Characterization of novel *KCNH2* mutations in type 2 long QT syndrome manifesting as seizures

Dagmar I Keller MD^{1,2}, Julie Grenier MSc³, Georges Christé PhD⁴, Frédérique Dubouloz PhD², Stefan Osswald MD¹, Marijke Brink PhD², Eckhard Ficker PhD⁵, Mohamed Chahine PhD³

DI Keller, J Grenier, G Christé, et al. Characterization of novel *KCNH2* mutations in type 2 long QT syndrome manifesting as seizures. *Can J Cardiol* 2009;25(8):455-462.

BACKGROUND: Long QT syndrome (LQTS) is characterized by corrected QT interval prolongation leading to torsades de pointes and sudden cardiac death. LQTS type 2 (LQTS2) is caused by mutations in the *KCNH2* gene, leading to a reduction of the rapidly activating delayed rectifier K⁺ current and loss of human ether-à-go-go-related gene (hERG) channel function by different mechanisms. Triggers for life-threatening arrhythmias in LQTS2 are often auditory stimuli.

OBJECTIVES: To screen *KCNH2* for mutations in patients with LQTS2 on an electrocardiogram and auditory-induced syncope interpreted as seizures and sudden cardiac death, and to analyze their impact on the channel function *in vitro*.

METHODS: The *KCNH2* gene was screened for mutations in the index patients of three families. The novel mutations were reproduced *in vitro* using site-directed mutagenesis and characterized using the *Xenopus* oocyte expression system in voltage clamp mode.

RESULTS: Novel *KCNH2* mutations (Y493F, A429P and del234-241) were identified in the index patients with mostly typical LQTS2 features on their electrocardiograms. The biochemical data revealed a trafficking defect. The biophysical data revealed a loss of function when mutated hERG channels were coexpressed with the wild type.

CONCLUSIONS: In all families, at least one patient carrying the mutation had a history of seizures after auditory stimuli, which is a major trigger for arrhythmic events in LQTS2. Seizures are likely due to cardiac syncope as a consequence of mutation-induced loss of function of the rapidly activating delayed rectifier K⁺ current.

Key Words: *KCNH2*; Long QT syndrome type 2; Loss of I_{Kr} channel function; Potassium channel; Seizure; Sudden cardiac death

Long QT syndrome (LQTS) is an inherited electrical cardiac disorder characterized by prolongation of ventricular repolarization, leading to syncope and sudden cardiac death (SCD) by torsades de pointes (TdP) degenerating into ventricular fibrillation. To date, 10 LQTS phenotypes are known to be caused by mutations in the cardiac ion channels and their linker proteins (<http://www.fsm.it/cardmoc/>). The second most frequent LQTS phenotype is LQTS type 2 (LQTS2), accounting for 35% to 40% of the LQTS phenotypes and mutations. LQTS2 is caused by mutations in the *KCNH2* gene, encoding the alpha subunit of the human ether-à-go-go-related gene (hERG) potassium channel underlying the rapidly activating delayed rectifier K⁺ current (I_{Kr}). Functional studies of *KCNH2* mutations in *in vitro* models usually demonstrate a reduction of I_{Kr} by different biophysical mechanisms, most often due to trafficking defects (1). A dominant negative effect results from current reduction of the tetrameric channel complex.

Caractérisation des nouvelles mutations du *KCNH2* dans le syndrome du QT long de type 2 se manifestant par des convulsions

HISTORIQUE : Le syndrome du QT long (SQTL) se caractérise par une prolongation de l'intervalle QT corrigé entraînant des torsades de pointe et la mort subite d'origine cardiaque. Le SQTL de type 2 (SQTL2) est causé par des mutations du gène *KCNH2* entraînant une réduction du courant potassique à rectification retardée et une perte de fonction du canal hERG (pour *human ether-à-go-go-related gene*) par le biais de différents mécanismes. Les déclencheurs des arythmies gravissimes dans le SQTL2 sont souvent des stimuli auditifs.

OBJECTIFS : Procéder au dépistage des mutations du *KCNH2* chez les patients présentant un SQTL2 à l'électrocardiogramme et des syncopes induites par des stimuli auditifs se manifestant par des convulsions et la mort subite d'origine cardiaque et analyser leur impact sur le fonctionnement du canal hERG *in vitro*.

MÉTHODES : Le gène *KCNH2* a fait l'objet d'analyses de mutation chez les cas de référence de trois familles. Les nouvelles mutations ont été reproduites *in vitro* par mutagenèse dirigée et caractérisées par la technique du potentiel imposé à un fragment de membrane (*patch-clamp*) à l'aide du xénope oocyte comme système d'expression.

RÉSULTATS : De nouvelles mutations du *KCNH2* (Y493F, A429P et del234-241) ont été identifiées chez les cas indicateurs présentant les caractéristiques typiques du SQTL2 à l'électrocardiogramme. Les données biochimiques ont révélé des anomalies du transport. Les données biophysiques ont révélé un ralentissement fonctionnel en présence de coexpression des canaux hERG mutés et sauvages.

CONCLUSIONS : Chez toutes les familles, au moins un patient porteur de la mutation avait des antécédents de convulsions consécutives à des stimuli auditifs constituant un déclencheur important des arythmies dans le SQTL2. Les convulsions sont probablement attribuables à une syncope cardiaque, elle-même due à une dysfonction induite par des mutations du courant potassique à rectification retardée.

