Long-QT Syndrome–Associated Missense Mutations in the Pore Helix of the HERG Potassium Channel

Fu-De Huang, MD; Jun Chen, PhD; Monica Lin, MD; Mark T. Keating, MD; Michael C. Sanguinetti, PhD

Background—Mutations in the human ether-à-go-go–related gene (HERG) cause chromosome 7–linked long-QT syndrome (LQTS), an inherited disorder of cardiac repolarization that predisposes affected individuals to arrhythmia and sudden death.

Methods and Results—Here, we characterize the physiological consequences of 3 LQTS-associated missense mutations (V612L, T613M, and L615V) located in the pore helix of the HERG channel subunit. Mutant HERG subunits were heterologously expressed in Xenopus oocytes alone or in combination with wild-type HERG subunits. Two-microelectrode voltage-clamp techniques were used to record currents, and a single oocyte chemiluminescence assay was used to assay surface expression of epitope-tagged subunits. When expressed alone, V612L and T613M HERG subunits did not induce detectable currents, and L615V induced very small currents. Coexpression of mutant and wild-type HERG subunits caused a dominant-negative effect that varied for each mutation.

Conclusions—These findings define the physiological consequences of mutations in HERG that cause LQTS and indicate the importance of the pore helix of HERG for normal channel function. (Circulation. 2001;104:1071-1075.)

Key Words: arrhythmia ■ long-QT syndrome ■ ion channels

Mutations in several cardiac ion channel genes, including HERG, MIRP1, KVLQT1, MinK, and SCN5A, cause an inherited disorder of ventricular repolarization, the long-QT syndrome (LQTS).1–5 The hallmark clinical sign of this disease is prolongation of the QT interval on the body surface ECG, indicative of lengthened ventricular action potentials.6 Affected individuals have intermittent arrhythmia and an increased risk of sudden death due to ventricular fibrillation.7 The most common cause of LQTS is mutations in the genes that encode subunits of K+$^+$ channels that conduct the rapid (I_{kr}) and slow (I_{ks}) delayed rectifier K+$^+$ currents.8–11 The rapid delayed rectifier K+$^+$ channel is formed by coassembly of 4 HERG α-subunits and MIRP1 β-subunits.5 Dominant mutations in HERG are a common cause of the Romano-Ward form of LQTS.12 Although individual mutations in HERG are located throughout the protein, clustering of mutations has been found in a few regions. For example, the amino-terminal domain has 12 reported missense mutations.12 Subunits containing a missense mutation in this domain cause an accelerated rate of channel deactivation.13 The pore helix is another region of the HERG channel subunit that has a cluster of LQTS-associated mutations. The pore helix is located between the turret and the selectivity filter of K+$^+$ channel subunits and, on the basis of analysis of the KcsA channel,14 is hypothesized to coordinate a single K+$^+$ ion in the inner cavity of the channel. Missense mutations in 5 consecutive amino acids in the pore helix (Figure 1A) have been reported.12,15–17 The functional consequences of 2 of the LQTS-associated mutations located in the pore helix (Y611H and A614V) have been characterized and found to cause loss of function.18,19

Here, we report the effects of previously unstudied LQTS-associated missense mutations in 3 other amino acids (V612L, T613M, and L615V) located in the pore helix of the HERG subunit (Figure 1B). Two-microelectrode voltage clamp of Xenopus oocytes was used to characterize the functional expression of channels formed by mutant subunits alone or when coexpressed with wild-type (WT) HERG subunits. A single-cell chemiluminescence assay20 was used to compare the relative plasma membrane surface expression of WT and mutant HERG subunits. All 3 missense mutations caused loss of function associated with a decrease in surface expression of protein and had a variable dominant-negative effect when coexpressed with WT subunits.

Methods

Construction of HERG Mutations and In Vitro Transcription of cRNA

Three reported LQTS-associated HERG missense mutations that have not previously been characterized were prepared for this study:
Figure 1. Location of LQTS-associated missense mutations in pore helix of HERG channel subunit. A, Sequence of HERG pore helix and 5 residues (in italics) found to be mutated in LQTS. LQTS-associated changes of individual amino acids are shown in blue. B, Single KcsA K^+ channel subunit showing location of 3 missense mutations studied in this report. Green α-helix corresponds to S6 domain; outer helix (equivalent to S5 in HERG) and pore helix are shown in yellow.

