Clinical, Genetic, and Electrophysiologic Characteristics of a New Pas-Domain HERG Mutation (M124R) Causing Long QT Syndrome

Liat Shushi, M.Sc.,* Batsheva Kerem, Ph.D.,* Maya Goldmit, M.Sc.,* Asher Peretz, Ph.D.,‡ Bernard Attali, Ph.D.,‡ Aron Medina, M.D.,† Jeffrey A. Towbin, M.D.,§ Junko Kurokawa, M.Sc.,¶ Robert S. Kass, Ph.D.,¶ and Jesaia Benhorin, M.D.†

From the *Department of Genetics, The Life Sciences Institute and †Department of Cardiology, Bikur Cholim Hospital, The Hebrew University, Jerusalem, Israel; ‡Departments of Physiology and Pharmacology, Sackler Medical School, Tel-Aviv University, Israel; §Department of Pediatric Cardiology, Baylor College of Medicine, Houston, TX; and ¶Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York

Objectives: To describe the clinical, genetic, and electrophysiologic characteristics of a new PAS-domain HERG mutation (M124R) that has been identified in a single large Jewish family with Long QT syndrome (LQTS).

Background: Many previously reported HERG mutations causing LQTS are located either in the C-terminus, or in the pore region. Relatively fewer clinical data are available on N-terminus (PAS-domain) mutation carriers.

Methods: Clinical data were available in 76 family members (aged 1–93 years, 69 alive) over 18 years of follow-up, while electrocardiographic data were available in 57, and genetic data in 45 family members. Cellular electrophysiology was assessed in transfected Chinese Hamster Ovary (CHO) cells using the whole-cell patch-clamp technique.

Results: Thirty-six family members were phenotypically categorized as nonaffected, 3 as equivocal, and 20 as affected. Mean QTc was 410 ± 23 , 440 ± 10 , and 498 ± 41 ms, respectively, in these three subgroups. Eight out of 20 affected family members were symptomatic: five had only syncope, two had aborted cardiac arrest, and one sudden death. Genetic analyses identified the M124R point mutation in all affected members tested ($n = 16$), while all those tested with nonaffected ($n = 26$) and equivocal ($n = 3$) phenotype did not carry the mutation. The M124R mutation reduced the HERG tail-current density by 65%, significantly accelerated the deactivation kinetics, and caused a negative shift in the voltage dependence of activation.

Conclusions: A new PAS-domain HERG mutation (M124R) was identified as causing LQTS in a large Jewish family, with high penetrance and frequent disease-related symptoms. This mutation markedly decreased the tail-current density and accelerated the deactivation kinetics of the HERG channel in transfected CHO cells.

A.N.E. 2005;10(3):334–341

long QT syndrome; HERG-mutation; PAS-domain

The hereditary long QT syndrome (LQTS) is a familial disorder characterized by prolonged ventricular repolarization and a propensity for syncope and arrhythmic sudden death.^{1–3} Multiple mutations have been identified in LQTS in several cardiac potassium channel genes [KvLQT1, KCNH2 [HERG], KCNE1, KCNE2, KCNJ2],^{4–8} one sodium channel gene (SCN5A),⁹ and one calcium

signaling gene (ANKB).¹⁰ Many HERG mutations that have been reported so far are located in the C-terminus or in the pore region of the HERG channel. Relatively fewer clinical data are available on N-terminus (PAS-domain) mutation carriers.¹¹

The purpose of this study was to describe the clinical, genetic, and electrophysiologic characteristics of a new HERG PAS-domain mutation

Address for reprints: Jesaia Benhorin, M.D., The Heiden Department of Cardiology, Bikur Cholim Hospital, P.O. Box 492, Jerusalem 91004, Israel. Fax: 972-2-625-1747; E-mail: benhorin@md2.huji.ac.il

This study was supported in part by research grants HL-33843 and HL-51618 from the National Institutes of Health, Bethesda, MD.

(M124R) that has been recently identified in a single large Jewish family with LQTS.

METHODS

Study Population

The study population consisted of a single large Iranian-Jewish family that has been clinically followed by us for the last 18 years (Fig. 1B). There were 76 family members (43 males, and 33 females, aged 1–93 years), of whom 69 are currently alive. Phenotypic characterization was based on our previously published electrocardiographic criteria.¹² In short, an affected status was determined, if the calculated QTc interval exceeded 0.45 seconds in adult males (>16 years), 0.47 seconds in adult females, and 0.46 seconds in children (<16 years, males and females). A nonaffected status was determined, if the calculated QTc was <0.43 seconds in adult males, <0.45 seconds in adult females, and <0.44 seconds in children. An equivocal phenotypic status was determined, if the calculated QTc was between the above mentioned cutoffs for each age and gender subset.