LQTS2 is diagnosed on a 12-lead surface electrocardiogram (ECG) showing a prolonged corrected QT (QTc) interval with low T-wave amplitude and a double-notched T-wave morphology (2,3). Specific triggers – auditory stimuli and noise in LQTS2 – can precipitate arrhythmic events in patients with LQTS (3). TdP can lead to syncope but can also degenerate into ventricular fibrillation, leading to SCD.

The aim of the present study was to determine the phenotypes and genotypes of three families with a history of seizures and SCD with typical LQTS2 on a 12-lead surface ECG. Mutations analysis of *KCNH2* revealed three novel mutations – Y493F, A429P and del234-241. *In vitro* expression of the mutations showed a loss of hERG potassium channel function by reduction of the current, suggesting a dominant negative effect. The common clinical feature in the families was the occurrence of seizure triggered by noise (alarm

¹Cardiology Department; ²Cardiology Research Laboratories, University Hospital of Basel, Basel, Switzerland; ³Le centre de recherche Université Laval Robert-Giffard, Québec, Québec; ⁴Institut National de la Santé et de la Recherche Médicale, Lyon, France; ⁵Case Western Reserve University, Cleveland, Ohio, USA

Correspondence and reprints: Dr Mohamed Chahine, Le centre de recherche Université Laval Robert-Giffard, 2601 chemin de la Canardière, Québec, Québec G1J 2G3. Telephone 418-663-5747 ext 4723, fax 418-663-8756, e-mail mohamed.chahine@phc.ulaval.ca

Received for publication December 7, 2008. Accepted January 27, 2009

Figure 1) Representative examples of recordings of currents in response to a classical two-pulse protocol. Non Inj Noninjected oocyte; WT Wild type

clock or telephone ringing). Biochemical and biophysical mechanisms leading to a decrease in I_{K_r} are discussed.

METHODS

Clinical evaluation and molecular genetics

Written informed consent was obtained from participating members of families 1 to 3 in accordance with the study protocol approved by the local ethics committee of the University Hospital of Basel (Basel, Switzerland). The investigation conformed to the principles outlined in the Declaration of Helsinki. Participating members underwent detailed clinical examinations including consecutive 12-lead surface ECGs.

Genomic DNA was extracted from peripheral lymphocytes. In the index patients of families 1 to 3, all coding exons of *KCNH2* were amplified by polymerase chain reaction using primers designed in intronic flanking sequences. Denaturing high-performance liquid chromatography was performed on DNA amplification products in at least one temperature condition. Abnormal denaturing high-performance liquid chromatography profiles were analyzed by sequence reaction in both strands of the exon, using a BigDye Terminator mix (Applied Biosystems USA) and analyzed by cycle sequencing using an automated laser fluorescent DNA sequencer (ABI Prism 377; Applied Biosystems). After identification, the specific mutations were directly searched in the family members by polymerase chain reaction and sequenced in both strands as described above. Mutations were absent in chromosomes from 200 normal subjects.

Mutagenesis

hERG mutations were generated using site-directed mutagenesis and were performed on the hERG-pGH19 (6.9 kb) construct provided by Dr Gail A Robertson (University of Wisconsin – Madison Medical School in Madison, Wisconsin, USA). The following mutagenetic sense and antisense primers, respectively, were used:

For hERG/del234-241:

5'-GCACGCAGCGCCGCGC ↓ AGATCGCGCAGGCACTG-3'
5'-CAGTGCCTGCGCGATCT ↑ GCGCGGCGCTGCGTGC-3'

For hERG/A429P:

5'-CTTCACACCTACTCGCCTGCCTTCCTGCTGAAGG-3'
5'-CCTTCAGCAGGAAGGCAGGCGAGTAGGGTGTGAAG-3'

For hERG/Y493F:

5'-CGCATCGCCGTCCACTTCTTCAAGGGCTGGTTC-3'
5'-GAACCAGCCCTTGAAGAAAGTGGACGGCGATGCG-3'

Mutagenesis was conducted according to the Quick Change kit (Stratagene, USA) and the mutated sites were confirmed by automatic sequencing. Capped messenger RNA (mRNA) of wild type (WT) and mutated hERG was produced using the SP6 mMESSAGE mMACHINE (Ambion Inc, USA).

Oocyte preparation and expression of hERG potassium channels

The preparation of *Xenopus* oocytes was described previously (4). Briefly, oocytes were subjected to collagenase treatment (2 mg/mL over 2.5 h to 3 h), stage V or VI oocytes were microinjected with 5 ng capped mRNA encoding either WT hERG, mutant hERG or both. The oocytes were maintained at 18°C in a twofold diluted solution of GIBCO Leibovitz's L-15 medium (GIBCO, USA) enriched with 15 mM HEPES (pH 7.6, adjusted with sodium hydroxide), 1 mM glutamine and 50 µg/µL gentamycin. Oocytes were used for experiments one to three days after injection. The macroscopic potassium currents from the mRNA-microinjected oocytes were recorded using a voltage-clamp technique with two 3 M potassium chloride-filled microelectrodes. Membrane potential was controlled by a Warner oocyte clamp (Warner Instrument Corporation, USA). Voltage commands were generated by computer using pCLAMP software version 7 (Axon Instruments Inc, USA). Currents were filtered at 2 kHz (−3 dB; four-pole Bessel filter).

Solutions

The Ringer's bathing solution contained 116 mM sodium chloride, 2 mM potassium chloride, 1.8 mM calcium chloride, 1.0 mM magnesium chloride and 5 mM HEPES (pH was adjusted to 7.6 at 22°C with sodium hydroxide). All experiments were performed at room temperature (22°C).