V612L, T613M, and L615V. Mutations were introduced into WT HERG cDNA by the megaprimer method. The mutant construct was then subcloned into the pSP64 (Promega) plasmid expression vector. Constructs were characterized by restriction mapping and DNA sequence analyses. cRNA for injection into oocytes was prepared with SP6 Capscribe (Boehringer Mannheim) according to the manufacturer’s protocol as previously described.24,25 HERG inactivation was determined by use of a 3-pulse voltage-pulse protocol.26 The Holding potential for all experiments was −70 mV.

Results

Expression of Mutant Channels Alone

The current induced by injection of 10 ng cRNA/oocyte of WT HERG (Figure 2A) was almost twice as large as that induced by injection of 5 ng cRNA/oocyte. For example, at −0 mV, the peak current during a 1-second pulse was 72% larger in oocytes injected with 10 ng than with 5 ng of cRNA (Figure 2B).

The lack of functional channel expression could be caused by a defect in protein processing and reduced trafficking to the surface membrane. Therefore, we monitored surface expression of WT and mutant HA-tagged HERG proteins. Oocytes were injected with 5 ng of cRNA encoding HA-tagged HERG subunits and incubated for 3 days before

Chemiluminescence Assay for Surface Expression of HERG Protein

To monitor surface expression of channels, a modified hemagglutinin protein (HA) epitope was inserted between G516 and S517 located within the S3–S4 extracellular loop of WT or mutant HERG subunits. The amino acid sequence of the HA-tagged region was GISAYGITYPYDVPDYAI. The chemiluminescence assay was performed as described by Zerangue et al.20 Relative light units were counted for 4 seconds with an MLX microtiter plate luminometer.

Figure 2. Representative currents recorded from oocytes expressing WT or mutant HERG channels. A, Currents recorded from an oocyte 3 days after injection with 10 ng WT HERG cRNA. Currents were elicited with 1-second pulses to test potentials of −70 to +40 mV. Tail currents were measured at −70 mV. B, Current–voltage relationships for WT HERG channel current elicited with 1-second pulses to indicated test potential in oocytes injected with 5 (●) or 10 (○) ng cRNA (n=10 for 10 ng; n=10 for 5 ng). C, Currents recorded from oocytes injected with 30 ng of specified mutant HERG cRNA.
Coexpression of WT and Mutant HERG Channel Subunits

The mutations in HERG studied here were inherited in a dominant manner. Therefore, we determined whether mutant subunits caused a dominant-negative effect when coexpressed with WT HERG subunits. For these experiments, oocytes were injected with 5 ng WT HERG cRNA or 5 ng WT HERG + 5 ng mutant HERG cRNA. Under these conditions, a combination of equal amounts of WT cRNA with cRNA encoding a loss-of-function mutant that did not interact with WT subunits was expected to elicit the same amount of HERG channel current as observed for oocytes injected with 5 ng WT HERG cRNA alone. Currents were recorded 3 days later for each group of oocytes, all harvested at the same time from the same frog. Currents were elicited with 1-second pulses applied every 15 seconds to a test potential ranging from −70 to +60 mV from a holding potential of −80 mV. Deactivating (tail) currents were measured at −70 mV. Examples of currents recorded from oocytes expressing either WT HERG subunits alone or WT + T613M HERG subunits are shown in Figure 4A and 4B. For all 3 mutations, the peak current during the test pulse (Figure 4C) and the peak tail current at −70 mV (Figure 4D) were significantly reduced in oocytes expressing mutant + WT HERG subunits compared with oocytes expressing only WT HERG subunits. These findings indicated that all 3 mutant subunits were capable of interaction with WT HERG subunits. The dominant-negative effect of the LQTS-associated mutation was greatest for T613M, intermediate for V612L, and least for L615V HERG. Note that the current magnitudes plotted in Figure 4C and 4D can also be compared with the current induced by 10 ng WT HERG cRNA plotted in Figure 2B.

We previously reported that LQTS-associated missense mutations in the amino-terminus of HERG accelerated channel deactivation. Therefore, we determined the effect of each mutation on the biexponential time course of HERG current deactivation for voltages from −120 to −60 mV. The rate of deactivation in oocytes expressing WT + L615V HERG subunits was not different from that for WT HERG, but deactivation was slower for the other 2 mutant HERG subunits at −60 and −70 mV (Figure 4E and 4F).

