  Leu Ser Ala Asp Met  Ala Thr Val Leu
1050                 1055                 1060

cag  ctg cta cag agg cag  atg acg ctg gtc ccg  ccc gcc tac agt       3250
Gln  Leu Leu Gln Arg Gln  Met Thr Leu Val Pro  Pro Ala Tyr Ser
1065                 1070                 1075

gct  gtg acc acc ccg ggg  cct ggc ccc act tcc  aca tcc ccg ctg       3295
Ala  Val Thr Thr Pro Gly  Pro Gly Pro Thr Ser  Thr Ser Pro Leu
1080                 1085                 1090

ttg  ccc gtc agc ccc ctc  ccc acc ctc acc ttg  gac tcg ctt tct       3340
Leu  Pro Val Ser Pro Leu  Pro Thr Leu Thr Leu  Asp Ser Leu Ser
1095                 1100                 1105

cag  gtt tcc cag ttc atg  gcg tgt gag gag ctg  ccc ccg ggg gcc       3385
Gln  Val Ser Gln Phe Met  Ala Cys Glu Glu Leu  Pro Pro Gly Ala
1110                 1115                 1120

cca  gag ctt ccc caa gaa  ggc ccc aca cga cgc  ctc tcc cta ccg       3430
Pro  Glu Leu Pro Gln Glu  Gly Pro Thr Arg Arg  Leu Ser Leu Pro
1125                 1130                 1135

ggc  cag ctg ggg gcc ctc  acc tcc cag ccc ctg  cac aga cac ggc       3475
Gly  Gln Leu Gly Ala Leu  Thr Ser Gln Pro Leu  His Arg His Gly
1140                 1145                 1150

tcg  gac ccg ggc agt tag tggggctgcc cagtgtggac acgtggctca            3523
Ser  Asp Pro Gly Ser
1155

cccagggatc aaggcgctgc tgggccgctc cccttggagg ccctgctcag gaggccctga    3583

ccgtggaagg ggagaggaac tcgaaagcac agctcctccc ccagcccttg ggaccatctt    3643

ctcctgcagt cccctgggcc ccagtgagag gggcaggggc agggccggca gtaggtgggg    3703

cctgtggtcc ccccactgcc ctgagggcat tagctggtct aactgcccgg aggcacccgg    3763
```

-continued

```
ccctgggcct taggcacctc aaggactttt ctgctattta ctgctcttat tgttaaggat   3823

aataattaag gatcatatga ataattaatg aagatgctga tgactatgaa taataaataa   3883

ttatcctgag gagaaaa                                                  3900


<210> SEQ ID NO 2
<211> LENGTH: 1159
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Met Pro Val Arg Arg Gly His Val Ala Pro Gln Asn Thr Phe Leu Asp
1               5                   10                  15

Thr Ile Ile Arg Lys Phe Glu Gly Gln Ser Arg Lys Phe Ile Ile Ala
            20                  25                  30

Asn Ala Arg Val Glu Asn Cys Ala Val Ile Tyr Cys Asn Asp Gly Phe
        35                  40                  45

Cys Glu Leu Cys Gly Tyr Ser Arg Ala Glu Val Met Gln Arg Pro Cys
    50                  55                  60

Thr Cys Asp Phe Leu His Gly Pro Arg Thr Gln Arg Arg Ala Ala Ala
65                  70                  75                  80

Gln Ile Ala Gln Ala Leu Leu Gly Ala Glu Glu Arg Lys Val Glu Ile
                85                  90                  95

Ala Phe Tyr Arg Lys Asp Gly Ser Cys Phe Leu Cys Leu Val Asp Val
            100                 105                 110

Val Pro Val Lys Asn Glu Asp Gly Ala Val Ile Met Phe Ile Leu Asn
        115                 120                 125

Phe Glu Val Val Met Glu Lys Asp Met Val Gly Ser Pro Ala His Asp
    130                 135                 140

Thr Asn His Arg Gly Pro Pro Thr Ser Trp Leu Ala Pro Gly Arg Ala
145                 150                 155                 160

Lys Thr Phe Arg Leu Lys Leu Pro Ala Leu Leu Ala Leu Thr Ala Arg
                165                 170                 175

Glu Ser Ser Val Arg Ser Gly Gly Ala Gly Gly Ala Gly Ala Pro Gly
            180                 185                 190

Ala Val Val Val Asp Val Asp Leu Thr Pro Ala Ala Pro Ser Ser Glu
        195                 200                 205

Ser Leu Ala Leu Asp Glu Val Thr Ala Met Asp Asn His Val Ala Gly
    210                 215                 220