Genetic Analyses

DNA Extraction

Blood samples for genetic analysis were available in 45 family members: 16 affected, 26 non-affected, and 3 with equivocal phenotype. DNA was prepared according to a method described elsewhere.¹³

Polymerase Chain Reaction (PCR)

DNA samples were amplified by PCR using the oligonucleotide primers for the polymorphic markers D11S1318, D3S1100, and D7S636 tightly linked to KvLQT1, SCN5A, and HERG, respectively.^{14–16} For each pair of primers of all PCR reactions, annealing temperatures and elongation times were optimized in order to exclude PCR artifacts. PCR products were then analyzed by the ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA) using the GeneScan software. For mutation detection within the HERG gene, a DNA sample of an affected family member (Fig. 1B, patient no. 19) was amplified by PCR with oligonucleotide primers for all 16 exons encoding the HERG gene and their flanking intronic sequences.¹⁷ The PCR conditions were essentially as described by Splawski et al.¹⁷ optimized by

the MasterAmp™ PCR optimization kit (Epicentre Technologies, Madison, WI).

DNA Sequence Determination

PCR products of all the 16 exons encoding the HERG gene and their flanking intronic sequences were directly sequenced as described by Sanger et al.¹⁸ and analyzed using the ABI PRISM 377 DNA Sequencer.

Identification of the HERG Mutation by NlaIII Digestion

A partial sequence of HERG exon 3 was amplified using a 5' oligonucleotide primer for exon 3¹⁷ and the internal 3' oligonucleotide primer (listed 5'-3'): TGTCTTCTCCATCACCACC. PCR products were digested with NlaIII restriction endonuclease (BioLabs, Beverly, MA) and subjected to electrophoresis on 3.5% NuSieve GTG agarose gel.

Cellular Electrophysiology

CHO cells were plated on poly-D-lysine-coated glass coverslips in a 24-multiwell plate and grown in Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine, 10% fetal calf serum, and antibiotics. Transfection was performed using 1.5 μ L of lipofectamine (Gibco BRL, Carlsbad, CA) according to the manufacturer's protocol and with 0.5 μ g of the respective channel complementary DNA plasmids together with 0.5 μ g of pIRES-CD8 (provided by Dr. Patel) as a marker of transfection. Transfected cells were visualized 48 hours following transfection, using the anti-CD8 antibody-coated beads method.¹⁹ Current measurements were performed 48 hours following transfection, using the whole-cell configuration of the patch-clamp technique.²⁰ Signals were amplified using an Axopatch 200B patch-clamp amplifier (Axon Instruments), sampled at 500 Hz, and filtered at 200 Hz via a 4-pole Bessel low pass filter. Data were acquired using pClamp 6.0.2 software (Axon Instruments, Union City, CA) and an IBM-compatible 486 computer in conjunction with a DigiData 1200 interface (Axon Instruments). The patch pipettes were pulled from borosilicate glass (fiber filled) with a resistance of 4–8 M Ω and were filled with (in mM): 130 KCl, 1 MgCl₂, 5 KATP, 5 EGTA, 20 KOH, and 10 HEPES at pH 7.4. The external solution contained (in mM): 140 NaCl, 4 KCl, 1.8 CaCl₂, 1.2 MgCl₂, 11 glucose, and 5.5 HEPES at pH 7.4. All data were expressed as mean $\pm$ SEM.

Figure 1. (A) Twelve-lead ECG in patient no. 29, 27-year-old female M124R carrier. The T wave has a bifid configuration in several leads with a QTc of 495 ms. (B) Pedigree of LQTS family. Phenotypically affected individuals are represented by solid circles (females) or solid squares (males); phenotypically unaffected individuals by open circles and squares; individuals with equivocal LQTS status by gray circles and squares; those with unknown phenotypic status by a short diagonal line from top left to bottom right; and deceased individuals by a long diagonal line from top right to bottom left. Genotyped individuals are noted by consecutive Arabic numbers above and to the left of pedigree symbols; + and – below pedigree symbols denotes the presence or absence, respectively, of the M124R mutation; S below the M124R indicator denotes LQT-related symptoms. * = Proband.

