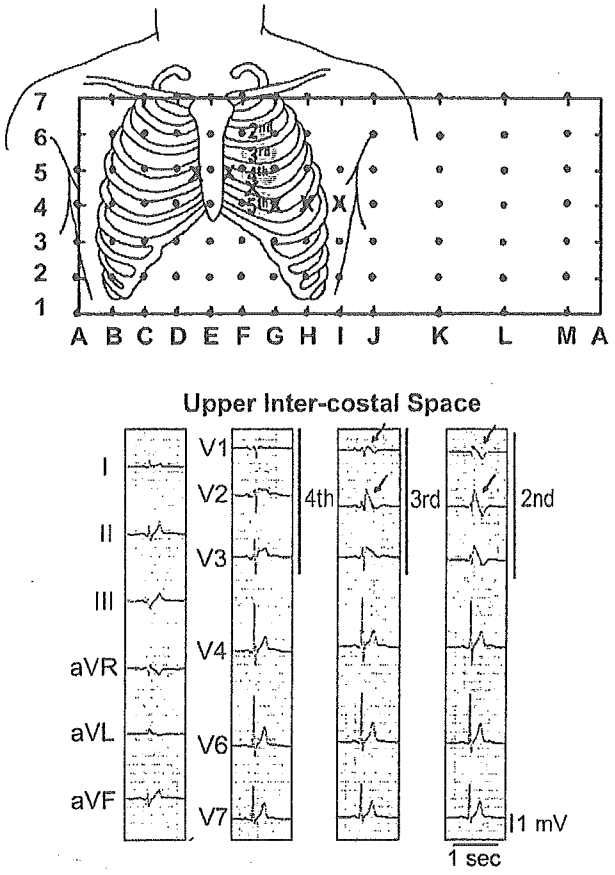

Figure 2. Shift of right precordial leads to 2nd and 3rd intercostal spaces unmasks a type 1 Brugada ECG. Top, Plot of 87 unipolar electrode sites (●) and of 6 precordial ECGs (✖). Eighty-seven lead points are arranged in a lattice-like pattern (13×7 matrix), except for 4 lead points on both midaxillary lines, and covered the entire thoracic surface. V_1 and V_2 leads of the ECG are located between D_5 and E_5 and between E_5 and F_5, respectively, whereas V_4, V_5, and V_6 are coincident with G_4, H_4, and I_4, respectively. Bottom, Twelve-lead ECGs in a patient with Brugada syndrome. Type 2 saddleback-type ST-segment elevation was observed in V_1 and V_2 of the standard 12-lead ECG (4th intercostal space), whereas typical type 1 coved-type ST-segment elevation was apparent in V_1 and V_2 recorded from the 2nd and 3rd intercostal spaces (←).

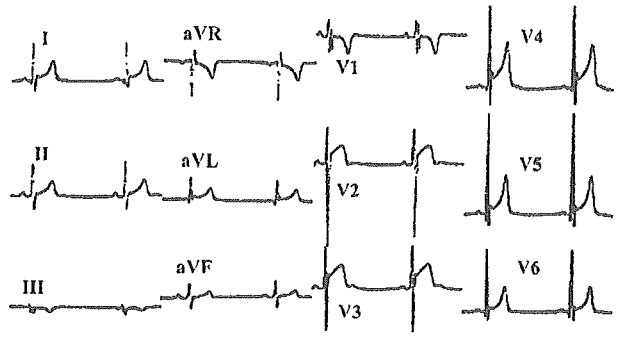

Figure 4. ECG of a well-trained, asymptomatic 24-year-old soccer player. ST-segment elevation is observed in V_2 to V_6 but with characteristics totally different from those seen in Brugada syndrome. A coved-type ST-segment elevation is not observed. A rounded or upsloping ST elevation in seen in V_2 and V_3, whereas V_4 to V_5 show a pattern resembling that commonly encountered in early repolarization syndrome.

clear, and the test is not without risk for provoking arrhythmic events.

Importantly, confounding factor or factors that could account for the ECG abnormality or syncope should be carefully excluded, including atypical right bundle-branch block, left ventricular hypertrophy, early repolarization, acute pericarditis, acute myocardial ischemia or infarction, pulmonary embolism, Prinzmetal angina,[21] dissecting aortic aneurysm,[22] various central and autonomic nervous system abnormalities,[23,24] Duchenne muscular dystrophy,[25] thiamin deficiency,[26] hyperkalemia,[22,27,28] hypercalcemia,[29,30] arrhythmogenic right ventricular dysplasia/cardiomyopathy,[31,32] pectus excavatum,[33] hypothermia,[34,35] and mechanical compression of the right ventricular outflow tract (RVOT) as occurs in mediastinal tumor[36] or hemopericardium.[37]

Of note, a Brugada-like ECG can occasionally appear for a brief period or for a period of several hours after direct-current cardioversion; it is not known whether these patients are gene carriers for Brugada syndrome.[38–40]

Another prominent confounding factor is the type of ST-segment elevation encountered in well-trained athletes (Figure 4), which is distinguished by an upslope rather than a

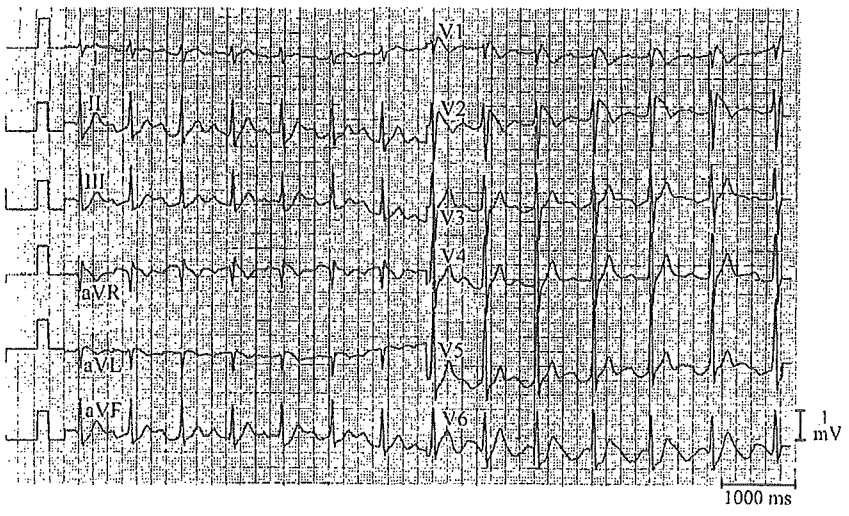

Figure 3. 12-lead ECG of a 40-year-old resuscitated man. The type 1 ECG shows typical repolarization as well as depolarization abnormalities. The former consists of ST-segment elevation in leads V_1 and V_2 (coved type, type 1). Depolarization abnormalities are present as PQ prolongation (270 ms), prolonged QRS width (120 ms), and S waves in leads I, II, and III. P wave duration is also wide.