Leu Gly Pro Ala Glu Glu Arg Arg Ala Leu Val Gly Pro Gly Ser Pro
225                 230                 235                 240

Pro Arg Ser Ala Pro Gly Gln Leu Pro Ser Pro Arg Ala His Ser Leu
                245                 250                 255

Asn Pro Asp Ala Ser Gly Ser Ser Cys Ser Leu Ala Arg Thr Arg Ser
            260                 265                 270

Arg Glu Ser Cys Ala Ser Val Arg Arg Ala Ser Ser Ala Asp Asp Ile
        275                 280                 285

Glu Ala Met Arg Ala Gly Val Leu Pro Pro Pro Pro Arg His Ala Ser
    290                 295                 300

Thr Gly Ala Met His Pro Leu Arg Ser Gly Leu Leu Asn Ser Thr Ser
305                 310                 315                 320

Asp Ser Asp Leu Val Arg Tyr Arg Thr Ile Ser Lys Ile Pro Gln Ile
                325                 330                 335

Thr Leu Asn Phe Val Asp Leu Lys Gly Asp Pro Phe Leu Ala Ser Pro
            340                 345                 350
```

-continued

```
Thr Ser Asp Arg Glu Ile Ile Ala Pro Lys Ile Lys Glu Arg Thr His
        355                 360                 365

Asn Val Thr Glu Lys Val Thr Gln Val Leu Ser Leu Gly Ala Asp Val
    370                 375                 380

Leu Pro Glu Tyr Lys Leu Gln Ala Pro Arg Ile His Arg Trp Thr Ile
385                 390                 395                 400

Leu His Tyr Ser Pro Phe Lys Ala Val Trp Asp Trp Leu Ile Leu Leu
                405                 410                 415

Leu Val Ile Tyr Thr Ala Val Phe Thr Pro Tyr Ser Ala Ala Phe Leu
            420                 425                 430

Leu Lys Glu Thr Glu Glu Gly Pro Pro Ala Thr Glu Cys Gly Tyr Ala
        435                 440                 445

Cys Gln Pro Leu Ala Val Val Asp Leu Ile Val Asp Ile Met Phe Ile
    450                 455                 460

Val Asp Ile Leu Ile Asn Phe Arg Thr Thr Tyr Val Asn Ala Asn Glu
465                 470                 475                 480

Glu Val Val Ser His Pro Gly Arg Ile Ala Val His Tyr Phe Lys Gly
                485                 490                 495

Trp Phe Leu Ile Asp Met Val Ala Ala Ile Pro Phe Asp Leu Leu Ile
            500                 505                 510

Phe Gly Ser Gly Ser Glu Glu Leu Ile Gly Leu Leu Lys Thr Ala Arg
        515                 520                 525

Leu Leu Arg Leu Val Arg Val Ala Arg Lys Leu Asp Arg Tyr Ser Glu
    530                 535                 540

Tyr Gly Ala Ala Val Leu Phe Leu Leu Met Cys Thr Phe Ala Leu Ile
545                 550                 555                 560

Ala His Trp Leu Ala Cys Ile Trp Tyr Ala Ile Gly Asn Met Glu Gln
                565                 570                 575

Pro His Met Asp Ser Arg Ile Gly Trp Leu His Asn Leu Gly Asp Gln
            580                 585                 590

Ile Gly Lys Pro Tyr Asn Ser Ser Gly Leu Gly Gly Pro Ser Ile Lys
        595                 600                 605

Asp Lys Tyr Val Thr Ala Leu Tyr Phe Thr Phe Ser Ser Leu Thr Ser
    610                 615                 620

Val Gly Phe Gly Asn Val Ser Pro Asn Thr Asn Ser Glu Lys Ile Phe
625                 630                 635                 640

Ser Ile Cys Val Met Leu Ile Gly Ser Leu Met Tyr Ala Ser Ile Phe
                645                 650                 655

Gly Asn Val Ser Ala Ile Ile Gln Arg Leu Tyr Ser Gly Thr Ala Arg
            660                 665                 670

Tyr His Thr Gln Met Leu Arg Val Arg Glu Phe Ile Arg Phe His Gln
        675                 680                 685

Ile Pro Asn Pro Leu Arg Gln Arg Leu Glu Glu Tyr Phe Gln His Ala
    690                 695                 700

Trp Ser Tyr Thr Asn Gly Ile Asp Met Asn Ala Val Leu Lys Gly Phe
705                 710                 715                 720

Pro Glu Cys Leu Gln Ala Asp Ile Cys Leu His Leu Asn Arg Ser Leu
                725                 730                 735

