

Brief Report

MOLECULAR DIAGNOSIS OF THE INHERITED LONG-QT SYNDROME IN A WOMAN WHO DIED AFTER NEAR-DROWNING

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DROWNING accounts for more accidental deaths in children and adolescents than all other causes except motor vehicle accidents.¹⁻³ Many of these fatalities are attributed to lack of supervision, trauma, alcohol or drug use, or seizures. However, an appreciable number of drownings have no satisfactory explanation. In these situations, cardiac arrhythmias, particularly those associated with the long-QT syndrome, may be an important consideration. The long-QT syndrome comprises a group of genetically distinct arrhythmogenic cardiovascular disorders, each resulting from a mutation in one of five genes encoding cardiac ion channels or auxiliary ion-channel subunits: *KVLQT1* (at the LQT1 locus), *HERG* (at LQT2), *SCN5A* (at LQT3), *hKCNE1* (encoding minimal potassium-channel beta subunit [minK], at LQT5), and *hKCNE2* (encoding minK-related peptide 1 [MiRP-1], at LQT6).^{4,5} As compared with other exertional activities, swimming seems to be particularly arrhythmogenic in patients with the long-QT syndrome.⁶⁻⁸

We report the results of postmortem molecular testing and the identification of a novel *KVLQT1* mutation in a 19-year-old woman who had been asymptomatic but who died after a near-drowning. Because of this molecular-test finding at autopsy, we were able to confirm the presence of the disease-causing mutation in several of the decedent's first-degree relatives. This molecular-test result led to the treatment of an asymptomatic sibling.

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CASE REPORT

On August 18, 1998, an apparently healthy 19-year-old woman was exercising in a local fitness center in northern Iowa. Over the previous several days, she had reported influenza-like gastrointestinal symptoms. After her weight-lifting routine, she sat in a hot tub and then swam laps in a lap pool that was 1.2 m (4 ft) deep. She was discovered face down at the bottom of the pool. The submersion time was estimated to be five minutes.

Cardiopulmonary resuscitation was initiated, and the woman was electrically defibrillated twice, but without the return of a pulse. She was transported to the local hospital, where she was given epinephrine and was defibrillated successfully. After resuscitation, her score on the Glasgow Coma Scale was 5 of 15. The woman was then transferred by helicopter to Saint Mary's Hospital at the Mayo Clinic in Rochester, Minnesota. When she arrived three hours later, she was already intubated and had pinpoint pupils, no response to painful stimuli, and bilateral decorticate posturing. She never regained consciousness, and she died 12 days later.

The immediate cause of death at autopsy was listed as anoxic encephalopathy after near-drowning. In addition, there was evidence of recent myocardial ischemia of moderate severity involving the circumferential subendocardial region of the left ventricle, severe pulmonary congestion, and acute renal tubular necrosis.

Because of some unusual features of this case, a pathologist involved with the autopsy froze a portion of myocardium and consulted the principal investigator. Namely, the woman was reportedly a good swimmer and yet drowned in only 1.2 m of water. There was no personal history or history among members of the woman's immediate family (her mother, who was 49 years old; her father, who was 52; and her three sisters, who were 27, 22, and 18) of seizures, syncope, or palpitations. There was no history in the extended family of any unexplained sudden death or accidents attributable to blackout spells. The woman had no history of drug or alcohol use, and the results of drug screening on admission were negative. Radiographs of the cervical spine and computed tomographic scans of the head were both normal.

Intriguingly, an electrocardiogram obtained four hours after the near-drowning revealed marked QT prolongation, with a QT interval corrected for heart rate (QTc) of 0.60 second^{1/2} (Fig. 1). This finding, however, was associated with electrolyte abnormalities that included hypokalemia, hypocalcemia, and hypomagnesemia, as well as acute anoxic brain injury. Each of these factors individually may cause QT prolongation. Despite correction of the electrolyte levels, the woman's QT prolongation persisted. In addition, she had several episodes of unsustained ventricular tachycardia that occurred in the setting of hypomagnesemia. These arrhythmias were terminated with intravenous infusions of magnesium sulfate. It was clinically impossible to determine whether the cardiac dysfunction and electrical instability preceded and caused the near-drowning or resulted from it.