Analysis of electrophysiological data

A standard two-pulse protocol was applied from a holding potential at −80 mV. A 2 s prepulse (P1) to various voltage potentials (V_{P1}) from −80 mV to +60 mV in 10 mV increments was followed by a 2 s test pulse (P2) to −60 mV (V_{P2}). The current magnitude at the end of the first pulse (I_{P1}) and the peak current amplitude shortly after the beginning of the second pulse (I_{P2}) were measured.

Accounting for the voltage dependence of the endogenous current:

The endogenous current (I_{ENDO}) was recorded in noninjected oocytes (eg, Figure 1A, bottom panel). I_{ENDO} was measured at the end of P1 (I_{ENDO1}) and at the time of peak hERG current in P2 (I_{ENDO2}). Both measurements were well accounted for by exponential functions of V_{P1} in P1:

$$I_{ENDO1} = g_{ENDO} * \exp(V_{P1}/k_{1V}) \quad (1)$$

$$I_{ENDO2} = g_{ENDO} * fr * \exp(V_{P1}/k_{2V}) \quad (2)$$

where fr is a factor between 0 and −1, reflecting the remaining I_{ENDO} during P2 after being activated during P1 and taking into account that the reversal voltage of I_{ENDO} is positive to the voltage in P2 (ie, −60 mV). g_{ENDO} is the endogenous conductance.

Figure 2A shows the fitting functions 1 and 2 adjusted to the averaged I_{ENDO} data sets yielding the following values:

$g_{\text{ENDO}}=3.44$ nA; $\text{fr}=-0.288$; $k_{1V}=26.8$ mV; and $k_{2V}=23.6$ mV. Functions were included in the next procedure to account for the I_{ENDO} that could corrupt data analysis of the hERG current.

Global fitting analysis of features of the hERG current: The voltage dependence of total oocyte currents I_{P1} and I_{P2} may respectively be described by the following expressions:

$$y1 = I_{\text{ENDO1}}(V_{P1}) + g_{\text{hERG}} * (V_{P1} - E_{\text{rev}}) * h_{\text{inf}}(V_{P1}) * r(V_{P1}) \quad (3)$$

$$y2 = I_{\text{ENDO2}}(V_{P1}) + g_{\text{hERG}} * (V_{P2} - E_{\text{rev}}) * h_{\text{inf}}(V_{P1}) \quad (4)$$

Where the product:

$$g_{\text{hERG}} * (V_{P1} - E_{\text{rev}}) \quad (5)$$

expresses the voltage dependence of the fully activated and non-inactivated hERG current. g_{hERG} is the maximal conductance of the hERG current and E_{rev} is the reversal voltage. This expresses the ohmic behaviour of the open hERG channel (5).

$h_{\text{inf}}(V_{P1})$ is the voltage-dependent value of the activation variable at the end of P1:

$$h_{\text{inf}}(V_{P1}) = 1/(1 + \exp((V_{P1} - V_{\text{ha}})/k_a)) \quad (6)$$

where V_{ha} is the one-half activation potential and k_a is the slope factor.

$r(V_{P1})$ is the voltage-dependent rectification function:

$$r(V_{P1}) = 1/(1 + \exp((V_{P1} - V_{\text{hr}})/k_r)) \quad (7)$$

where V_{hr} is the one-half rectification potential and k_r is the slope factor.

I_{ENDO1} and I_{ENDO2} are the endogenous oocyte currents that respectively correspond to functions 1 and 2, where k_{1V} and k_{2V} were both set at a value of 25 mV.

A global fitting procedure simultaneously adjusted functions $y1$ and $y2$ by a least-squares algorithm (simplex) to the experimental series of data couples: I_{P1} versus V_{P1} and I_{P2} versus V_{P1} of each cell. The reversal voltage of the hERG current (E_{rev}) was fixed at -77 mV. Parameters g_{ENDO} , fr , g_{hERG} , V_{ha} , k_a , V_{hr} and k_r were adjusted for the best simultaneous fit of both data sets. The values for g_{hERG} , V_{ha} , k_a , V_{hr} and k_r are reported in Table 1.

An example of the fitting is given in Figure 2B, showing the adequacy of the functions to represent the data sets.

This analysis is consistent with the theoretical description of Sanguinetti et al (6).

Western blot analysis

Antibodies used in the present study have been described previously (7). Briefly, the anti-hERG polyclonal antibody used in Western blots was generated in rabbits against a glutathione S-transferase fusion protein containing the last 112 amino acids of hERG (residues 1048 to 1159). hERG antiserum was either purified on an affinity column consisting of a short carboxylic acid-terminal peptide corresponding to hERG residues 1102 to 1121 (TLTLDLSLQVSQFMACEELP) or the entire fusion protein. Briefly, human embryonic kidney/hERG cells were solubilized for 1 h at 4°C in lysis buffer containing 150 mM sodium chloride, 1 mM EDTA and 50 mM Tris at a pH of 8.8 with 1% Triton X-100 and protease inhibitors (Complete, Roche Diagnostics, USA). Proteins were separated on sodium dodecyl sulfate polyacrylamide gels, transferred to polyvinylidene difluoride membranes and developed using hERG basic antibody followed by ECL Plus (GE Healthcare Life Sciences, USA).

Statistical analysis

A Student's t test on two independent samples was performed to compare parameters of each of the mutated hERG currents with those of the WT.