Leu Gln His Cys Lys Pro Phe Arg Gly Ala Thr Lys Gly Cys Leu Arg
            740                 745                 750

Ala Leu Ala Met Lys Phe Lys Thr Thr His Ala Pro Pro Gly Asp Thr
        755                 760                 765
```

-continued

```
Leu Val His Ala Gly Asp Leu Leu Thr Ala Leu Tyr Phe Ile Ser Arg
    770                 775                 780

Gly Ser Ile Glu Ile Leu Arg Gly Asp Val Val Val Ala Ile Leu Gly
785                 790                 795                 800

Lys Asn Asp Ile Phe Gly Glu Pro Leu Asn Leu Tyr Ala Arg Pro Gly
                805                 810                 815

Lys Ser Asn Gly Asp Val Arg Ala Leu Thr Tyr Cys Asp Leu His Lys
            820                 825                 830

Ile His Arg Asp Asp Leu Leu Glu Val Leu Asp Met Tyr Pro Glu Phe
        835                 840                 845

Ser Asp His Phe Trp Ser Ser Leu Glu Ile Thr Phe Asn Leu Arg Asp
    850                 855                 860

Thr Asn Met Ile Pro Gly Ser Pro Gly Ser Thr Glu Leu Glu Gly Gly
865                 870                 875                 880

Phe Ser Arg Gln Arg Lys Arg Lys Leu Ser Phe Arg Arg Arg Thr Asp
                885                 890                 895

Lys Asp Thr Glu Gln Pro Gly Glu Val Ser Ala Leu Gly Pro Gly Arg
            900                 905                 910

Ala Gly Ala Gly Pro Ser Ser Arg Gly Arg Pro Gly Gly Pro Trp Gly
        915                 920                 925

Glu Ser Pro Ser Ser Gly Pro Ser Ser Pro Glu Ser Ser Glu Asp Glu
    930                 935                 940

Gly Pro Gly Arg Ser Ser Ser Pro Leu Arg Leu Val Pro Phe Ser Ser
945                 950                 955                 960

Pro Arg Pro Pro Gly Glu Pro Pro Gly Gly Glu Pro Leu Met Glu Asp
                965                 970                 975

Cys Glu Lys Ser Ser Asp Thr Cys Asn Pro Leu Ser Gly Ala Phe Ser
            980                 985                 990

Gly Val Ser Asn Ile Phe Ser Phe  Trp Gly Asp Ser Arg  Gly Arg Gln
        995                 1000                 1005

Tyr Gln  Glu Leu Pro Arg Cys  Pro Ala Pro Thr Pro  Ser Leu Leu
    1010                 1015                 1020

Asn Ile  Pro Leu Ser Ser Pro  Gly Arg Arg Pro Arg  Gly Asp Val
    1025                 1030                 1035

Glu Ser  Arg Leu Asp Ala Leu  Gln Arg Gln Leu Asn  Arg Leu Glu
    1040                 1045                 1050

Thr Arg  Leu Ser Ala Asp Met  Ala Thr Val Leu Gln  Leu Leu Gln
    1055                 1060                 1065

Arg Gln  Met Thr Leu Val Pro  Pro Ala Tyr Ser Ala  Val Thr Thr
    1070                 1075                 1080

Pro Gly  Pro Gly Pro Thr Ser  Thr Ser Pro Leu Leu  Pro Val Ser
    1085                 1090                 1095

Pro Leu  Pro Thr Leu Thr Leu  Asp Ser Leu Ser Gln  Val Ser Gln
    1100                 1105                 1110

Phe Met  Ala Cys Glu Glu Leu  Pro Pro Gly Ala Pro  Glu Leu Pro
    1115                 1120                 1125

Gln Glu  Gly Pro Thr Arg Arg  Leu Ser Leu Pro Gly  Gln Leu Gly
    1130                 1135                 1140

Ala Leu  Thr Ser Gln Pro Leu  His Arg His Gly Ser  Asp Pro Gly
    1145                 1150                 1155

Ser
```

-continued

```
<210> SEQ ID NO 3
<211> LENGTH: 3191
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (325)..(2784)