TABLE 1. Drug-Induced Brugada-Like ECG Patterns

I. Antiarrhythmic drugs
  1. Na^+ channel blockers
    Class IC drugs (flecainide,[16,51,55,117,118] pilsicainide,[119,120] propafenone[121])
    Class IA drugs (ajmaline,[16,122] procainamide,[18,19] disopyramide,[4,19] cibenzoline[123])
  2. Ca^{2+} channel blockers
    Verapamil
  3. β-Blockers
    Propranolol, etc
II. Antianginal drugs
  1. Ca^{2+} channel blockers
    Nifedipine, diltiazem
  2. Nitrate
    Isosorbide dinitrate, nitroglycerine[124]
  3. K^+ channel openers
    Nicorandil
III. Psychotropic drugs
  1. Tricyclic antidepressants
    Amitriptyline,[125,126] nortriptyline,[77] desipramine,[75] clomipramine[76]
  2. Tetracyclic antidepressants
    Maprotiline[125]
  3. Phenothiazine
    Perphenazine,[125] cyamemazine[127]
  4. Selective serotonin reuptake inhibitors
    Fluoxetine[126]
IV. Other drugs
  1. Dimenhydrinate[78]
  2. Cocaine intoxication[79,128]
  3. Alcohol intoxication

downslope and by remaining largely unaffected by challenge with a sodium channel blocker. In addition, a variety of drugs have been reported to produce a Brugada-like ST-segment elevation (Table 1), although it is not yet clear whether or to what extent a genetic predisposition may be involved. The inclusion of drug categories in Table 1 should not be interpreted to imply that other members of the same "class" necessarily produce similar effects.

Although most cases of Brugada syndrome display right precordial ST-segment elevation, isolated cases of inferior lead[41] or left precordial lead[42] ST-segment elevation have been reported in Brugada-like syndromes; in some cases they have been associated with *SCN5A* mutations.[43]

The type 2 ST-segment elevation has a saddleback appearance with a high takeoff ST-segment elevation of ≥2 mm, a trough displaying ≥1 mm ST elevation, and then either a positive or biphasic T wave (Figure 1). Type 3 has either a saddleback or coved appearance with an ST-segment elevation of <1 mm. Type 2 and type 3 ECG are not diagnostic of the Brugada syndrome. These 3 patterns may be observed spontaneously in serial ECG tracings from the same patient or after the introduction of specific drugs. The diagnosis of Brugada syndrome is also considered positive when a type 2 (saddleback pattern) or type 3 ST-segment elevation is observed in >1 right precordial lead under baseline conditions and conversion to the diagnostic type 1 pattern occurs after sodium channel blocker administration (ST-segment elevation should be ≥2 mm). One or more of the clinical criteria described above also should be present. Drug-induced conversion of type 3 to type 2 ST-segment elevation is considered inconclusive for a diagnosis of Brugada syndrome.

TABLE 2. Drugs Used to Unmask Brugada Syndrome

Drug	Dosage and Administration
Ajmaline	1 mg/kg over 5 min, IV
Flecainide	2 mg/kg over 10 min, IV (400 mg, PO)
Procainamide	10 mg/kg over 10 min, IV
Pilsicainide	1 mg/kg over 10 min, IV

Placement of the right precordial leads in a superior position (up to the second intercostal space above normal) can increase the sensitivity of the ECG for detecting the Brugada phenotype in some patients, both in the presence or absence of a drug challenge (Figure 2).[44,45] Although previous reports suggested that none of the control patients displayed type 1 ST elevation when the V_1 to V_2 leads were displaced upward,[44,45] a prospective study with a larger number of controls will be required to exclude the possibility of false-positive results via this method.

A slight prolongation of the QT interval is sometimes observed in association with ST-segment elevation in Brugada syndrome.[46–48] The QT interval is prolonged more in the right precordial leads than it is in the left precordial leads, presumably because of a preferential prolongation of action potential duration in right ventricular epicardium secondary to accentuation of the action potential notch.[49] Depolarization abnormalities (Figure 3), including prolongation of P wave duration and PR and QRS intervals, are frequently observed, particularly in patients linked to *SCN5A* mutations.[50] PR prolongation likely reflects HV conduction delay.[46]

In addition to Brugada syndrome, ST-segment elevation is associated with a wide variety of benign as well as malignant pathophysiological conditions. A differential diagnosis is at times difficult, particularly when the degree of ST-segment elevation is relatively small and the specificity of sodium channel blockers such as flecainide, ajmaline, procainamide, disopyramide, propafenone, and pilsicainide[18,48,51] to identify patients at risk is uncertain. The recommended dosages are listed in Table 2. The test should be monitored with a continuous ECG recording (a speed of 10 mm/s can be used throughout the test period, interposed with recordings at 25 or 50 mm/s) and should be terminated when the diagnostic type 1 Brugada ECG develops, the ST segment in type 2 ECG increases by ≥2 mm, premature ventricular beats or other arrhythmias develop, or QRS widens to ≥130% of baseline. Intravenous sodium channel blockers always should be administered with great caution and infused slowly (as recommended in Table 1), closely monitored, and performed in a setting that is fully equipped for resuscitation. Particular caution should be exercised in patients with a preexisting

atrial or ventricular conduction (or both) disturbance (eg, suspected cases of Lev or Lenègre disease) or in the presence of wide QRS, wide P waves, or prolonged PR intervals (ie, infranodal conduction disease) to avoid the risk of precipitating complete AV block. Mechanoelectrical dissociation has been encountered in isolated cases. Isoproterenol and sodium lactate may be effective antidotes in this setting.