RESULTS

Clinical Data

Twelve-lead electrocardiograms (1–10 per individual over 18 years of follow-up) were available in 57 family members, of whom 36 were categorized

(according to their mean QTc in all available ECGs) as nonaffected, 18 as affected, and 3 as equivocal (Fig. 1B). Their QTc (mean ± SD, ms) was 410 ± 23, 498 ± 41, and 440 ± 10, respectively. A representative 12-lead ECG of an affected family member is depicted in figure 1A. Figure 1B depicts two

Table 1. Clinical Events Among Symptomatic Affected Family Members

Pedigree No.	Current Age (Years)	Sex	Type of Symptoms	Symptomatic Events (No.)	Age at First Event (Years)	Age at Last Event (Years)	Beta-Blocker Therapy	Pacemaker
16	60	M	S	1	22	22	—	—
18	55	F	S	5	17	21	+	—
19	55	F	S	5	16	22	—	—
28	25	F	S; SCD	2	24	25	—	—
32	39	M	S	5	11	16	—	—
33	34	F	ACA; S	2	26	27	+	+
37	19	M	S	1	12	12	+	+
41	33	M ^a	S; ACA	2	18	19	+	+

Multiple symptoms per individual are listed according to their sequence of occurrence.

S = Syncope; ACA = Aborted cardiac arrest; SCD = Sudden cardiac death.

^aProband.

additional family members marked as phenotypically affected, although ECG was not available for both (spouse of patient no. 2, an obligate carrier and patient no. 28, LQT-related sudden death at 25 years of age). Among the 20 affected family members (18 by ECG) eight were symptomatic (four males and four females). The mean QTc of symptomatic affected family members was 522 ± 53 ms, while that of asymptomatic affected family members was 478 ± 9 ms. Their clinical events are summarized in Table 1. Five had only syncopal episodes that first occurred at 11–22 years of age, while two had aborted cardiac arrest (one male at 18 years and one female at 26 years), and one had sudden cardiac death (female, at 25 years). A specific trigger of cardiac events (acoustic, early morning phone call) was identified in only two cases: preceding both syncope and aborted cardiac arrest in one, and sudden cardiac death in the other (Fig. 1B, patient nos. 33 and 28, respectively). Only four of the eight symptomatic family members were treated by beta-blockers, three of whom also had a pacemaker implanted at the time the beta-blockers were started. They have all remained asymptomatic since their last event for 7–35 years. Three symptomatic family members refused beta-blocker therapy and have remained asymptomatic since their last event for the past 23–28 years. Of the 12 asymptomatic affected family members, only 2 (Fig. 1B, patient nos. 29 [Fig. 1A] and 39) were prophylactically treated with beta-blockers. One of them also had a pacemaker implanted at the time the beta-blockers were started. They both remained asymptomatic for 7–16 years after the beta-blockers were started.

Genetic Analyses

Linkage Analysis

Linkage analysis was performed according to Hasstedt and Moll.²¹ No linkage was found between the disease and the KvLQT1 or SCN5A markers, while the disease was found to be linked to an allele of the HERG marker. This allele was found in 16 phenotypically affected family members and not found in 26 phenotypically nonaffected family members and three with an equivocal phenotype.

Identification of the HERG Mutation

Direct sequencing of the 16 exons encoding the HERG gene and their flanking intronic sequences was performed on DNA of an affected family member (Fig. 1, patient no. 19) and compared with the available sequences in GeneBank. The analysis revealed four base substitutions. Three were single nucleotide polymorphisms, which do not change the amino acid sequence of the protein (C->T at position 1467, A->G at position 1692, and T->C at position 1956) and were all previously reported as polymorphisms in other families.^{22,23} One base substitution, T->G at nucleotide 370 within exon 3 (Fig. 2A), is expected to cause a nonconservative change in position 124 of the HERG protein, from Methionine to Arginine (M124R) in a highly conserved region of the HERG channel (Fig. 2B), indicating the significance of this region to the normal function of the HERG channel. The M124R mutation was found to destroy a restriction site of the NlaIII endonuclease. Hence, 45 family members (Fig. 1B) were analyzed for the mutation by

amplification of sequences within exon 3 and analysis of the NlaIII restriction site. The M124R mutation was found in all 16 LQT-affected family members, while all 26 nonaffected and the three individuals with an equivocal phenotype did not carry the mutation. The mean OTc of mutation car-

riers was 501 ± 43 ms, while that of noncarriers was 413 ± 22 ms. The M124R mutation was not found in 100 chromosomes obtained from healthy individuals of Jewish-Iranian origin (obtained from the National Laboratory for the Genetics of Israeli Populations). Furthermore, the M124R mutation was

not found in 16 unrelated small Israeli families with LQT-affected individuals, in whom the molecular basis for the LQTS is unknown.