<400> SEQUENCE: 3

ccggctggag gagctgaggt tccgagtgcg gccgctgctg ggctggcggg cgggcagagc     60

acggcaccct ggcagcaggg cccacgccac ggggccatgg gcagctcgag ccaggcaggc    120

tgctgcccac gcttactgcc agggtgaccc cagccctggg gcccagccac aaccaccctg    180

gcttcatgcc aggggctgct ctggttgcca gtcggccagc ctcgggggtg cagcctgggc    240

tgggactgct gctggggtgc aggtgaggca gtggccgggc cctcaggccc cagggcaggc    300

aggctgcagg gagccaagtc ctcc atg gcg gcc cca gcc ggg aag gcg agc       351
                          Met Ala Ala Pro Ala Gly Lys Ala Ser
                          1               5

agg aca ggg gct ctg cgg ccc agg gcc cag aaa ggc cgg gtg agg cgg      399
Arg Thr Gly Ala Leu Arg Pro Arg Ala Gln Lys Gly Arg Val Arg Arg
10                  15                  20                  25

gcc gtg cgc atc tcc agc ctc gtg gcc cag gag gtc ctg tcc ctg ggc      447
Ala Val Arg Ile Ser Ser Leu Val Ala Gln Glu Val Leu Ser Leu Gly
                30                  35                  40

gcc gac gtg ctg cct gag tac aag ctg cag gca ccg cgc atc cac cgc      495
Ala Asp Val Leu Pro Glu Tyr Lys Leu Gln Ala Pro Arg Ile His Arg
            45                  50                  55

tgg acc atc ctg cat tac agc ccc ttc aag gcc gtg tgg gac tgg ctc      543
Trp Thr Ile Leu His Tyr Ser Pro Phe Lys Ala Val Trp Asp Trp Leu
        60                  65                  70

atc ctg ctg ctg gtc atc tac acg gct gtc ttc aca ccc tac tcg gct      591
Ile Leu Leu Leu Val Ile Tyr Thr Ala Val Phe Thr Pro Tyr Ser Ala
    75                  80                  85

gcc ttc ctg ctg aag gag acg gaa gaa ggc ccg cct gct acc gag tgt      639
Ala Phe Leu Leu Lys Glu Thr Glu Glu Gly Pro Pro Ala Thr Glu Cys
90                  95                  100                 105

ggc tac gcc tgc cag ccg ctg gct gtg gtg gac ctc atc gtg gac atc      687
Gly Tyr Ala Cys Gln Pro Leu Ala Val Val Asp Leu Ile Val Asp Ile
                110                 115                 120

atg ttc att gtg gac atc ctc atc aac ttc cgc acc acc tac gtc aat      735
Met Phe Ile Val Asp Ile Leu Ile Asn Phe Arg Thr Thr Tyr Val Asn
            125                 130                 135

gcc aac gag gag gtg gtc agc cac ccc ggc cgc atc gcc gtc cac tac      783
Ala Asn Glu Glu Val Val Ser His Pro Gly Arg Ile Ala Val His Tyr
        140                 145                 150

ttc aag ggc tgg ttc ctc atc gac atg gtg gcc gcc atc ccc ttc gac      831
Phe Lys Gly Trp Phe Leu Ile Asp Met Val Ala Ala Ile Pro Phe Asp
    155                 160                 165

ctg ctc atc ttc ggc tct ggc tct gag gag ctg atc ggg ctg ctg aag      879
Leu Leu Ile Phe Gly Ser Gly Ser Glu Glu Leu Ile Gly Leu Leu Lys
170                 175                 180                 185

act gcg cgg ctg ctg cgg ctg gtg cgc gtg gcg cgg aag ctg gat cgc      927
Thr Ala Arg Leu Leu Arg Leu Val Arg Val Ala Arg Lys Leu Asp Arg
                190                 195                 200

tac tca gag tac ggc gcg gcc gtg ctg ttc ttg ctc atg tgc acc ttt      975
Tyr Ser Glu Tyr Gly Ala Ala Val Leu Phe Leu Leu Met Cys Thr Phe
            205                 210                 215

gcg ctc atc gcg cac tgg cta gcc tgc atc tgg tac gcc atc ggc aac     1023
Ala Leu Ile Ala His Trp Leu Ala Cys Ile Trp Tyr Ala Ile Gly Asn
        220                 225                 230
```

-continued

```
atg gag cag cca cac atg gac tca cgc atc ggc tgg ctg cac aac ctg         1071
Met Glu Gln Pro His Met Asp Ser Arg Ile Gly Trp Leu His Asn Leu
    235                 240                 245

ggc gac cag ata ggc aaa ccc tac aac agc agc ggc ctg ggc ggc ccc         1119
Gly Asp Gln Ile Gly Lys Pro Tyr Asn Ser Ser Gly Leu Gly Gly Pro
250                 255                 260                 265

tcc atc aag gac aag tat gtg acg gcg ctc tac ttc acc ttc agc agc         1167
Ser Ile Lys Asp Lys Tyr Val Thr Ala Leu Tyr Phe Thr Phe Ser Ser
                270                 275                 280