Patients at high risk for drug-induced AV block, such as older adults with syncope, should be administered sodium channel blockers in an electrophysiological study (EPS) environment after the insertion of a temporary pacing electrode. For other individuals, especially younger patients, sodium blocker challenge can be safely performed as a bedside test, provided the drug is discontinued as soon as excessive ST-segment elevation, QRS widening, or ventricular ectopy is observed.

Differentiation From ARVC and Other Structural Heart Diseases

A subpopulation of arrhythmogenic right ventricular cardiomyopathy (ARVC) patients have been found to display an ST-segment elevation and polymorphic VT that is characteristic of Brugada syndrome.[52] In addition, a case has been reported in which a patient with a Brugada syndrome phenotype required heart transplantation because of untreatable arrhythmias[52] and in whom severe fibrosis of the right ventricle was subsequently reported. These facts notwithstanding, the vast majority of patients with Brugada syndrome possess a structurally normal heart, which is consistent with the notion that this is a primary electrical heart disease.[53] It is not unreasonable to speculate that fibrosis and myocarditis, however mild, may occur and may exacerbate or indeed trigger events in patients with Brugada syndrome, although definitive evidence in support of this hypothesis is lacking. It is worth noting that recent studies suggest that some *SCN5A* defects may be capable of causing fibrosis in the conduction system and ventricular myocardium.[54]

ARVC and Brugada syndrome are distinct clinical entities both with regard to clinical presentation and genetic predisposition.[4] The only gene thus far linked to Brugada syndrome is *SCN5A*, the gene that encodes for the α subunit of the cardiac sodium channel, whereas ARVC has been linked to 10 different chromosomal loci and 3 putative genes independent of those responsible for Brugada syndrome.[55,56] Only the ARVC5 locus has been mapped to a region that overlaps with the second locus for Brugada syndrome, but no gene has been identified as yet.[57,58] Imaging techniques such as ECG, angiography, MRI, and radionuclide scintigraphy show no evidence of overt structural heart disease in patients with Brugada syndrome, whereas ARVC patients characteristically display right ventricular morphological and functional changes (eg, global dilatation, bulgings/aneurysms, and wall motion abnormalities). Ventricular arrhythmias in ARVC are most commonly monomorphic VT (left bundle-branch block type), which is often precipitated by catecholamines or exercise and accounts for sudden death in young competitive athletes.[59] In contrast, ST-segment elevation and arrhythmias in patients with Brugada syndrome are enhanced by vagotonic agents or β-adrenergic blockers, and polymorphic VT occurs most commonly during rest or sleep.[60] In contrast to those in Brugada syndrome, the ECG abnormalities in ARVC are not dynamic and display a constant T-wave inversion, epsilon waves, and, in the progressive stage, reduction of the R amplitude. These are largely unaffected by sodium channel blocker administration.[4]

Electron beam computed tomography has uncovered wall motion abnormalities in a series of patients with Brugada syndrome.[61] Although these contractile abnormalities are commonly considered pathognomonic of structural disease, recent studies[62,63] suggest that such contractile dysfunction can result from loss of the action potential dome in regions of the right ventricular epicardium and thus may be unrelated to any type of morphological defect. Loss of the dome leads to contractile dysfunction because the entry of calcium into the cells is greatly diminished and sarcoplasmic reticulum calcium stores are depleted. Signal-averaged ECG recordings have demonstrated late potentials in patients with Brugada syndrome, especially in the anterior wall of the RVOT,[64,65] and recordings from the epicardial surface of the anterior wall of the RVOT have revealed delayed potentials.[66] Although these types of potentials are commonly considered to be representative of the delayed activation of the myocardium secondary to structural defects, recent studies suggest that in the case of Brugada syndrome these late and delayed potentials may represent the delayed second upstroke of the epicardial action potential or local phase 2 reentry.[63] Late potentials also may reflect intraventricular conduction delays associated with *SCN5A* defects. Delayed contractile activation of the right ventricle in patients with Brugada syndrome[67] likewise may reflect delayed impulse propagation or, alternatively, a delayed second upstroke and action potential dome in the right ventricular epicardium.

Genetic Factors Underlying Brugada Syndrome

Inheritance of Brugada syndrome occurs via an autosomal dominant mode of transmission. The first and only gene to be linked to Brugada syndrome is *SCN5A*, the gene that encodes for the α subunit of the cardiac sodium channel gene.[68] More than 80 mutations in *SCN5A* have been linked to the syndrome since 2001.[69–73] About 2 dozen of these mutations have been studied in expression systems and shown to result in loss of function because of failure of the sodium channel to express; a shift in the voltage and time dependence of sodium channel current (I_{Na}) activation, inactivation, or reactivation; entry of the sodium channel into an intermediate state of inactivation from which it recovers more slowly; or accelerated inactivation of the sodium channel. A second locus on chromosome 3, close to but apart from the *SCN5A* locus, was linked recently to Brugada syndrome[57] in a large pedigree in which the syndrome is autosomal dominant inherited and associated with progressive conduction disease, a low sensitivity to procainamide, and a relatively benign prognosis. *SCN5A* mutations account for ≈18% to 30% of Brugada syndrome cases. A higher incidence of *SCN5A* mutations has been reported in familial than in sporadic cases.[74] Of note, negative *SCN5A* results do not rule out causal gene mutations because, in general, the promoter region, cryptic splicing