Despite the entirely asymptomatic personal and family histories, we conjectured that perhaps the observed QT prolongation stemmed from a predisposing genetic defect that caused the woman's ultimately fatal near-drowning. Using a piece of the frozen myocardium obtained during the autopsy, we undertook molecular genetic screening for ion-channel mutations known to cause the long-QT syndrome.

METHODS

A research protocol aimed at identifying ion-channel mutations in patients with possible long-QT syndrome or unexplained sudden death was reviewed and approved by the Mayo Foundation's institutional review board. After written consent had been obtained from the woman's parents, genomic DNA was extracted and isolated from a piece of frozen left ventricular tissue with the use of standard phenol-chloroform extraction. DNA was also extracted from peripheral-blood lymphocytes in specimens obtained from family members, with the use of a DNA extraction kit (Pur-gene, Gentra Systems, Minneapolis).⁹

Using the full-length sequences and previously described primers

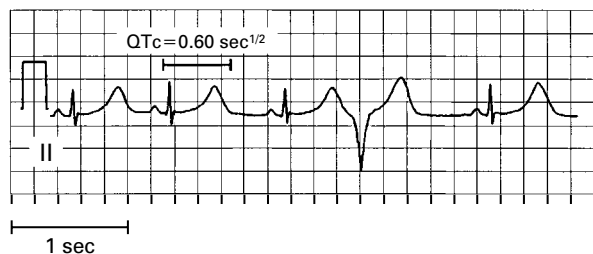


Figure 1. Electrocardiogram from a 19-Year-Old Woman after Near-Drowning.

A portion of the lead II tracing from a standard 12-lead electrocardiogram recorded at 25 mm per second reveals marked prolongation of the QT interval. This electrocardiogram was recorded approximately four hours after the patient's near-drowning. QTc denotes corrected QT interval.

for introns and exons of *KVLQT1*, *HERG*, *hKCNE1*, and *SCN5A*, we screened all the exons previously reported to contain mutations causing the long-QT syndrome (Fig. 2).^{10,11} This method involved exon-specific amplification by the polymerase chain reaction and direct manual sequence analysis (ThermoSequenase, Amersham Life Sciences, Cleveland) with ³³P-labeled dideoxy nucleotide triphosphates.⁸ No mutations were identified by this exon-targeted approach. We then proceeded to screen each gene from end to end, beginning with *KVLQT1*, since mutation of that gene is the most common molecular basis of the long-QT syndrome. The screening of exon 1 yielded a novel mutation. Because of DNA-sequence compressions occurring in this guanine- and cytosine-rich region, modifications of the standard protocol, including the use of 7-deaza-2'-deoxyguanosine triphosphate and formamide, were required to delineate precisely the 9-bp deletion.

After the mutation was identified, blood samples were obtained from members of the woman's immediate family, who gave their written informed consent, to determine whether this mutation was sporadic or familial. The presence of the mutation in the *KVLQT1* gene in other family members was confirmed by single-strand conformation polymorphism analysis, again with use of