Cellular Electrophysiology

The HERG channel is known to produce an inwardly rectifying K^+ current, which results from rapid channel inactivation.^{24,25} To study the naturally occurring point mutation M124R, we expressed the HERG channel in CHO cells and used the whole-cell configuration of the patch-clamp technique. Representative WT currents are shown in Figure 3A left. In this experiment, we used a tail protocol in which the cells were stepped to a conditioning pulse of +40 mV (1 second) and then stepped to a test pulse (3 seconds) ranging from -120 to +40 mV, evoking the measured tail currents. As compared to WT the point mutation M124R reduced the HERG tail-current density by about 65%, from 26.38 ± 2.50 (pA/pF $\pm$ SEM, $n = 27$) to 8.92 ± 1.94 (pA/pF $\pm$ SEM, $n = 22$) for -20 mV tail voltage (Figs. 3A and B). In addition, there was a small but significant ($P < 0.05$) negative shift (6.8 mV) of the voltage dependence of activation as a result of the M124R mutation. The Boltzman fitting values were $V_{50} = +5.18 \pm 1.12$ (mV $\pm$ SEM, $n = 12$) slope = -8.51 ± 0.43 (mV/e-fold $\pm$ SEM) and $V_{50} = -1.62 \pm 3.04$ (mV $\pm$ SEM, $n = 5$), slope = -8.74 ± 0.51 (mV/e-fold $\pm$ SEM), for WT and M124R, respectively (Fig. 3C). The deactivation process was markedly altered by this mutation. Tail-current analysis revealed that the M124R mutation accelerated significantly the deactivation kinetics, as shown in the normalized traces in Figures 3D and 3E. The decay of the tail current was best fitted using two exponentials, τ fast and τ slow. For example, when the membrane was stepped to a tail voltage of -60 mV, τ fast was reduced from 178 ± 14 (ms $\pm$ SEM, $n = 27$) to 72 ± 6 (ms $\pm$ SEM, $n = 20$), and τ slow was reduced from 850 ± 90 to 358 ± 29 (ms $\pm$ SEM, $n = 20$) for WT and M124R, respectively (Fig. 3E).

DISCUSSION

This study identified a new PAS-domain HERG mutation (M124R) among a large Jewish family with LQTS, which causes a change in a highly conserved region of the HERG channel. In a cellular electrophysiologic model, the M124R mutation decreased the tail-current density by 65%, signif-

Figure 3. HERG currents of WT and M124R mutation in transfected CHO cells. (A) HERG tail currents recorded from WT (left panel) and M124R mutant (right panel) expressed in CHO transfected cells. The membrane potential was stepped from a holding potential of -85 to +40 mV (1 second) and then tail currents were elicited by test pulses to voltages ranging from -120 to +40 mV in 20 mV increments (3 seconds). (B) Tail-current (-40 mV, 4 seconds) density (pA/pF) plotted against the pre-pulse voltage (mV, 4 seconds) for WT (open squares) and M124R mutant (closed squares). (C) Normalized tail current (I/I_{max} , -20 mV, 3 seconds) plotted against pre-pulses ranged from -50 to +50 mV (10 mV increments, 2 seconds). (D) Normalized tail current (-40 mV) of WT and M124R in transfected cells. (E) Fast (left panel) and slow (right panel) time constants of deactivation plotted against tail voltage of WT (open squares) and M124R mutant (closed squares) taken from the experiments presented in Figure 3A.

icantly accelerated the deactivation kinetics, and caused a negative shift of the voltage dependence of activation of the HERG channel.

The identification of a new mutation in the studied family is not surprising, since different Jewish subgroups tended to live as relatively isolated

populations until recent times, thus specific genetic defects are expected to be found in their gene pool.