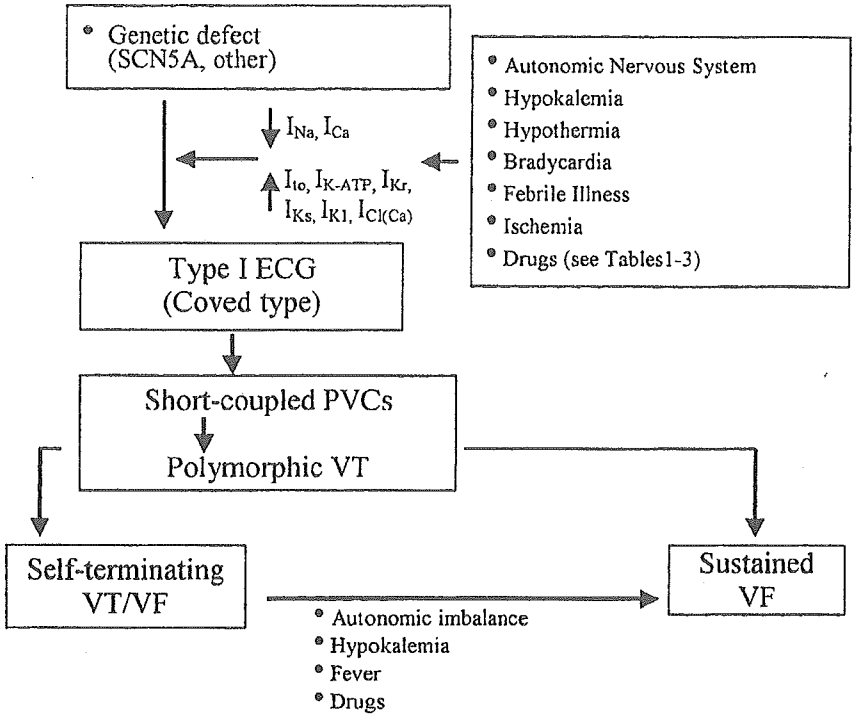

Figure 5. Factors predisposing to the ECG and arrhythmic manifestations of Brugada syndrome.

mutations, or the presence of gross rearrangements is not investigated.

At present, knowledge of a specific mutation may not provide guidance in formulating a diagnosis or determining a prognosis. Genetic testing is recommended, however, to support the clinical diagnosis, for early detection of relatives at potential risk, and to advance through research our understanding of the genotype–phenotype relationship.

Modulating and Precipitating Factors

The ECG manifestations of congenital Brugada syndrome are often concealed but can be unmasked or modulated by sodium channel blockers, a febrile state, vagotonic agents, α-adrenergic agonists, β-adrenergic blockers, tricyclic or tetracyclic antidepressants, a combination of glucose and insulin, hyperkalemia, hypokalemia, hypercalcemia, and alcohol and cocaine toxicity (Figure 5).[17–19,75–81] These agents may also induce acquired forms of Brugada syndrome (Table 1). Until a definitive list of drugs to avoid in Brugada syndrome is formulated, the list of agents in Table 1 may provide some guidance.

Acute myocardial infarction or ischemia from vasospasm involving the RVOT mimics ST-segment elevation similar to that in Brugada syndrome. This effect is likely the result of a depression of calcium channel current (I_{Ca}) and the activation of ATP-sensitive potassium channel current ($I_{K\text{-}ATP}$) during ischemia, and it suggests that patients with congenital and possibly acquired forms of Brugada syndrome may be at a higher risk for ischemia-related sudden cardiac death.[82]

VF and sudden death in Brugada syndrome usually occur at rest and at night. Figure 6 shows the circadian pattern of 64 VF episodes in 19 SUNDS patients treated with ICD. Circadian variation of sympathovagal balance, hormones, and other metabolic factors are likely to contribute to this circadian pattern. Bradycardia resulting from altered autonomic balance or other factors may contribute to the initiation of arrhythmia.[83–85]

Wichter et al demonstrated an abnormal ^{123}I-*m*-iodobenzylguanidine (^{123}I-MIBG) uptake in 8 (47%) of 17 patients with Brugada syndrome, but 0 in the control group.[86] Segmental reduction of ^{123}I-MIBG occurred in the inferior and the septal left ventricular walls, indicating presynaptic sympathetic dysfunction. It is noteworthy that imaging of the right ventricle, particularly the RVOT, is difficult with this

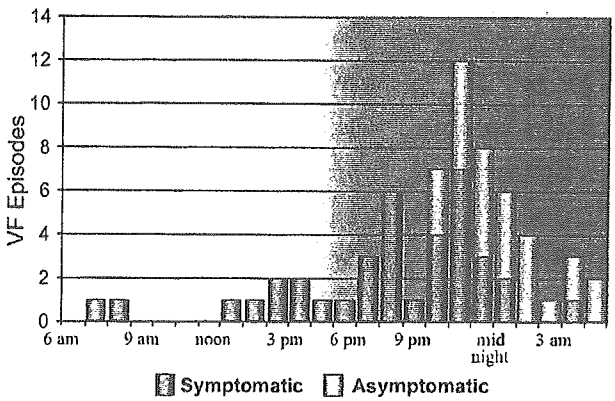

Figure 6. Circadian pattern of VF episodes in patients with Brugada syndrome. A nocturnal increase of VF episodes was found in 19 Thai-SUNDS patients with Brugada syndrome who received an ICD. All VF episodes were detected and documented by ICD interrogation. Interestingly, a significant number of VF episodes were asymptomatic because these episodes occurred while the patients were asleep (between 10 PM and early-morning hours) and they did not experience ICD discharges.

technique, so insufficient information is available about sympathetic function in the regions known to harbor the arrhythmogenic substrate. Moreover, it remains unclear what role the reduced uptake function plays in the arrhythmogenesis of Brugada syndrome. If the RVOT is similarly affected, then this defect may indeed alter the sympathovagal balance in favor of the development of an arrhythmogenic substrate.[87,88]

Hypokalemia has been implicated as a contributing cause of the prevalence of SUNDS in northeastern Thailand, where potassium deficiency is endemic.[81,89] Serum potassium in this northeastern population is significantly lower than that of the population in Bangkok, which lies in the central part of Thailand, where potassium is abundant in food.

The 1990 report of the Thai Ministry of Public Health found an association between a large meal of glutinous ("sticky") rice or carbohydrates ingested on the night of death in patients with SUNDS.[89] Consistent with this observation, a recent study by Nogami et al found that glucose and insulin could unmask the Brugada ECG.[80]

Dumaine et al first demonstrated that premature inactivation of the sodium channel in *SCN5A* mutations associated with Brugada syndrome is a function of temperature[90] and suggested that a febrile state may unmask Brugada syndrome. Indeed, several case reports have emerged recently demonstrating that febrile illness could unmask Brugada syndrome and precipitate VF.[20,91–95] Anecdotal data point to hot baths as a possible precipitating factor. Of note, northeastern Thailand, where Brugada syndrome is most prevalent, is known for its hot climate.