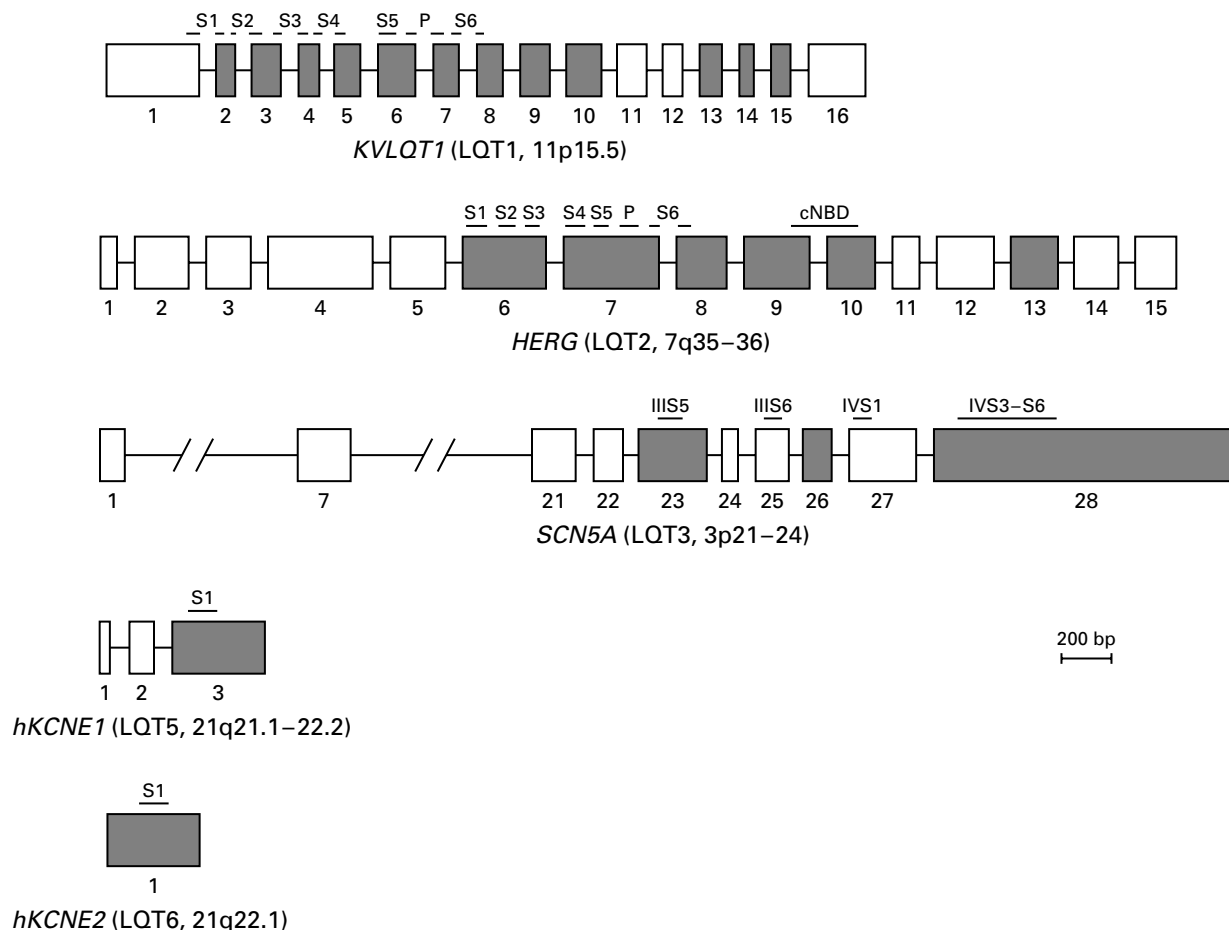


Figure 2. Mutations That Cause the Long-QT Syndrome.

The five genes involved in the long-QT syndrome are shown, with the exons labeled by number and drawn to scale. Shading indicates exons in which mutations have been reported previously. Labels above the genes indicate where the transmembrane regions (S1 through S6), pore (P), and cyclic nucleotide-binding domain (cNBD) of the ion channels are encoded. Roman numerals indicate the subunit domains. Labels in parentheses below the genes provide the locus and chromosome map location of each gene. Primers used to amplify the exons have been described previously.^{5,10,11}