RESULTS

Identification of three novel *KCNH2* mutations and family screening

Three novel *KCNH2* mutations were identified in the index patients

Figure 2 **A** Endogenous current (I_{ENDO}) values (filled circles) at the end of the prepulse (P1) (I_{ENDO1}) and at the time of peak human ether-à-go-go-related gene (hERG) current at the beginning of the test pulse (P2) (I_{ENDO2}). The solid curves of I_{ENDO1} and I_{ENDO2} result from the fit by functions 1 and 2 (Methods), respectively. **B** Example of the global fitting with functions 3 and 4 (Methods), respectively, of data series of current measurements at the end of P1 (I_{P1}) and current measurements at peak hERG current during P2 (I_{P2}) as a function of voltage in P1 (V_{P1}). **C** and **E** Currents measured at the end of P1 were plotted after subtraction of I_{ENDO} (as evaluated by the global fitting procedure) to show the hERG current and allow visual comparison of the voltage-dependent rectification. **D** and **F** After subtraction of I_{ENDO} from peak current values measured at the beginning of P2, currents for each cell were normalized to the maximum value at positive voltages, allowing visual comparison of steady-state activation versus voltage relations. WT Wild type

of the families (patient II-1 of family 1, patient II-1 of family 2 and patient II-2 of family 3). In patient II-1 of family 1 (Figure 3A), a heterozygous change of tyrosine (TAC) to phenylalanine (TTC) resulted in the missense mutation Y493F (Figure 3B). Y493F is localized at the end of the S2 to S3 linker region of the hERG potassium channel. In patient II-1 of family 2 (Figure 4A), a heterozygous change of alanine (GCT) to proline (CCT) resulted in the A429P mutation (Figure 4B). A429P is localized at the beginning of S1 to S2 of the hERG potassium channel. A deletion in exon 2 (del234-241 TGCCGCGC in codon 78) was identified in patient II-2 of family 3 (Figures 5A and 5B). This mutation, at the beginning of the N-terminal, introduces a stop codon 62 amino acids past codon 78. During family screening, Y493F was identified in individual III-1 of family 1, A429P in individuals III-1 and IV-2 of family 2, and del234-241 in individuals II-1 and III-3 of family 3.

Phenotypes of the families

Family 1: The index patient II-1 (male, born 1951) was diagnosed with seizures in childhood. The seizures always occurred in the

TABLE 1

Mean ($\pm$ SEM) values of parameters evaluated from the global fitting procedure applied to data recorded in oocytes expressing either the wild type (WT) human ether-à-go-go-related gene (hERG) or one of the mutants alone or in an equal parts mixture

	n	g_{hERG} (nA/mV)	Activation		Rectification	
			V_{ha} (mV)	k_a (mV)	V_{hr} (mV)	k_r (mV)
WT	5	36.0 $\pm$ 3.6	-20.5 $\pm$ 1.3	-7.50 $\pm$ 0.16	-53.0 $\pm$ 1.6	16.80 $\pm$ 0.55
WT + del234-241	6	18.2 $\pm$ 4.9*	-19.9 $\pm$ 0.9	-8.10 $\pm$ 0.17*	-54.3 $\pm$ 1.6	17.08 $\pm$ 0.73
WT + A429P	4	17.9 $\pm$ 3.1*	-23.4 $\pm$ 1.9	-9.44 $\pm$ 0.95**	-46.0 $\pm$ 4.7	17.28 $\pm$ 0.81
WT + Y493F	3	18.4 $\pm$ 4.3*	-22.4 $\pm$ 1.0	-8.65 $\pm$ 0.14**	-47.9 $\pm$ 1.5	16.98 $\pm$ 0.26
Y493F	7	2.4 $\pm$ 0.2***	-32.1 $\pm$ 0.8***	-9.34 $\pm$ 0.15***	-52.0 $\pm$ 1.4	20.12 $\pm$ 0.79*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. g_{hERG} Maximal conductance of the cell; k_a Corresponding slope factor of the voltage for one-half activation; k_r Corresponding slope factor of the voltage for one-half rectification; n Number of eggs used in each series; V_{ha} Voltage for one-half activation; V_{hr} Voltage for one-half rectification

Figure 3) A Pedigree of family 1. The index patient is marked by an arrow. Individuals with black squares/circles carry the mutation and the clinical phenotype with symptoms. Patient III-2 died from sudden death at 14 years of age; no genotype or electrocardiogram (ECG) data were available (grey circle). **B** Identification of the Y493F mutation in exon 6 of KCNH2. A heterozygous change of tyrosine (TAC) to phenylalanine (TTC) resulted in the missense mutation Y493F. **C** ECG of the index patient (II-1) with typical type 2 long QT syndrome with a corrected QT interval prolongation of 525 ms and a low-amplitude T-wave with double notch

morning after auditory stimuli. A diagnosis of LQTS2 was made after presyncope at 54 years of age. A 12-lead ECG showed typical LQTS2 with a QTc of 525 ms (Figure 3C). Family history revealed that his father (I-1) died from rhythm problems at 52 years of age. The daughter of the index patient (III-2) died from SCD at 14 years of age; she was diagnosed with seizures in early childhood. The son of the index patient (III-1, born in 1981) was diagnosed with seizures at nine years of age; he was treated with carbamazepine. After 18 years of age, he no longer had seizures and was still taking carbamazepine. LQTS2 was diagnosed during family screening with a QTc of 539 ms and typical T-wave morphology (data not shown). Patients II-1 and III-1 both showed LQTS2 on the ECG and carried the Y493F KCNH2 mutation. Both received an implantable cardioverter defibrillator (ICD). No ICD discharges were documented in either patient until present.