ctc acc agt gtg ggc ttc ggc aac gtc tct ccc aac acc aac tca gag         1215
Leu Thr Ser Val Gly Phe Gly Asn Val Ser Pro Asn Thr Asn Ser Glu
            285                 290                 295

aag atc ttc tcc atc tgc gtc atg ctc att ggc tcc ctc atg tat gct         1263
Lys Ile Phe Ser Ile Cys Val Met Leu Ile Gly Ser Leu Met Tyr Ala
        300                 305                 310

agc atc ttc ggc aac gtg tcg gcc atc atc cag cgg ctg tac tcg ggc         1311
Ser Ile Phe Gly Asn Val Ser Ala Ile Ile Gln Arg Leu Tyr Ser Gly
    315                 320                 325

aca gcc cgc tac cac aca cag atg ctg cgg gtg cgg gag ttc atc cgc         1359
Thr Ala Arg Tyr His Thr Gln Met Leu Arg Val Arg Glu Phe Ile Arg
330                 335                 340                 345

ttc cac cag atc ccc aat ccc ctg cgc cag cgc ctc gag gag tac ttc         1407
Phe His Gln Ile Pro Asn Pro Leu Arg Gln Arg Leu Glu Glu Tyr Phe
                350                 355                 360

cag cac gcc tgg tcc tac acc aac ggc atc gac atg aac gcg gtg ctg         1455
Gln His Ala Trp Ser Tyr Thr Asn Gly Ile Asp Met Asn Ala Val Leu
            365                 370                 375

aag ggc ttc cct gag tgc ctg cag gct gac atc tgc ctg cac ctg aac         1503
Lys Gly Phe Pro Glu Cys Leu Gln Ala Asp Ile Cys Leu His Leu Asn
        380                 385                 390

cgc tca ctg ctg cag cac tgc aaa ccc ttc cga ggg gcc acc aag ggc         1551
Arg Ser Leu Leu Gln His Cys Lys Pro Phe Arg Gly Ala Thr Lys Gly
    395                 400                 405

tgc ctt cgg gcc ctg gcc atg aag ttc aag acc aca cat gca ccg cca         1599
Cys Leu Arg Ala Leu Ala Met Lys Phe Lys Thr Thr His Ala Pro Pro
410                 415                 420                 425

ggg gac aca ctg gtg cat gct ggg gac ctg ctc acc gcc ctg tac ttc         1647
Gly Asp Thr Leu Val His Ala Gly Asp Leu Leu Thr Ala Leu Tyr Phe
                430                 435                 440

atc tcc cgg ggc tcc atc gag atc ctg cgg ggc gac gtc gtc gtg gcc         1695
Ile Ser Arg Gly Ser Ile Glu Ile Leu Arg Gly Asp Val Val Val Ala
            445                 450                 455

atc ctg ggg aag aat gac atc ttt ggg gag cct ctg aac ctg tat gca         1743
Ile Leu Gly Lys Asn Asp Ile Phe Gly Glu Pro Leu Asn Leu Tyr Ala
        460                 465                 470

agg cct ggc aag tcg aac ggg gat gtg cgg gcc ctc acc tac tgt gac         1791
Arg Pro Gly Lys Ser Asn Gly Asp Val Arg Ala Leu Thr Tyr Cys Asp
    475                 480                 485

cta cac aag atc cat cgg gac gac ctg ctg gag gtg ctg gac atg tac         1839
Leu His Lys Ile His Arg Asp Asp Leu Leu Glu Val Leu Asp Met Tyr
490                 495                 500                 505

cct gag ttc tcc gac cac ttc tgg tcc agc ctg gag atc acc ttc aac         1887
Pro Glu Phe Ser Asp His Phe Trp Ser Ser Leu Glu Ile Thr Phe Asn
                510                 515                 520

ctg cga gat acc aac atg atc ccg ggc tcc ccc ggc agt acg gag tta         1935
Leu Arg Asp Thr Asn Met Ile Pro Gly Ser Pro Gly Ser Thr Glu Leu
            525                 530                 535

gag ggt ggc ttc agt cgg caa cgc aag cgc aag ttg tcc ttc cgc agg         1983
Glu Gly Gly Phe Ser Arg Gln Arg Lys Arg Lys Leu Ser Phe Arg Arg
        540                 545                 550
```

-continued