Risk Stratification and Current Recommendations

Risk stratification aimed at the identification of patients at risk for sudden death is an important goal of research teams worldwide.[71,96–98] Brugada et al[96] found that patients initially presenting with aborted sudden death are at the highest risk for a recurrence (69% at 54±54 months of follow-up), whereas patients presenting with syncope and a spontaneously appearing type 1 ECG have a recurrence rate of 19% at 26±36 months of follow-up. An 8% occurrence of cardiac events was observed in initially asymptomatic patients. This adverse prognosis was not observed in a population of similar size by Priori et al,[70] although the diagnostic criteria applied in the 2 studies may have been different in that the report by Priori et al does not specify a requirement for a coved-type ECG (type 1) in ≥1 precordial leads as a means to diagnose Brugada syndrome. Among asymptomatic patients, those at highest risk displayed the type 1 ECG spontaneously; patients in whom ST-segment elevation appeared only after provocation with sodium channel blockers appeared to be at minimal or no risk for arrhythmic events. Taken together, the data indicate that asymptomatic Brugada patients at highest risk are men with inducible VT/VF and a spontaneously elevated ST segment (type 1 ECG).[96]

Recent studies have suggested that combined ECG markers may be helpful in risk stratification. Atarashi et al used the width of the S wave and the ST-segment elevation magnitude, whereas Morita et al combined ST-segment elevation and the presence of late potentials.[99,100] The value of these combined markers remains to be tested in a prospective study.

Brugada et al[96] suggested that among asymptomatic patients, the inducibility of VT/VF during EPS may forecast risk. Studies by Priori et al,[70] Kanda et al,[97] and Eckardt et al,[98] however, failed to find an association between inducibility and recurrence of VT/VF among both asymptomatic and symptomatic patients with Brugada syndrome. These discrepancies may result from differences in patient characteristics and the use of nonstandardized or noncomparable stimulation protocols.[13] The adverse prognosis and higher predictive value of inducibility by Brugada et al may, at least in part, be due to more demanding criteria for diagnosing patients with Brugada syndrome.

It is noteworthy that programmed electrical stimulation–induced VF is observed in 6% to 9% of apparently healthy individuals and may represent a false-positive and nonspecific response, particularly when aggressive stimulation protocols are used.[101]

A protocol involving up to 3 extrastimuli applied to the right ventricular apex at cycle lengths ≥200 ms is recommended. If not inducible from the right ventricular apex, then stimulation may be applied to the RVOT. The predictive value of EPS is based largely on right ventricular apex stimulation; the value of RVOT pacing for risk stratification is not known. Although inducibility in experimental models is most readily achieved with epicardial stimulation,[88,102] clinical data involving this approach are limited.[103] Clearly, additional studies are needed to define further the risk stratification strategy for asymptomatic patients.

A recent study by Brugada et al[104] reported on 547 individuals diagnosed with Brugada syndrome who had had no previous cardiac arrest. In 124 patients, the abnormal ECG was identified after ≥1 episode of syncope, and in 423 individuals, the abnormal ECG was identified during routine ECG screening or during study because they were family members of patients with the syndrome. Structural disease was ruled out in all patients. This study, which evaluated the clinical outcome of the largest population of patients with Brugada syndrome thus far reported, reached the following conclusions:

1. Patients have a relatively high risk for sudden arrhythmic death, even in the absence of a history of cardiac arrest: 8.2% experienced sudden death or at least one documented episode of VF during a mean follow-up of 24±33 months. Individuals with a spontaneously abnormal type 1 ECG carried a 7.7-fold higher risk of developing an arrhythmic event during a lifetime as compared with individuals in whom the ECG diagnostic of Brugada syndrome was evident only after sodium channel blocker challenge.
2. Male gender is another risk factor for sudden death. Men had a 5.5-fold higher risk of sudden death than did women.
3. Programmed electrical stimulation that induces a sustained ventricular arrhythmia is the strongest marker of risk, associated with an 8-fold higher risk of (aborted) sudden death than in noninducible patients.
4. Familial forms of the disease are not associated with a worse prognosis than are sporadic cases because a positive

TABLE 3. Device and Pharmacological Considerations for Therapy in Brugada Syndrome

Devices	
√	ICD—only established effective therapy
?	Ablation or cryosurgery
?	Pacemaker
Drugs	
×	Amiodarone: does not protect[108]
↗	β-Blockers: do not protect[105]
√	β-Adrenergic agonists (isoproterenol[19,44])
√	Phosphodiesterase inhibitors (cilostazol[116])
↘	Class IC antiarrhythmics (flecainide, propafenone): contraindicated
	Class IA antiarrhythmics
×	Procainamide: contraindicated
?	Disopyramide[129]
√	Quinidine[88,110,111,130]
?	Tedisamil
√	I_{to} blockers: cardioselective and ion channel–specific

family history of Brugada syndrome did not predict outcome.

Therapeutic Recommendations for Brugada Syndrome

The important strides in the identification and characterization of Brugada syndrome during the past decade notwithstanding, progress relative to therapy has been less impressive. The various device and pharmacological therapies tested clinically or suggested on the basis of experimental evidence are listed in Table 3. Currently, an ICD is the only proven effective treatment for the disease.[105,106] Of 690 patients with Brugada syndrome included in a multicenter registry, 258 received an ICD because of a suspected high risk of sudden arrhythmic death. The stored electrograms were reviewed to assess the efficacy of the device by analyzing the number of patients that had an appropriate defibrillation of at least one episode of VF. The patients' mean age at implantation was 42±13.5 years, and 210 (81.3%) of these were men. A total of 160 (62%) patients were symptomatic before establishing the diagnosis; 120 patients (48.4%) had a family history of sudden death, a familial Brugada ECG pattern, or both. A sustained ventricular arrhythmia was induced during the EPS in 198 patients (76.7%). During a mean follow-up of 2.5 years (median 2), 1 patient died during an electrical storm, but 69 (26.7%) patients had at least one appropriate defibrillation. The cumulative efficacy of the device was 18%, 24%, 32%, 36%, and 38% at 1, 2, 3, 4, and 5 years of follow-up, respectively (Figure 7).