Data from electroencephalograms (EEGs) were available for patient III-1. Under therapy with carbamazepine, the EEG before ICD

Figure 4) A Pedigree of family 2. The index patient is marked by an arrow. Individuals with black circles carry the mutation and the clinical phenotype with symptoms. Individual III-1 (grey circle) carries the mutation but has no symptoms. **B** Identification of the A429P mutation in exon 6 of KCNH2. A heterozygous change of alanine (GCT) to proline (CCT) resulted in the A429P mutation. **C** Electrocardiogram of the index patient (II-1) with typical type 2 long QT syndrome with a corrected QT interval prolongation of 462 ms and a low-amplitude T-wave with double notch

implantation showed normal baseline activity with minor functional bitemporo-occipital disturbance.

Family 2: The index patient II-1 (female, born in 1930) was diagnosed with LQTS2 at 75 years of age, with TdP during anesthesia to fix her broken arm. She was diagnosed with auditory reflex epilepsy in childhood with syncope always occurring after telephone ringing. No EEG data were available. Since childhood, she was treated with phenytoin and had approximately 10 syncope until 40 years of age. From 40 to 75 years of age, she had no symptoms and was still taking phenytoin. The TdP described above occurred while on phenytoin medication. Her most recent 12-lead ECG showed typical LQTS2 with a QTc of 462 ms (Figure 4C). During family screening, her only daughter (III-1, born in 1970) was diagnosed with LQTS2 with a QTc of 476 ms (data not shown). In contrast, she had no symptoms. The daughter of patient III-1 (IV-2, born in 1999) had one syncope while standing. Patients II-1, III-1

and IV-2 carried the A429P *KCNH2* mutation; they are all being treated with a beta-blocker. On clinical follow-up examinations, no arrhythmic events were noticed.

Family 3: In family 3, patient I-2 (female) died at 43 years of age after a telephone rang. The index patient II-2 (male, born in 1966) suffered from syncope during sports in childhood. His 12-lead ECG showed a QTc of 467 ms with a broad T-wave, not typical for LQTS2 (Figure 5C). In contrast, his brother (II-1, born in 1965) suffered from seizures in childhood and showed typical LQTS2 on the 12-lead ECG, with a QTc of 464 ms (Figure 5C). During family screening, the daughter (III-3, born in 1992) of the index patient was diagnosed with LQTS2 on the ECG, with a history of dizziness. In patients II-2, II-1 and III-3, the *KCNH2* mutation del234-241 was found. Patients II-2 and II-1 received an ICD, while patient III-3 is being treated with beta-blockers. Until present, no arrhythmic events could be documented on ICD interrogation and clinical follow-ups. No EEG data are available for this family.

Macroscopic recordings of hERG currents

As shown in Figure 1A, the expression of mutation hERG/Y493F yielded a considerably lower current than the WT. When the mutant hERG was coexpressed with the WT using equal amounts of RNA in each oocyte, the current amplitude was reduced to approximately one-half of that in the WT. The two other mutations (A429P and del234-241), when expressed alone, failed to express more than the I_{ENDO} observed in noninjected oocytes (Figures 1B and 1C, middle panels). When coexpressed with WT hERG (Figures 1B and 1C, bottom panels), each of them developed currents that were approximately one-half smaller than when the WT was expressed alone.

To accurately evaluate the macroscopic properties of these currents, a global fitting procedure was used to evaluate steady-state activation versus voltage relation and apparent steady-state rectification versus voltage relation by simultaneously fitting theoretical functions to current versus voltage data sets as measured at the end of P1 and at peak current at the beginning of P2 (see Methods). A function accounting for I_{ENDO} , which was derived from analysis of the I_{ENDO} in noninjected oocytes, automatically prevented distortions of the hERG currents (see Methods).

After applying this procedure to data sets from each of the cells, the I_{ENDO} was subtracted and average hERG current-voltage relations were plotted in for currents I_{P1} and I_{P2} (Figures 2C, 2D, 2E and 2F). This allows direct comparison of the voltage-dependent features between mutated hERG coexpressed with the WT and the WT hERG expressed alone. The average values of the individual parameters resulting from the global fitting analysis are reported for each expression case in Table 1.

Coexpression of mutation del234-241 with the WT resulted in a twofold reduction in the current at the end of P1 (Figure 2C), in agreement with the one-half lower maximal hERG conductance (Table 1). This is in line with the absence of changes in activation (Figure 2D and Table 1) and rectification parameters (Table 1). When mutation A429P was coexpressed with the WT, the current during P1 only decreased by 22% from that of the WT (Figure 2C), whereas the maximal hERG conductance was reduced twofold (Table 1). This is the result of the combination of a negative shift in the voltage for one-half activation (V_{ha}) (Figure 2D and Table 1) and a positive shift in the voltage for one-half rectification (V_{hr}) (Table 1). Both of these changes, although not statistically significant, caused a larger availability of the current, while their respective slope factors k_a and k_r did not differ from those of the WT (Table 1). Of the three mutations studied here, only mutation Y493F yielded a current when expressed alone. Although the maximal apparent current at the end of P1 was only 40% less than in the WT (Figure 2E), the maximal conductance was six times lower than that of the WT (Table 1). This discrepancy resulted from a shift of the activation curve by 12 mV in the hyperpolarizing direction (Figure 2F and

Figure 5) A Pedigree of family 3. The index patient is marked by an arrow. Individuals with black squares/circles carry the mutation and the clinical phenotype with symptoms. Individual I-2 died suddenly at 43 years of age after telephone ringing; no genetic data were available (grey circle). **B** Identification of del234-241 in exon 2 of *KCNH2* (del234-241 TGCCGCGC in codon 78). **C** Electrocardiograms (ECGs) of patients II-2 and II-1. The ECG of II-2 does not show typical type 2 long QT syndrome (LQTS2); the T-wave is broad and a double notch is absent, with a corrected QT interval (QTc) of 467 ms. The ECG of II-1 shows typical LQTS2 with QTc prolongation of 464 ms and a low-amplitude T-wave with double notch