Voltage Dependence of HERG Channel Activation and Inactivation

We first determined whether coexpression of WT and mutant HERG subunits altered the voltage dependence of channel activation by applying 30-second test pulses to potentials
**Discussion**

Five missense mutations that cause inherited LQTS have been identified in the pore helix domain of HERG.\textsuperscript{12,15–17} Two of these mutations (Y611H and A614V) were characterized previously and shown to not induce detectable currents when expressed alone in heterologous expression systems. The loss of function of Y611H HERG when expressed in HEK293 cells was caused by failure of the protein to be transported to the surface membrane.\textsuperscript{18} Further analysis of Y611H HERG indicated that mutant subunits undergo core glycosylation in the endoplasmic reticulum but then fail to be transported to the Golgi apparatus for further processing and instead are rapidly degraded. These findings suggest that the Y611H mutation causes HERG subunits to misfold. Loss of function of A614V HERG when expressed in *Xenopus* oocytes is also likely to be caused by abnormal protein processing. When coexpressed with WT HERG subunits in oocytes, however, A614V HERG subunits caused a moderate dominant-negative effect and a −9-mV shift in the voltage dependence of inactivation of the heteromultimeric HERG channel.\textsuperscript{19} Thus, A614V HERG subunits can coassemble with WT HERG subunits to form a heteromultimeric channel with altered gating properties. In the present study, we characterized 3 other LQTS-associated missense mutations (V612L, T613M, and L615V) found in the pore helix of HERG.

All 3 mutations studied here prevented normal trafficking of the HERG protein to the surface membrane, as shown by the decrease in cell-surface chemiluminescence of HA-tagged mutant subunits compared with HA-tagged WT HERG subunits. Two of the mutations (V612L and L615V) would not a priori have been expected to cause loss of function. Although Leu and Val differ by only a single methyl group, apparently such a slight change can disrupt the pore helix sufficiently to disrupt protein trafficking. Although we have no direct evidence, it is likely that V612L, T613M, and L615V, like Y611H, cause channel misfolding, retention in the endoplasmic reticulum, and rapid degradation. The slight decreases in deactivation rate observed for oocytes expressing WT subunits plus V612L or T613M HERG subunits would tend to increase rather than decrease HERG current during a cardiac action potential. This would be offset, however, by the decrease in current caused by the dominant-negative effect of these mutations. The suppression of current resulting from coexpression of WT and mutant subunits differed for the 3 mutations. When expressed alone, V612L and T613M subunits caused a similar loss of function as did wild-type HERG, whereas L615V, like Y611H, caused channel misfolding, retention in the endoplasmic reticulum, and rapid degradation.

The voltage dependence of HERG channel inactivation was determined with a 3-pulse protocol, in which the voltage of the second pulse (prepulse) was varied from −140 to +10 mV (Figure 5C). The V/2 and slope factor for WT HERG were −80.7±4.1 mV and 21.8±1.1 mV, respectively. Although the V/2 values differed by only 6 mV, ANOVA showed that the relative inactivation-voltage relationship was significantly (P<0.001) more negative in oocytes expressing WT+T613M HERG and significantly (P<0.005) more positive in oocytes expressing WT+L615V HERG subunits (Figure 5D). These shifts were in opposite directions to the shifts observed for the voltage dependence of activation.

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decrease in current beyond that resulting from a dominant-negative–induced suppression of channel function.

L615V caused less dominant-negative suppression of WT HERG current than V612L or T613M HERG. Unlike these other mutant subunits, L615V subunits alone were capable of limited coassembly, trafficking, and insertion into the plasma membrane to form functional homotetrameric channels. L615V also induced a negative shift in the voltage dependence of activation and a positive shift in the voltage dependence of inactivation of heteromultimeric channels. Opposite to what was observed for T613M, these shifts would cause an increase in current magnitude compared with WT homomultimeric channels. L615V also caused a dominant-negative effect on channel function, however, so the net result of coexpression of L615V+WT HERG subunits was an ~50% reduction in current magnitude compared with WT HERG channels.

In summary, all 5 of the identified LQTS-associated missense mutations located in the pore helix of HERG cause a loss of function and a dominant-negative effect. Some mutations also caused small alterations in the steady-state voltage dependence of channel gating of heteromultimeric channels. It is also likely that each mutant subunit differed in its ability to form stable heteromultimeric complexes with WT subunits and thereby allow trafficking to the membrane of channels with slightly altered function as detected by changes in voltage dependence of gating. These findings indicate the importance of the structural integrity of the pore helix of HERG for normal channel function. This study confirms previous reports13,18,19,26–30 that LQTS-associated HERG mutations cause long QT syndrome.

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References


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