Indeed, a variety of genetic diseases have a significantly higher prevalence among Jewish than among non-Jewish populations. Moreover, a specific repertoire of mutations causing common diseases in the general population has been reported among Jews.²⁶

The studied family had several specific phenotypic features, only some of which are rather unusual, and therefore could be interpreted as mutation specific. The QTc of symptomatic carriers ($n = 8$, 522 ± 53 ms) was more prolonged than that of asymptomatic carriers ($n = 10$, 478 ± 9 ms), as has been previously reported regarding several LQTS genotypes.²⁷ There was a very high penetrance level (all carriers had prolonged QTc) in comparison to previous reports that included carriers of several other HERG mutations,²⁸ and a rather low rate of equivocal QTc (5.8%) among the whole family. Acoustic triggering (early morning phone call) of cardiac events was present in two symptomatic carriers (25%), a rate which is similar to that previously reported in HERG-related LQTS.²⁹ Beta-blocker therapy was recommended to all eight symptomatic M124R-carriers, yet three of them refused. In three of the five carriers, who were treated with beta-blockers, a pacemaker was implanted concomitantly. Beta-blocker therapy was also given to two asymptomatic carriers (one with a pacemaker) who had a QTc > 0.55 seconds. All treated carriers remained asymptomatic for 5–30 years following the initiation of therapy. All untreated carriers remained asymptomatic as well, over an average follow-up of 18 years. This good clinical response to beta-blockers on one hand, and the lack of clinical events beyond the age of 26 years even in untreated carriers, is probably related to the complex age dependency of cardiac events in LQTS.²⁷

In a previous study, Moss et al. reported that the arrhythmic risk in carriers of pore mutations was significantly higher than that in carriers of nonpore mutations in the HERG channel.³⁰ Such finding should influence considerations regarding the practical contribution of genotyping and the desired aggressiveness of therapeutic interventions by mutation in HERG-related LQTS. In that previous study, nonpore mutations included C-terminus and N-terminus mutations. It is therefore important to note that in the current study among M124R carriers (an N-terminus mutation), the arrhythmic risk was not low at all; 8 out of 20 carriers (40%) were

symptomatic (at 11–26 years of age), while three symptomatic carriers (15%) had aborted cardiac arrest or sudden death as their first symptom (at 18–26 years of age).

From a structural and functional point of view, the M124R mutation resides in a strategic location of the PAS domain. The crystal structure of the HERG N-terminus revealed the design of a basic helix-loop-helix, typical of the PAS domains found in sensory transduction proteins.³¹ The HERG PAS structure is endowed with five-stranded antiparallel β -sheet packed against a long strand of a coil and a single turn of a 3_{10} helix.³¹ The residue M124 is located over a domain surface within a hydrophobic patch having significant solvent accessibility. It was previously suggested that the hydrophobic patch in the PAS structure may provide an interface for interaction with the S4–S5 linker of HERG, which in turn would regulate the channel deactivation process.³¹ It is likely that the hydrophobic environment of the patch is maintained through the packing of hydrophobic surfaces. Thus, the M124R mutation is expected to disturb the hydrophobic surface of patch. This strategic location of M124 in HERG reasonably accounts for the impact that the mutation has on channel deactivation. It is unclear, however, by which mechanism the M124R mutant decreases the HERG current density. A PAS domain mutation (T65P) was previously shown to exhibit a trafficking defect in addition to its impact on channel deactivation.^{32,33} It remains to be clarified whether the M124R mutation affects the processing of the channel or the unitary channel conductance. Clearly, the increased arrhythmic risk reported in this study is probably mutation specific and might be related to pleiotropic impacts on HERG channel function.

REFERENCES

1. Ward OC. A new familial cardiac syndrome in children. *J Ir Med Assoc* 1964;54:103–106.
2. Schwartz PJ, Periti M, Malliani A. The long QT syndrome. *Am Heart J* 1975;89:378–390.
3. Moss AJ, Schwartz PJ, Crampton RS, et al. The long QT syndrome. Prospective longitudinal study of 328 families. *Circulation* 1991;84:1136–1144.
4. Wang Q, Curran ME, Splawski I, et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet* 1996;12:17–23.
5. Curran ME, Splawski I, Timothy KW, et al. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* 1995;80:795–803.
6. Splawski I, Tristani-Firouzi M, Lehmann MH, et al. Mutations in the hminK gene cause long QT syndrome and suppress IKs function. *Nat Genet* 1997;17:338–340.