Table 1). There was a slight but significant increase (1.85 mV) in the value of the negative slope factor k_a , whereas, for the apparent rectification versus voltage, only the slope factor k_r was affected, ie, increased by 3.3 mV (Table 1). When mutation Y493F was coexpressed with the WT, the maximal macroscopic current I_{P1} was only 27% less than that of the WT alone (Figure 2E), whereas the maximal hERG conductance was lowered by one-half compared with the WT alone (Table 1). This may be related to a shift by 6 mV in the depolarizing direction of V_{hr} , which, although not statistically significant, caused an increase in the availability of the current (Figure 2E), while the slope factor k_r of the rectification curve did not change (Table 1). The voltage for V_{ha} was not significantly affected, whereas the absolute value of the slope factor k_a was increased by 1.2 mV (Table 1).

Western blot analysis of hERG mutations

Figure 6 shows Western blots of the hERG mutations compared with the WT. The hERG antibody used is described in the Methods section. As shown, hERG Y493F produces a small amount of fully glycosylated cell surface hERG (160 kDa). This is possibly a hypomorphic mutation that may be rescued by low incubation temperature or a hERG blocker such as astemizole. However, hERG A429P can only be detected as a core glycosylated, endoplasmic reticulum-retained protein of approximately 135 kDa at 37°C.

Interestingly, the hERG deletion (del234-241) quite surprisingly produces fully glycosylated protein, as shown in the blot. Evidently, the amount of protein is severely reduced compared with the WT or even with the other missense mutations shown on this blot.

DISCUSSION

We report three novel *KCNH2* mutations in three families with LQTS2 and a history of seizures with loud noise as the common trigger. In vitro expression of the *KCNH2* mutations Y493F, A429P and del234-241 showed a loss of hERG potassium current function by reduction of the current, suggesting a trafficking defect.

Figure 6) Western blot comparing human ether-à-go-go-related gene (hERG) wild type (WT) and hERG/Y493F, hERG/A429P and hERG/del234-241 transiently expressed in human embryonic kidney (HEK) 293 cells using FuGENE (Roche Applied Science, USA). HEK293 cells were transfected with 1 µg of complementary DNA. hERG WT is expressed as a fully glycosylated, mature 160 kDa form (fg) and a core glycosylated, endoplasmic reticulum resident 135 kDa form (cg). hERG/Y493F expresses considerably less fg hERG protein. For A429P, the protein in fg form is absent. hERG/del234-241 expresses considerably less hERG protein, either as the fg or cg form, than WT hERG protein

Genotype-phenotype correlation and triggers for arrhythmia

Except for patient II-2 of family 3, all affected family members showed typical LQTS2 ECG phenotypes, consistent with the positive KCNH2 genotype. The ECG of patient II-2 of family 3 shows a broad T-wave with a delayed onset of the upstroke part of the T-wave without double notch and low amplitude, which is more consistent with an ECG of LQTS type 1 or type 3 (Figure 5C) (2). No mutations in the responsible genes *KCNQ1* (LQTS type 1) and *SCN5A* (LQTS type 3) were identified in this patient (data not shown). An important observation in LQTS is the change of the ECG phenotype appearance by recording serial ECGs. Still, in our patient, repetitive ECGs did not show the typical LQTS2 phenotype. This may be due to additional genetic and other factors influencing the phenotype caused by the major gene mutation.

In at least one member of all families, seizures were diagnosed in childhood. Unfortunately, no EEG data were recorded from this time. Seizures always occurred after telephone or alarm clock ringing, usually in the morning. This is consistent with the known triggers for arrhythmias in LQTS2 (3).

Mutations in the Y493 residue of *KCNH2* have previously been found. The first mutation was Y493X in a family with LQTS (8). Lupoglazoff et al (9) described a heterozygous Y493C mutation in a patient with fetal bradycardia. Functional studies are not available for these mutations.

The interpretation of seizures in these families is likely due to cardiac syncope as a consequence of malignant ventricular arrhythmias. EEG data are available from only one patient, which showed minor functional bitemporo-occipital disturbance. In this patient, the question arises whether the underlying primary cause of seizure may be due to a channel mutation in the brain. In a recent paper by Gurnett and Hedges (10), susceptibility genes for human epilepsy include genes coding for neuronal sodium channels (*SCN1A*, *SCN1B*, *SCN2A*), calcium channels (*CACNA1A*, *CACNA1H*, *CACNB4*) and potassium channels (*KCNQ2*, *KCNQ3*, *KCNA1*), genes for acetylcholine and gamma-aminobutyric acid receptors, and other genes. Mutations in *KCNQ2* and *KCNQ3* alter the structure of the pore region and/or carboxylic acid-terminal cytoplasmic domain (for review, refer to the

report by Lehmann-Horn and Jerkat-Rott [11]). These alterations lead to a potential loss or gain of function, or dominant negative effects. The *hERG* gene is expressed in the heart, where it is responsible for the I_{Kr} current, but transcript variants encoding distinct isoforms have been identified in the brain and skeletal muscle tissues (12,13). The properties of hERG channels match with the gating properties of ether-à-go-go (*eag*)-related-potassium channels (14). The sequence of the *hERG* gene is similar to that of the *Drosophila eag* gene. The mutant phenotype of the *eag* gene was initially attributed to an increase in neuronal excitability and transmitter release at the neuromuscular junction (15). This observation may explain a possible link between seizure and cardiac arrhythmias.