Recommendations for ICD implantation are summarized in Figure 8. Symptomatic patients displaying the type 1 Brugada ECG (either spontaneously or after sodium channel blockade) who present with aborted sudden death should receive an ICD without additional need for EPS. Similar patients presenting with related symptoms such as syncope, seizure, or nocturnal agonal respiration also should undergo ICD implantation

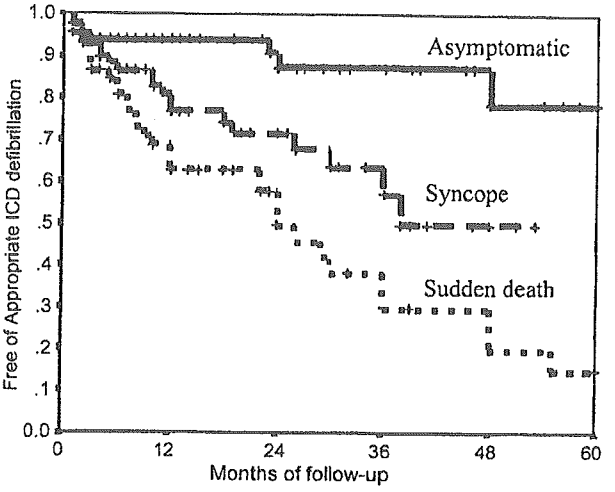

Figure 7. Kaplan-Meier curve of effectiveness of the ICD in 258 patients with ECG pattern of Brugada syndrome according to symptoms. Data are from the multicenter registry of 690 patients with Brugada syndrome.

after noncardiac causes of these symptoms have been carefully ruled out. EPS is recommended in symptomatic patients only for the assessment of supraventricular arrhythmias. Asymptomatic patients displaying a type 1 Brugada ECG (either spontaneously or after sodium channel blockade) should undergo EPS if a family history of sudden cardiac death is suspected to be the result of Brugada syndrome. EPS is justified when the family history is negative for sudden cardiac death if the type 1 ECG occurs spontaneously. If inducible for ventricular arrhythmia, then the patient should receive an ICD. Asymptomatic patients who have no family history and who develop a type 1 ECG only after sodium channel blockade should be closely followed up.

As additional data become available, these recommendations will require further refinement. Until more specific data are available, our recommendation with regard to patients who manifest a spontaneous type 1 ECG only after placement of the right precordial leads in superior positions is to treat them no differently from patients exhibiting a spontaneous type 1 ECG with the leads in the standard positions.

ICD implantation may not be an adequate solution for infants and young children or for patients who reside in regions of the world where an ICD is cost prohibitive. Although, in general, arrhythmias and sudden cardiac death occur during sleep or at rest and have been associated with slow heart rates, a potential therapeutic role for cardiac pacing remains largely unexplored. Data relative to a cryosurgical approach or the use of ablation therapy are limited. A recent report by Haissaguerre and coworkers[107] points to focal radiofrequency ablation as a potentially valuable tool in controlling arrhythmogenesis by focal ablation of the ventricular premature beats that trigger VT/VF in Brugada syndrome.

The pharmacological approach to therapy, based on experimental data, has been tailored to a rebalancing of currents that are active during the early phases of the epicardial action potential in the right ventricle to reduce the magnitude of the

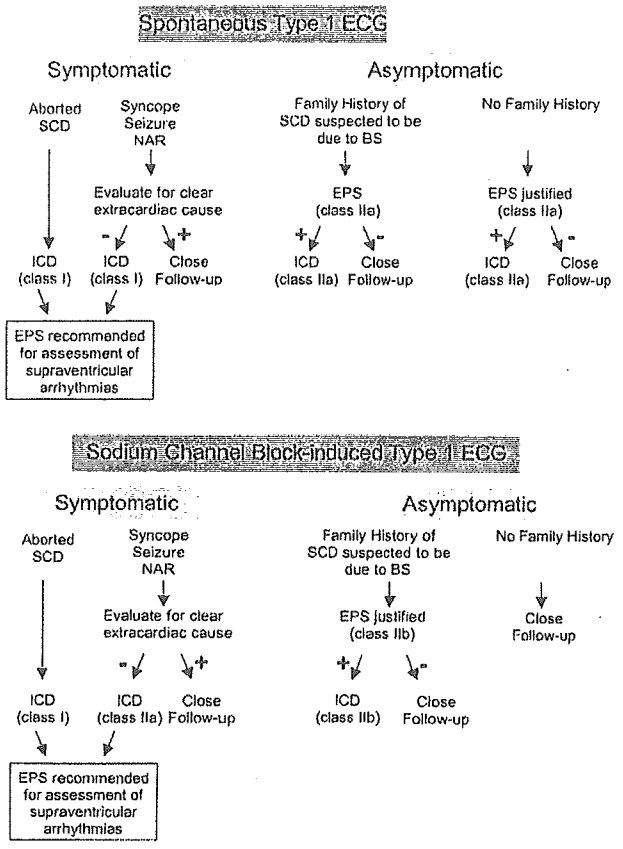

Figure 8. Indications for ICD implantation in patients with Brugada syndrome. Class I designation indicates clear evidence that the procedure or treatment is useful or effective; Class II, conflicting evidence about usefulness or efficacy; Class IIa, weight of evidence is in favor of usefulness or efficacy; and Class IIb, usefulness or efficacy is less well established. BS indicates Brugada syndrome; NAR, nocturnal agonal respiration; and SCD, sudden cardiac death.