```
cgc acg gac aag gac acg gag cag cca ggg gag gtg tcg gcc ttg ggg     2031
Arg Thr Asp Lys Asp Thr Glu Gln Pro Gly Glu Val Ser Ala Leu Gly
    555                 560                 565

ccg ggc cgg gcg ggg gca ggg ccg agt agc cgg ggc cgg ccg ggg ggg     2079
Pro Gly Arg Ala Gly Ala Gly Pro Ser Ser Arg Gly Arg Pro Gly Gly
570                 575                 580                 585

ccg tgg ggg gag agc ccg tcc agt ggc ccc tcc agc cct gag agc agt     2127
Pro Trp Gly Glu Ser Pro Ser Ser Gly Pro Ser Ser Pro Glu Ser Ser
                590                 595                 600

gag gat gag ggc cca ggc cgc agc tcc agc ccc ctc cgc ctg gtg ccc     2175
Glu Asp Glu Gly Pro Gly Arg Ser Ser Ser Pro Leu Arg Leu Val Pro
            605                 610                 615

ttc tcc agc ccc agg ccc ccc gga gag ccg ccg ggt ggg gag ccc ctg     2223
Phe Ser Ser Pro Arg Pro Pro Gly Glu Pro Pro Gly Gly Glu Pro Leu
        620                 625                 630

atg gag gac tgc gag aag agc agc gac act tgc aac ccc ctg tca ggc     2271
Met Glu Asp Cys Glu Lys Ser Ser Asp Thr Cys Asn Pro Leu Ser Gly
    635                 640                 645

gcc ttc tca gga gtg tcc aac att ttc agc ttc tgg ggg gac agt cgg     2319
Ala Phe Ser Gly Val Ser Asn Ile Phe Ser Phe Trp Gly Asp Ser Arg
650                 655                 660                 665

ggc cgc cag tac cag gag ctc cct cga tgc ccc gcc ccc acc ccc agc     2367
Gly Arg Gln Tyr Gln Glu Leu Pro Arg Cys Pro Ala Pro Thr Pro Ser
                670                 675                 680

ctc ctc aac atc ccc ctc tcc agc ccg ggt cgg cgg ccc cgg ggc gac     2415
Leu Leu Asn Ile Pro Leu Ser Ser Pro Gly Arg Arg Pro Arg Gly Asp
            685                 690                 695

gtg gag agc agg ctg gat gcc ctc cag cgc cag ctc aac agg ctg gag     2463
Val Glu Ser Arg Leu Asp Ala Leu Gln Arg Gln Leu Asn Arg Leu Glu
        700                 705                 710

acc cgg ctg agt gca gac atg gcc act gtc ctg cag ctg cta cag agg     2511
Thr Arg Leu Ser Ala Asp Met Ala Thr Val Leu Gln Leu Leu Gln Arg
    715                 720                 725

cag atg acg ctg gtc ccg ccc gcc tac agt gct gtg acc acc ccg ggg     2559
Gln Met Thr Leu Val Pro Pro Ala Tyr Ser Ala Val Thr Thr Pro Gly
730                 735                 740                 745

cct ggc ccc act tcc aca tcc ccg ctg ttg ccc gtc agc ccc ctc ccc     2607
Pro Gly Pro Thr Ser Thr Ser Pro Leu Leu Pro Val Ser Pro Leu Pro
                750                 755                 760

acc ctc acc ttg gac tcg ctt tct cag gtt tcc cag ttc atg gcg tgt     2655
Thr Leu Thr Leu Asp Ser Leu Ser Gln Val Ser Gln Phe Met Ala Cys
            765                 770                 775

gag gag ctg ccc ccg ggg gcc cca gag ctt ccc caa gaa ggc ccc aca     2703
Glu Glu Leu Pro Pro Gly Ala Pro Glu Leu Pro Gln Glu Gly Pro Thr
        780                 785                 790

cga cgc ctc tcc cta ccg ggc cag ctg ggg gcc ctc acc tcc cag ccc     2751
Arg Arg Leu Ser Leu Pro Gly Gln Leu Gly Ala Leu Thr Ser Gln Pro
    795                 800                 805

ctg cac aga cac ggc tcg gac ccg ggc agt tag tggggctgcc cagtgtggac   2804
Leu His Arg His Gly Ser Asp Pro Gly Ser
810                 815

acgtggctca cccagggatc aaggcgctgc tgggccgctc cccttggagg ccctgctcag   2864

gaggccctga ccgtggaagg ggagaggaac tcgaaagcac agctcctccc ccagcccttg   2924

ggaccatctt ctcctgcagt cccctgggcc ccagtgagag gggcaggggc agggccggca   2984

gtaggtgggg cctgtggtcc ccccactgcc ctgagggcat tagctggtct aactgcccgg   3044

aggcacccgg ccctgggcct taggcacctc aaggactttt ctgctattta ctgctcttat   3104
```

```
tgttaaggat aataattaag gatcatatga ataattaatg aagatgctga tgactatgaa   3164

taataaataa ttatcctgag gagaaaa                                         3191


<210> SEQ ID NO 4
<211> LENGTH: 819
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Ala Ala Pro Ala Gly Lys Ala Ser Arg Thr Gly Ala Leu Arg Pro
1               5                   10                  15