Pathophysiological implications of mutated hERG macroscopic current properties

Evaluation of the pathophysiological consequences is based on the changes of properties of macroscopic currents developed when each mutation is coexpressed with the WT hERG, a situation that mimics the heterozygous condition seen in the present mutations (see Results).

None of the two mutated hERG del234-241 and A429P produced any current when expressed alone in oocytes (Figure 1). Although hERG del234-241 generated no currents, some expression could be seen on the Western blot for this deletion mutant. This was consistent in the four Western blots performed. More experiments are required to clarify this issue. Compared with WT injected oocytes, the one-half lower whole-cell maximal conductance derived for oocytes injected with either WT + del234-241 or WT + A429P (Table 1) was not accompanied by significant changes in activation and rectification properties.

It should be noted that, at the most negative voltages, activation is incomplete at the end of the first pulse of the two-pulse protocol. Whether this would cause a misevaluation of the characteristics of the activation versus voltage relationship was tested in a computer model of hERG current in which a function of the time constant of activation versus voltage was set to reproduce the properties of the WT hERG current. We found that incomplete activation caused a less than 0.6 mV positive shift in V_{ha} and an absolute underestimation by less than 0.1 of K_a .

While part of the lower conductance may be due to nonconducting or nontrafficking of the homotetramers formed with the mutated proteins alone, this part of the conductance should not exceed 1/16th of the maximal current if all other tetramers would traffic and conduct as the WT tetramer. As was previously reported for a number of hERG mutations, the lower conductance may be due to a negative dominant effect of the mutated form of hERG on the WT (6,16). Such a decrease in hERG conductance may suffice to account for the propensity of the subjects bearing these mutations to generate arrhythmias, as was confirmed by model simulations (17,18), due to a delayed repolarization of the ventricular action potential.

Regarding the changes caused by mutation Y493F, the situation is not as simple. A very large decrease in conductance when the mutation is expressed alone in oocytes suggests that, when coexpressed with the WT, a small part of the decrease in whole-cell conductance is due to homotetramers, again either less conductive or in lower numbers at the membrane (eg, partially defective trafficking). The whole-cell maximal conductance when mutation Y493F was coexpressed with the WT was one-half smaller than for the WT alone. For this mutation, when coexpressed with the WT, only a slight increase in the absolute value of the slope factor of the activation versus voltage curve was noted. Whether the combination of these effects would cause a diminished hERG current response during an action potential ought to be tested within a ventricular cell model.

This was performed using a ventricular cell model that was developed recently (19). None of the kinetic changes related to voltage dependence of activation and apparent rectification were able to affect the duration of the action potential (Figure 7A) or the amplitude and time-course of the hERG current (Figure 7B). When the decrease by

Figure 7 Model study of the effects of changes in human ether-à-go-go-related gene (hERG) properties as induced by the mutations when coexpressed with the wild type (WT) on the action potential time course (**A**) and on the underlying hERG current of the model cell (**B**). When compared with the WT alone (thick black line), if only the changes in activation and rectification due to mutation Y493F are introduced (WT + Y493F, no conductance change, thin grey line), no change is observed in the shape of the action potential (**A**) or the time course of the rapidly activating delayed rectifier K^+ current in the model (**B**), as evidenced by the thin grey line appearing in the middle of the thick black line. When, in turn, a twofold reduction in the whole cell conductance of hERG is included (thin black line), the action potential duration at 90% repolarization is lengthened by approximately 10%

one-half of the whole-cell hERG conductance was inserted into the model, a 9% lengthening of the action potential duration at a 90% repolarization level was found. This is the likely cause of the observed long QT condition and the resulting predisposition to arrhythmias and associated syncope.

Influence of antiepileptic drugs on the hERG potassium channel

In the families described in the present report, two patients were treated with antiepileptic drugs: patient III-1 of family 1 (carbamazepine) and patient II-1 of family 2 (phenytoin). Both phenytoin and carbamazepine have been shown to induce concentration-dependent cardiac arrhythmogenic potential (20). For phenytoin, the mechanism is believed to be a cardiac I_{Kr} blocking potential in vitro (21). The demonstrated loss of channel function caused by the A429P mutation could be theoretically aggravated by the additional in vitro I_{Kr} blocking potential of phenytoin in the patient carrying the mutation. Surprisingly, the patient had no cardiac symptoms or seizures for almost 35 years while on phenytoin. Phenytoin used for the treatment of seizures exerts its anticonvulsant action by inhibiting voltage-dependent sodium and calcium channels.

Alternatively, phenytoin was shown in vivo to efficiently treat malignant ventricular arrhythmias associated with myocardial infarction (22), whereas in myocardial infarction, the function of the hERG channel is not affected. In addition, fosphenytoin, a prodrug of phenytoin, is known to prolong the QT interval and have a possible risk for TdP (<http://www.arizonacert.org/>). In our patient, the QT interval remained prolonged after stopping phenytoin. Thus, it remains unclear why the patient was asymptomatic for many years while on phenytoin and finally developed TdP while still taking the

drug. This observation may be consistent with the wide phenotype heterogeneity of LQTS2 influenced by a complex genotype.