action potential notch, restore the action potential dome, or both (Table 3). Antiarrhythmic agents such as amiodarone and β-blockers have been shown to be ineffective.[108] Class IC antiarrhythmic drugs (eg, flecainide and propafenone) and class IA agents (eg, procainamide) are contraindicated for reasons enumerated previously. Specific class IA agents such as quinidine and tedisamil, however, may exert a therapeutic action because of their I_{to}-blocking properties. Because the presence of a prominent transient outward current, I_{to}, in the right ventricle is at the heart of the mechanism underlying Brugada syndrome, any agent that inhibits this current may be protective. Cardioselective and I_{to}-specific blockers are not available. The only agent on the US market with significant I_{to}-blocking properties is quinidine. It is for this reason that it was suggested that this agent may be of therapeutic value in Brugada syndrome.[109] Studies have shown quinidine to be effective in restoring the epicardial action potential dome, thus normalizing the ST segment and preventing phase 2 reentry and polymorphic VT in experimental models of Brugada syndrome.[87] Clinical evidence of the effectiveness of quinidine in normalizing ST-segment elevation in patients with Brugada syndrome has been reported (see Figure 1),[110,111] although clinical trials designed to assess the efficacy of this agent are limited.[112] Relatively high doses of quinidine are recommended (1200 to 1500 mg/d). Agents that boost the L-type calcium current, such as isoproterenol, may be useful as well.[69,87] Both types of agents (I_{to} blocker and agents that augment I_{Ca}) have been shown to be effective in normalizing ST-segment elevation in patients with Brugada syndrome and in controlling "electrical storms," particularly in children.[44,110,111,113,114] Other than the studies by Belhassen and coworkers involving quinidine, none have as yet demonstrated long-term efficacy in the prevention of sudden cardiac death.[110,115] The most recent addition to the pharmacological armamentarium is a phosphodiesterase III inhibitor, cilostazol,[116] which normalizes the ST segment most likely by augmenting the calcium current (I_{Ca}), as well as by reducing I_{to} secondary to an increase in heart rate. Finally, an experimental antiarrhythmic agent, tedisamil, with potent action to block I_{to} among other outward currents has been suggested as a therapeutic candidate.[69] Tedisamil may be more potent than quinidine because it lacks the relatively strong inward current–blocking actions of quinidine. The development of a cardioselective and I_{to}-specific blocker would be a most welcome addition to the limited therapeutic armamentarium available to combat this disease. Appropriate clinical trials are needed to establish the effectiveness of all of the above pharmacological agents as well as the possible role of pacemakers in some forms of the disease.[117–130]

Disclosures

This study was supported by unrestricted educational grants from Medtronic Inc, Guidant Inc, St. Jude Medical, and the Ramon Brugada Senior Foundation, and by grants HL47678 and HL61669 from the National Heart, Lung, and Blood Institute (C.A., R.B.), the American Heart Association (R.B., C.A.), the DFG (Schu1082/3-1; E.S.-B.), and the New York State and Florida Grand Lodges F. & A. M.

References

1. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. *J Am Coll Cardiol.* 1992; 20:1391–1396.
2. Antzelevitch C, Brugada P, Brugada J, Brugada R, Shimizu W, Gussak I, Perez Riera AR. Brugada syndrome: a decade of progress. *Circ Res.* 2002;91:1114–1118.
3. Wilde AA, Antzelevitch C, Borggrefe M, Brugada J, Brugada R, Brugada P, Corrado D, Hauer RN, Kass RS, Nademanee K, Priori SG, Towbin JA; Study Group on the Molecular Basis of Arrhythmias of the European Society of Cardiology. Proposed diagnostic criteria for the Brugada syndrome. *Eur Heart J.* 2002;23:1648–1654.
4. Wilde AA, Antzelevitch C, Borggrefe M, Brugada J, Brugada R, Brugada P, Corrado D, Hauer RN, Kass RS, Nademanee K, Priori SG, Towbin JA; Study Group on the Molecular Basis of Arrhythmias of the European Society of Cardiology. Proposed diagnostic criteria for the Brugada syndrome: consensus report. *Circulation.* 2002;106: 2514–2519.
5. Nademanee K, Veerakul G, Nimmannit S, Chaowakul V, Bhuripanyo K, Likittanasombat K, Tunsanga K, Kuasirikul S, Malasit P, Tansupasawadikul S, Tatsanavivat P. Arrhythmogenic marker for the sudden unexplained death syndrome in Thai men. *Circulation.* 1997;96: 2595–2600.
6. Brugada P, Brugada R, Antzelevitch C, Nademanee K, Towbin J, Brugada J. The Brugada syndrome. In: Gussak I, Antzelevitch C, eds. *Cardiac Repolarization. Bridging Basic and Clinical Science.* Totowa, NJ: Humana Press; 2003:427–446.