Arg Ala Gln Lys Gly Arg Val Arg Arg Ala Val Arg Ile Ser Ser Leu
            20                  25                  30

Val Ala Gln Glu Val Leu Ser Leu Gly Ala Asp Val Leu Pro Glu Tyr
        35                  40                  45

Lys Leu Gln Ala Pro Arg Ile His Arg Trp Thr Ile Leu His Tyr Ser
    50                  55                  60

Pro Phe Lys Ala Val Trp Asp Trp Leu Ile Leu Leu Leu Val Ile Tyr
65                  70                  75                  80

Thr Ala Val Phe Thr Pro Tyr Ser Ala Ala Phe Leu Leu Lys Glu Thr
                85                  90                  95

Glu Glu Gly Pro Pro Ala Thr Glu Cys Gly Tyr Ala Cys Gln Pro Leu
            100                 105                 110

Ala Val Val Asp Leu Ile Val Asp Ile Met Phe Ile Val Asp Ile Leu
        115                 120                 125

Ile Asn Phe Arg Thr Thr Tyr Val Asn Ala Asn Glu Glu Val Val Ser
    130                 135                 140

His Pro Gly Arg Ile Ala Val His Tyr Phe Lys Gly Trp Phe Leu Ile
145                 150                 155                 160

Asp Met Val Ala Ala Ile Pro Phe Asp Leu Leu Ile Phe Gly Ser Gly
                165                 170                 175

Ser Glu Glu Leu Ile Gly Leu Leu Lys Thr Ala Arg Leu Leu Arg Leu
            180                 185                 190

Val Arg Val Ala Arg Lys Leu Asp Arg Tyr Ser Glu Tyr Gly Ala Ala
        195                 200                 205

Val Leu Phe Leu Leu Met Cys Thr Phe Ala Leu Ile Ala His Trp Leu
    210                 215                 220

Ala Cys Ile Trp Tyr Ala Ile Gly Asn Met Glu Gln Pro His Met Asp
225                 230                 235                 240

Ser Arg Ile Gly Trp Leu His Asn Leu Gly Asp Gln Ile Gly Lys Pro
                245                 250                 255

Tyr Asn Ser Ser Gly Leu Gly Gly Pro Ser Ile Lys Asp Lys Tyr Val
            260                 265                 270

Thr Ala Leu Tyr Phe Thr Phe Ser Ser Leu Thr Ser Val Gly Phe Gly
        275                 280                 285

Asn Val Ser Pro Asn Thr Asn Ser Glu Lys Ile Phe Ser Ile Cys Val
    290                 295                 300

Met Leu Ile Gly Ser Leu Met Tyr Ala Ser Ile Phe Gly Asn Val Ser
305                 310                 315                 320

Ala Ile Ile Gln Arg Leu Tyr Ser Gly Thr Ala Arg Tyr His Thr Gln
                325                 330                 335

Met Leu Arg Val Arg Glu Phe Ile Arg Phe His Gln Ile Pro Asn Pro
            340                 345                 350
```

-continued

```
Leu Arg Gln Arg Leu Glu Glu Tyr Phe Gln His Ala Trp Ser Tyr Thr
        355                 360                 365

Asn Gly Ile Asp Met Asn Ala Val Leu Lys Gly Phe Pro Glu Cys Leu
    370                 375                 380

Gln Ala Asp Ile Cys Leu His Leu Asn Arg Ser Leu Leu Gln His Cys
385                 390                 395                 400

Lys Pro Phe Arg Gly Ala Thr Lys Gly Cys Leu Arg Ala Leu Ala Met
                405                 410                 415

Lys Phe Lys Thr Thr His Ala Pro Pro Gly Asp Thr Leu Val His Ala
            420                 425                 430

Gly Asp Leu Leu Thr Ala Leu Tyr Phe Ile Ser Arg Gly Ser Ile Glu
        435                 440                 445

Ile Leu Arg Gly Asp Val Val Val Ala Ile Leu Gly Lys Asn Asp Ile
    450                 455                 460

Phe Gly Glu Pro Leu Asn Leu Tyr Ala Arg Pro Gly Lys Ser Asn Gly
465                 470                 475                 480