CONCLUSION

The present paper describes auditory-induced cardiac syncope in three families with LQTS2 carrying one of the following hERG mutations: Y493F, A429P or del234-241. The biochemical data revealed a trafficking defect, while the biophysical data revealed a loss of function of hERG channels. Together, the clinical, biochemical and biophysical data demonstrate the severity of the disease in patients carrying these mutations. Patients with seizures of unclear origin following auditory stimuli should be evaluated for malignant arrhythmias in the context of LQTS.

FUNDING: DI Keller was supported by grants from the Swiss Heart Foundation and the 'Stiftung für Kardiovaskuläre Forschung' University Hospital of Basel. The present study was supported by grants from the Heart and Stroke Foundation of Québec and the Canadian Institutes of Health Research (MT-13181). Dr G Christé received support from the Fédération des Maladies Orphelines (Paris, France) and from the Centre Jacques Cartier (Lyon, France). Dr M Chahine is a JC Edwards Foundation Senior Investigator.

ACKNOWLEDGEMENTS: We thank Dr V Fressart (UF Cardiogénétique/Myogénétique, GH Pitié-Salpêtrière, Paris, France) for the verification of the mutations, and Dr B Biedermann, Dr P Rickenbacher and Dr S Ruegg (University Hospital of Basel and Bruderholz Hospital, Switzerland) for providing clinical data.

REFERENCES

- Dumaine R, Antzelevitch C. Molecular mechanisms underlying the long QT syndrome. *Curr Opin Cardiol* 2002;17:36-42.
- Zhang L, Timothy KW, Vincent GM, et al. Spectrum of ST-T-wave patterns and repolarization parameters in congenital long-QT syndrome: ECG findings identify genotypes. *Circulation* 2000;102:2849-55.
- Schwartz PJ, Priori SG, Spazzolini C, et al. Genotype-phenotype correlation in the long-QT syndrome: Gene-specific triggers for life-threatening arrhythmias. *Circulation* 2001;103:89-95.
- Chahine M, Chen LQ, Barchi RL, Kallen RG, Horn R. Lidocaine block of human heart sodium channels expressed in *Xenopus* oocytes. *J Mol Cell Cardiol* 1992;24:1231-6.
- Wang S, Liu S, Morales MJ, Strauss HC, Rasmusson RL. A quantitative analysis of the activation and inactivation kinetics of HERG expressed in *Xenopus* oocytes. *J Physiol* 1997;502:45-60.
- Sanguinetti MC, Curran ME, Spector PS, Keating MT. Spectrum of HERG K^+ -channel dysfunction in an inherited cardiac arrhythmia. *Proc Natl Acad Sci USA* 1996;93:2208-12. (Erratum in 1996;93:8796).
- Ficker E, Thomas D, Viswanathan PC, et al. Novel characteristics of a misprocessed mutant HERG channel linked to hereditary long QT syndrome. *Am J Physiol* 2000;279:H1748-56.
- Itoh T, Tanaka T, Nagai R, et al. Genomic organization and mutational analysis of HERG, a gene responsible for familial long QT syndrome. *Hum Genet* 1998;102:435-9.
- Lupoglazoff JM, Denjoy I, Villain E, et al. Long QT syndrome in neonates: Conduction disorders associated with HERG mutations

- and sinus bradycardia with *KCNQ1* mutations. *J Am Coll Cardiol* 2004;43:826-30.
10. Gurnett CA, Hadera P. New ideas in epilepsy genetics: Novel epilepsy genes, copy number alterations, and gene regulation. *Arch Neurol* 2007;64:324-8.
 11. Lehmann-Horn F, Jurkat-Rott K. Voltage-gated ion channels and hereditary disease. *Physiol Rev* 1999;79:1317-72.
 12. Wymore RS, Gintant GA, Wymore RT, Dixon JE, McKinnon D, Cohen IS. Tissue and species distribution of mRNA for the I_{Kr} -like K^+ channel, *erg*. *Circ Res* 1997;80:261-8.
 13. London B, Trudeau MC, Newton KP, 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* 1997;81:870-8.
 14. Trudeau MC, Warmke JW, Ganetzky B, Robertson GA. *HERG*, a human inward rectifier in the voltage-gated potassium channel family. *Science* 1995;269:92-5.
 15. Ganetzky B, Wu CF. Neurogenetic analysis of potassium currents in *Drosophila*: Synergistic effects on neuromuscular transmission in double mutants. *J Neurogenet* 1983;1:17-28.
 16. Sanguinetti MC, Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. *Nature* 2006;440:463-9.
 17. Roden DM, Balser JR. A plethora of mechanisms in the *HERG*-related long QT syndrome. Genetics meets electrophysiology. *Cardiovasc Res* 1999;44:242-6.
 18. Clancy CE, Rudy Y. Cellular consequences of HERG mutations in the long QT syndrome: Precursors to sudden cardiac death. *Cardiovasc Res* 2001;50:301-13.
 19. Pasek M, Simurda J, Orchard CH, Christie G. A model of the guinea-pig ventricular cardiac myocyte incorporating a transverse-axial tubular system. *Prog Biophys Mol Biol* 2008;96:258-80.
 20. Kenneback G, Ericson M, Tomson T, Bergfeldt L. Changes in arrhythmia profile and heart rate variability during abrupt withdrawal of antiepileptic drugs. Implications for sudden death. *Seizure* 1997;6:369-75.
 21. Danielsson BR, Lansdell K, Patmore L, Tomson T. Phenytoin and phenobarbital inhibit human *HERG* potassium channels. *Epilepsy Res* 2003;55:147-57.
 22. Khan IA, Gowda RM. Novel therapeutics for treatment of long-QT syndrome and torsade de pointes. *Int J Cardiol* 2004;95:1-6.