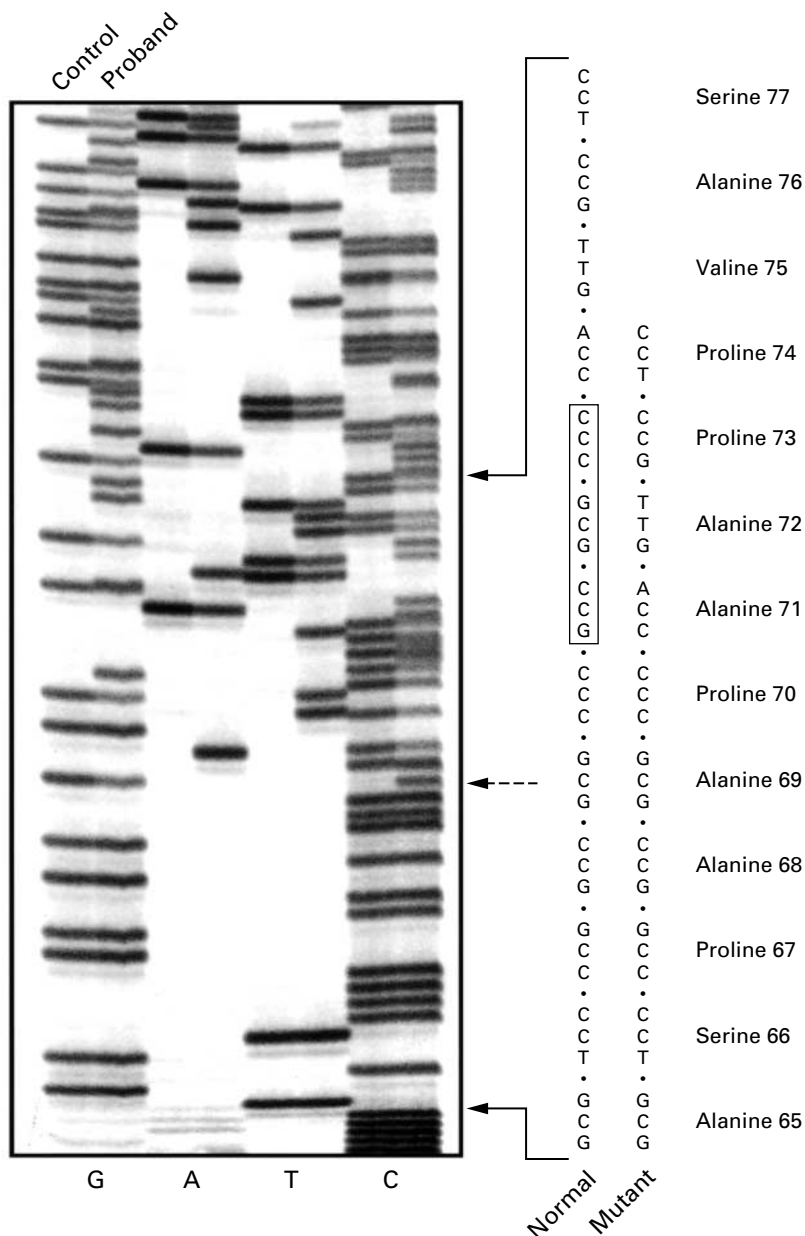


Figure 3. Identification of a Novel Mutation Causing the Long-QT Syndrome in Exon 1 of the Potassium-Channel Gene *KVLQT1*.

A portion of the DNA sequence of exon 1 that encodes some of the cytoplasmic N-terminal residues of the *KVLQT1* subunit is shown. DNA was extracted from a specimen of myocardium from the proband and from a control blood sample. The dashed arrow indicates the presence of a mutant allele in the proband's sequence. The nucleotide sequences of the region between the two solid arrows are shown on the right-hand side. The 9-bp deletion (shown enclosed by a box in the normal sequence) results in loss of alanine, alanine, and proline residues from positions 71 through 73. Dots separate triplet codons.

7-deaza-2'-deoxyguanosine triphosphate to demonstrate the deletion clearly.

RESULTS

Novel 9-bp Deletion in *KVLQT1*

Figure 3 depicts a portion of the DNA sequence from exon 1 of *KVLQT1*, showing a 9-bp deletion involving nucleotides 373 through 381. The mutation was discovered in DNA isolated from the woman's myocardium. This 9-bp deletion (GCCGCGCCC) results in an in-frame deletion of three amino acids (alanine, alanine, and proline residues from positions 71 through 73) in the cytoplasmic N-terminal region of the *KVLQT1* ion-channel subunit. This mutation was not present in blood samples from 100 control subjects, representing 200 alleles (data not shown).

Screening of Family Members

After identifying this deletion, we sought to determine whether it was a sporadic mutation or a first and fatal manifestation of the familial long-QT syndrome. The immediate family members were examined by 12-lead electrocardiography, and DNA isolated from samples of their blood was subjected to mutation detection by single-strand conformation polymorphism analysis (Fig. 4). The woman's maternal grandfather, mother, and 18-year-old sister also had the 9-bp deletion. The mother's screening electrocardiogram was diagnostic of the long-QT syndrome, with a mean calculated QTc from lead II of 0.53 second^{1/2}. The T-wave morphology, however, was not characteristic of the phenotype resulting from a mutation in *KVLQT1*¹² (data not shown).