Asp Val Arg Ala Leu Thr Tyr Cys Asp Leu His Lys Ile His Arg Asp
                485                 490                 495

Asp Leu Leu Glu Val Leu Asp Met Tyr Pro Glu Phe Ser Asp His Phe
            500                 505                 510

Trp Ser Ser Leu Glu Ile Thr Phe Asn Leu Arg Asp Thr Asn Met Ile
        515                 520                 525

Pro Gly Ser Pro Gly Ser Thr Glu Leu Glu Gly Gly Phe Ser Arg Gln
    530                 535                 540

Arg Lys Arg Lys Leu Ser Phe Arg Arg Arg Thr Asp Lys Asp Thr Glu
545                 550                 555                 560

Gln Pro Gly Glu Val Ser Ala Leu Gly Pro Gly Arg Ala Gly Ala Gly
                565                 570                 575

Pro Ser Ser Arg Gly Arg Pro Gly Gly Pro Trp Gly Glu Ser Pro Ser
            580                 585                 590

Ser Gly Pro Ser Ser Pro Glu Ser Ser Glu Asp Glu Gly Pro Gly Arg
        595                 600                 605

Ser Ser Ser Pro Leu Arg Leu Val Pro Phe Ser Ser Pro Arg Pro Pro
    610                 615                 620

Gly Glu Pro Pro Gly Gly Glu Pro Leu Met Glu Asp Cys Glu Lys Ser
625                 630                 635                 640

Ser Asp Thr Cys Asn Pro Leu Ser Gly Ala Phe Ser Gly Val Ser Asn
                645                 650                 655

Ile Phe Ser Phe Trp Gly Asp Ser Arg Gly Arg Gln Tyr Gln Glu Leu
            660                 665                 670

Pro Arg Cys Pro Ala Pro Thr Pro Ser Leu Leu Asn Ile Pro Leu Ser
        675                 680                 685

Ser Pro Gly Arg Arg Pro Arg Gly Asp Val Glu Ser Arg Leu Asp Ala
    690                 695                 700

Leu Gln Arg Gln Leu Asn Arg Leu Glu Thr Arg Leu Ser Ala Asp Met
705                 710                 715                 720

Ala Thr Val Leu Gln Leu Leu Gln Arg Gln Met Thr Leu Val Pro Pro
                725                 730                 735

Ala Tyr Ser Ala Val Thr Thr Pro Gly Pro Gly Pro Thr Ser Thr Ser
            740                 745                 750

Pro Leu Leu Pro Val Ser Pro Leu Pro Thr Leu Thr Leu Asp Ser Leu
        755                 760                 765
```

-continued

```
Ser Gln Val Ser Gln Phe Met Ala Cys Glu Glu Leu Pro Pro Gly Ala
    770                 775                 780

Pro Glu Leu Pro Gln Glu Gly Pro Thr Arg Arg Leu Ser Leu Pro Gly
785                 790                 795                 800

Gln Leu Gly Ala Leu Thr Ser Gln Pro Leu His Arg His Gly Ser Asp
                805                 810                 815

Pro Gly Ser
```

We claim:

1. A cultured mammalian cell line characterized in that cells in the line express an HERG1b subunit component comprising SEQ ID NO:4, wherein the cell line does not natively express the HERG1b subunit.

2. A cultured cell line as claimed in claim **1** further comprising an HERG1a subunit component comprising SEQ ID NO:2.

3. A cultured cell line as claimed in claim **1** wherein cells in the line comprise SEQ ID NO:3 from nucleotide 325 to nucleotide 2784.

4. A cultured cell line as claimed in claim **3** wherein the cell line further comprises SEQ ID NO:1 from nucleotide 14 to nucleotide 3493.

5. A cultured mammalian cell line that produces a membrane current, wherein cells in the line comprising a first heterologous polynucleotide that encodes HERG1a and a second heterologous polynucleotide that encodes HERG1b, the current being characterized as exhibiting rapid inactivation, slow deactivation under a step voltage protocol and sensitivity to a methanesulfonanilide drug, wherein the first polynucleotide comprises SEQ ID NO:1 from nucleotide 14 to nucleotide 3493, and wherein the second polynucleotide comprises SEQ ID NO:3 from nucleotide 325 to nucleotide 2784, and wherein the cell line does not natively express the first and second heterologous polynucleotides.

6. A cultured cell line as claimed in claim **5** wherein the cell line is a human cell line.

7. A cultured cell line as claimed in claim **6** wherein the human cell line is HEK-293 stably transformed with the first and second polynucleotides.

* * * * *