7. Abbott GW, Sesti F, Splawski I, et al. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 1999;97:175-187.
8. Tristani-Firouzi M, Jensen JL, Donaldson MR, et al. Functional and clinical characterization of KCNJ2 mutations associated with LQT7 (Andersen syndrome). *J Clin Invest* 2002;110:381-388.
9. Wang Q, Shen J, Splawski I, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* 1995;80:805-811.
10. Mohler PJ, Schott JJ, Gramolini AO, et al. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature* 2003;421:634-639.
11. Splawski I, Shen J, Timothy KW, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation* 2000;102:1178-1185.
12. Benhorin J, Kalman YM, Medina A, et al. Evidence of genetic heterogeneity in the long QT syndrome. *Science* 1993;260:1960-1962.
13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
14. Hu RJ, Lee MP, Connors TD, et al. A 2.5-Mb transcript map of a tumor-suppressing subchromosomal transferable fragment from 11p15.5, and isolation and sequence analysis of three novel genes. *Genomics* 1997;46:9-17.
15. Jiang C, Atkinson D, Towbin JA, et al. Two long QT syndrome loci map to chromosomes 3 and 7 with evidence for further heterogeneity. *Nat Genet* 1994;8:141-147.
16. Satler CA, Walsh EP, Vesely MR, et al. Novel missense mutation in the cyclic nucleotide-binding domain of HERG causes long QT syndrome. *Am J Med Genet* 1996;65:27-35.
17. Splawski I, Shen J, Timothy KW, et al. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. *Genomics* 1998;51:86-97.
18. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 1977;74:5463-5467.
19. Jurman ME, Boland LM, Liu Y, et al. Visual identification of individual transfected cells for electrophysiology using antibody-coated beads. *Biotechniques* 1994;17:876-881.
20. Hamill OP, Sakmann B. Multiple conductance states of single acetylcholine receptor channels in embryonic muscle cells. *Nature* 1981;294:462-464.
21. Hasstedt SJ, Moll PP. Estimation of genetic model parameters: Variables correlated with a quantitative phenotype exhibiting major locus inheritance. *Genet Epidemiol* 1989;6:319-332.
22. Akimoto K, Furutani M, Imamura S, et al. Novel missense mutation (G601S) of HERG in a Japanese long QT syndrome family. *Hum Mutat* 1998;(Suppl. 1):S184-S186.
23. Larsen LA, Christiansen M, Vuust J, et al. High-throughput single-strand conformation polymorphism analysis by automated capillary electrophoresis: Robust multiplex analysis and pattern-based identification of allelic variants. *Hum Mutat* 1999;13:318-327.
24. Smith PL, Baukrowitz T, Yellen G. The inward rectification mechanism of the HERG cardiac potassium channel. *Nature* 1996;379:833-836.
25. Zou A, Curran ME, Keating MT, et al. Single HERG delayed rectifier K⁺ channels expressed in xenopus oocytes. *Am J Physiol* 1997;272:H1309-H1314.
26. Kerem B, Chiba-Falek O, Kerem E. Cystic fibrosis in Jews: Frequency and mutation distribution. *Genet Test* 1997;1:35-39.
27. Zareba W, Moss AJ, Locati EH, et al. Modulating effects of age and gender on the clinical course of long QT syndrome by genotype. *J Am Coll Cardiol* 2003;42:103-109.
28. Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: Clinical impact. *Circulation* 1999;99:529-533.
29. Benhorin J, Moss AJ, Bak M, et al. Variable expression of long QT syndrome among gene carriers from families with five different HERG mutations. *Ann Noninvasive Electrocardiol* 2002;7:40-46.
30. Moss AJ, Zareba W, Kaufman ES, et al. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. *Circulation* 2002;105:794-799.
31. Morais CJH, Lee A, Cohen SL, et al. Crystal structure and functional analysis of the HERG potassium channel N terminus: A eukaryotic PAS domain. *Cell* 1998;95:649-655.
32. Paulussen A, Raes A, Matthijs G, et al. A novel mutation (T65P) in the PAS domain of the human potassium channel HERG results in the long QT syndrome by trafficking deficiency. *J Biol Chem* 2002;277:48610-48616.
33. Chen J, Zou A, Splawski I, et al. Long QT syndrome-associated mutations in the Per-Arnt-Sim (PAS) domain of HERG potassium channels accelerate channel deactivation. *J Biol Chem* 1999;274(15):10113-10118.