Effect of Genetic Testing on Clinical Management

The screening electrocardiogram from the 18-year-old sister revealed a QTc with borderline prolongation (0.45 second^{1/2}), and the automated diagnostic interpretation labeled it as normal (Fig. 5). We asked eight cardiologists who specialized in clinical electrophysiology and who were unaware of the results of the molecular tests to review the screening electrocardiograms and to make recommendations for the clinical care of this family, with particular attention to the care of the 18-year-old sister. On review of this sister's electrocardiogram, only one cardiologist judged it to be definitely abnormal; three considered it equivocal, and four considered it entirely normal. Indeed, half the cardiologists concluded that the sister should be considered unaffected. Only two of the eight cardiologists recommended beta-blocker therapy on the basis of this clinical information.

Subsequently, each family member's genotype was revealed, and the effect of genetic testing on cardiac care was determined. After the 18-year-old sister's genotype was disclosed, all eight cardiologists strongly recommended that she receive beta-blocker therapy,

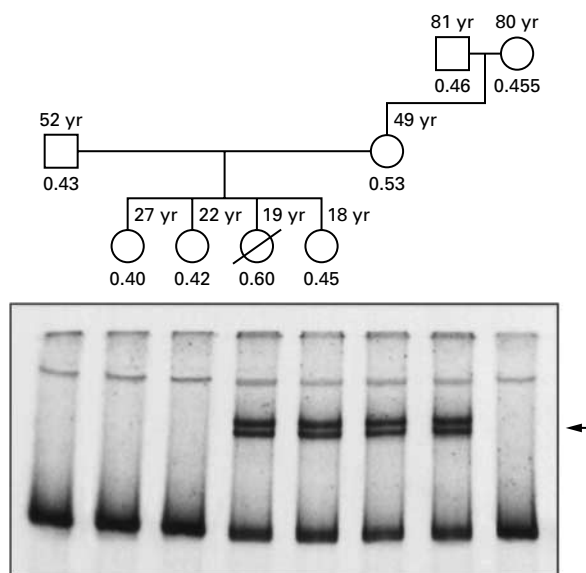


Figure 4. Detection of the Mutation in the Woman's Immediate Family Members.

Results of single-strand conformation polymorphism analyses for the 9-bp deletion are shown. The pedigree (squares represent males and circles females) is shown with the symbol for each family member aligned above the lane containing his or her amplified polymerase-chain-reaction product. The symbol for the proband (deceased) is shown with a slash mark. Each family member's age is indicated above his or her symbol, and the corrected QT interval from the screening electrocardiogram is shown (in seconds^{1/2}) below the symbol. The heteroduplex banding pattern indicative of the woman's mutation (arrow) is present in DNA from her 18-year-old sister, mother, and maternal grandfather.

and three of them stated that they would suggest the use of an internal cardioverter-defibrillator device.

DISCUSSION

This case, in which the long-QT syndrome was diagnosed by molecular analysis of an autopsy specimen, holds potentially great importance for forensic science. The postmortem identification of a novel mutation in the ion-channel gene *KVLQT1*, causing the long-QT syndrome, in a sample of the woman's myocardium provided a plausible mechanism to explain her near-drowning and death.

The resuscitation of this patient, though ultimately unsuccessful, suggests a potential mechanism for unexplained drownings. Specifically, her resuscitation allowed electrocardiographic documentation of QT prolongation, which was a notable finding, given the entirely asymptomatic personal and family history. Her death was the only apparent manifestation of the familial long-QT syndrome in this family. It is not yet known whether mutations in cardiac ion channels underlie a substantial number of unexplained drown-

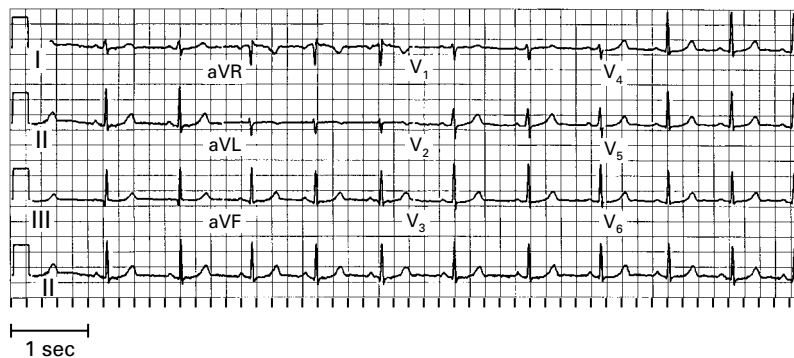


Figure 5. Screening Electrocardiogram from the Woman's Asymptomatic 18-Year-Old Sister.

A portion of a standard 12-lead electrocardiogram recorded at 25 mm per second is shown. The corrected QT interval was 0.45 second^{1/2}. At a heart rate of 66 beats per minute, the PR interval was 0.15 second, the QRS duration 0.08 second, and the uncorrected QT interval 0.43 second. The automated diagnostic interpretation labeled this electrocardiogram as normal.

ings. On the basis of the findings in this case, we have initiated a large, prospective study involving molecular screening for long-QT syndrome in such cases. In the meantime, use of 12-lead electrocardiography to screen persons from families in whom an unexplained fatal drowning has occurred may be worthwhile. Notably, in this case the decedent's mother was found to have a QTc diagnostic of the long-QT syndrome (0.53 second^{1/2}).

More important for this particular family, identification of this disease-causing mutation, which was traced back to the woman's maternal grandfather, provides a means of screening at least 60 extended relatives who may be at risk for a potentially fatal arrhythmia. Although molecular testing is currently unavailable as a routine clinical test, the importance of the test result in the recommended care of the 18-year-old sister clearly underscores the vital role of molecular genetic testing in the diagnosis of the long-QT syndrome.^{13,14} Without genetic testing, she would have been considered normal and would not have been treated. She also would not have received genetic counseling, including information about the 50 percent chance of passing this mutation to future offspring, the risks associated with swimming and other strenuous exertion, the list of medications to avoid, and the possible protective benefit of beta-blocker therapy.

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REFERENCES

- O'Flaherty JE, Pirie PL. Prevention of pediatric drowning and near-drowning: a survey of members of the American Academy of Pediatrics. *Pediatrics* 1997;99:169-74.
- Mizuta R, Fujita H, Osamura T, Kidowaki T, Kiyosawa N. Childhood drownings and near-drownings in Japan. *Acta Paediatr Jpn* 1993;35:186-92.
- Kallas HJ, O'Rourke PP. Drowning and immersion injuries in children. *Curr Opin Pediatr* 1993;5:295-302.
- Ackerman MJ. The long QT syndrome: ion channel diseases of the heart. *Mayo Clin Proc* 1998;73:250-69.
- Abbott GW, Sesti F, Splawski I, et al. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 1999; 97:175-87.
- Garson A Jr, Dick M II, Fournier A, et al. The long QT syndrome in children: an international study of 287 patients. *Circulation* 1993;87:1866-72.
- Moss AJ, Schwartz PJ, Crampton RS, et al. The long QT syndrome: prospective longitudinal study of 328 families. *Circulation* 1991;84:1136-44.
- Ackerman MJ, Schroeder JJ, Berry R, et al. A novel mutation in KVLQT1 is the molecular basis of inherited long QT syndrome in a near-drowning patient's family. *Pediatr Res* 1998;44:148-53.
- Anderson MA, Gusella JF. Use of cyclosporin A in establishing Epstein-Barr virus-transformed human lymphoblastoid cell lines. *In Vitro* 1984;20: 856-8.
- Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. *Genomics* 1998;51:86-97.
- Wang Q, Li Z, Shen J, Keating MT. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. *Genomics* 1996; 34:9-16.
- Moss AJ, Zareba W, Benhorin J, et al. ECG T-wave patterns in genetically distinct forms of the hereditary long QT syndrome. *Circulation* 1995;92:2929-34.
- Maron BJ, Moller JH, Seidman CE, et al. Impact of laboratory molecular diagnosis on contemporary diagnostic criteria for genetically transmitted cardiovascular diseases: hypertrophic cardiomyopathy, long-QT syndrome, and Marfan syndrome. *Circulation* 1998;98:1460-71.
- Priori SG, Barhanin J, Hauer RNW, et al. Genetic and molecular basis of cardiac arrhythmias: impact on clinical management. *Circulation* 1999; 99:518-28.