7. Miyasaka Y, Tsuji H, Yamada K, Tokunaga S, Saito D, Imuro Y, Matsumoto N, Iwasaka T. Prevalence and mortality of the Brugada-type electrocardiogram in one city in Japan. *J Am Coll Cardiol*. 2001;38:771–774.
8. Greer RW, Glancy DL. Prevalence of the Brugada electrocardiographic pattern at the Medical Center of Louisiana in New Orleans. *J La State Med Soc*. 2003;155:242–246.
9. Hermida JS, Lemoine JL, Aoun FB, Jarry G, Rey JL, Quiret JC. Prevalence of the Brugada syndrome in an apparently healthy population. *Am J Cardiol*. 2000;86:91–94.
10. Sudden, unexpected, nocturnal deaths among Southeast Asian refugees. *MMWR Morb Mortal Wkly Rep*. 1981;30:581–584, 589.
11. Vatta M, Dumaine R, Varghese G, Richard TA, Shimizu W, Aihara N, Nademanee K, Brugada R, Brugada J, Veerakul G, Li H, Bowles NE, Brugada P, Antzelevitch C, Towbin JA. Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada syndrome. *Hum Mol Genet*. 2002;11:337–345.
12. Morita H, Kusano-Fukushima K, Nagase S, Fujimoto Y, Hisamatsu K, Fujio H, Haraoka K, Kobayashi M, Morita ST, Nakamura K, Emori T, Matsubara H, Hina K, Kita T, Fukatani M, Ohe T. Atrial fibrillation and atrial vulnerability in patients with Brugada syndrome. *J Am Coll Cardiol*. 2002;40:1437–1444.
13. Eckardt L, Kirchhof P, Johna R, Haverkamp W, Breithardt G, Borggrefe M. Wolff-Parkinson-White syndrome associated with Brugada syndrome. *Pacing Clin Electrophysiol*. 2001;24:1423–1424.
14. Morita H, Fukushima-Kusano K, Nagase S, Miyaji K, Hiramatsu S, Banba K, Nishii N, Watanabe A, Kakishita M, Takenaka-Morita S, Nakamura K, Saito H, Emori T, Ohe T. Sinus node function in patients with Brugada-type ECG. *Circ J*. 2004;68:473–476.
15. Takehara N, Makita N, Kawabe J, Sato N, Kawamura Y, Kitabatake A, Kikuchi K. A cardiac sodium channel mutation identified in Brugada syndrome associated with atrial standstill. *J Intern Med*. 2004;255:137–142.
16. Bordachar P, Reuter S, Garrigue S, Cai X, Hocini M, Jais P, Haissaguerre M, Clementy J. Incidence, clinical implications and prognosis of atrial arrhythmias in Brugada syndrome. *Eur Heart J*. 2004;25:879–884.
17. Brugada P, Brugada J, Brugada R. Arrhythmia induction by antiarrhythmic drugs. *Pacing Clin Electrophysiol*. 2000;23:291–292.
18. Brugada R, Brugada J, Antzelevitch C, Kirsch GE, Potenza D, Towbin JA, Brugada P. Sodium channel blockers identify risk for sudden death in patients with ST-segment elevation and right bundle branch block but structurally normal hearts. *Circulation*. 2000;101:510–515.
19. Miyazaki T, Mitamura H, Miyoshi S, Soejima K, Aizawa Y, Ogawa S. Autonomic and antiarrhythmic drug modulation of ST segment elevation in patients with Brugada syndrome. *J Am Coll Cardiol*. 1996;27:1061–1070.
20. Antzelevitch C, Brugada R. Fever and Brugada syndrome. *Pacing Clin Electrophysiol*. 2002;25:1537–1539.
21. Wang K, Asinger RW, Marriott HJ. ST-segment elevation in conditions other than acute myocardial infarction. *N Engl J Med*. 2003;349:2128–2135.
22. Myers GB. Other QRS-T patterns that may be mistaken for myocardial infarction: IV. Alterations in blood potassium; myocardial ischemia; subepicardial myocarditis; distortion associated with arrhythmias. *Circulation*. 1950;2:75–93.
23. Abbott JA, Cheitlin MD. The nonspecific camel-hump sign. *JAMA*. 1976;235:413–414.
24. Hersch C. Electrocardiographic changes in head injuries. *Circulation*. 1961;23:853–860.
25. Perloff JK, Henze E, Schelbert HR. Alterations in regional myocardial metabolism, perfusion, and wall motion in Duchenne muscular dystrophy studied by radionuclide imaging. *Circulation*. 1984;69:33–42.
26. Read DH, Harrington DD. Experimentally induced thiamine deficiency in beagle dogs: clinical observations. *Am J Vet Res*. 1981;42:984–991.
27. Merrill JP, Levine HD, Somerville W, Smith S. Clinical recognition and treatment of acute potassium intoxication. *Ann Intern Med*. 1950;33:797–830.
28. Ortega-Carnicer J, Benezet J, Ruiz-Lorenzo F, Alcazar R. Transient Brugada-type electrocardiographic abnormalities in renal failure reversed by dialysis. *Resuscitation*. 2002;55:215–219.
29. Douglas PS, Carmichael KA, Palevsky PM. Extreme hypercalcemia and electrocardiographic changes. *Am J Cardiol*. 1984;54:674–675.
30. Sridharan MR, Horan LG. Electrocardiographic J wave of hypercalcemia. *Am J Cardiol*. 1984;54:672–673.
31. Corrado D, Nava A, Buja G, Martini B, Fasoli G, Oselladore L, Turrini P, Thiene G. Familial cardiomyopathy underlies syndrome of right bundle branch block, ST segment elevation and sudden death. *J Am Coll Cardiol*. 1996;27:443–448.
32. Corrado D, Basso C, Buja G, Nava A, Rossi L, Thiene G. Right bundle branch block, right precordial ST-segment elevation, and sudden death in young people. *Circulation*. 2001;103:710–717.
33. Kataoka H. Electrocardiographic patterns of the Brugada syndrome in right ventricular infarction/ischemia. *Am J Cardiol*. 2000;86:1056.
34. Osborn JJ. Experimental hypothermia: respiratory and blood pH changes in relation to cardiac function. *Am J Physiol*. 1953;175:389–398.
35. Noda T, Shimizu W, Tanaka K, Chayama K. Prominent J wave and ST segment elevation: serial electrocardiographic changes in accidental hypothermia. *J Cardiovasc Electrophysiol*. 2003;14:223.
36. Tarin N, Farre J, Rubio JM, Tunon J, Castro-Dorticos J. Brugada-like electrocardiographic pattern in a patient with a mediastinal tumor. *Pacing Clin Electrophysiol*. 1999;22:1264–1266.
37. Tomcsanyi J, Simor T, Papp L. Images in cardiology. Haemopericardium and Brugada-like ECG pattern in rheumatoid arthritis. *Heart*. 2002;87:234.
38. Kok LC, Mitchell MA, Haines DE, Mounsey JP, DiMarco JP. Transient ST elevation after transthoracic cardioversion in patients with hemodynamically unstable ventricular tachyarrhythmia. *Am J Cardiol*. 2000;85:878–881, A9.
39. Gurevitz O, Glikson M. Cardiac resynchronization therapy: a new frontier in the management of heart failure. *Isr Med Assoc J*. 2003;5:571–575.
40. Gurevitz O, Lipchenca I, Yaacoby E, Segal E, Perel A, Eldar M, Glikson M. ST-segment deviation following implantable cardioverter defibrillator shocks: incidence, timing, and clinical significance. *Pacing Clin Electrophysiol*. 2002;25:1429–1432.
41. Kalla H, Yan GX, Marinchak R. Ventricular fibrillation in a patient with prominent J (Osborn) waves and ST segment elevation in the inferior electrocardiographic leads: a Brugada syndrome variant? *J Cardiovasc Electrophysiol*. 2000;11:95–98.
42. Horigome H, Shigeta O, Kuga K, Isobe T, Sakakibara Y, Yamaguchi I, Matsui A. Ventricular fibrillation during anesthesia in association with J waves in the left precordial leads in a child with coarctation of the aorta. *J Electrocardiol*. 2003;36:339–343.
43. Potet F, Mabo P, Le Coq G, Probst V, Schott JJ, Airaud F, Guihard G, Daubert JC, Escande D, Le Marec H. Novel Brugada SCN5A mutation leading to ST segment elevation in the inferior or the right precordial leads. *J Cardiovasc Electrophysiol*. 2003;14:200–203.
44. Shimizu W, Matsuo K, Takagi M, Tanabe Y, Aiba T, Taguchi A, Suyama K, Kurita T, Aihara N, Kamakura S. Body surface distribution and response to drugs of ST segment elevation in Brugada syndrome: clinical implication of eighty-seven-lead body surface potential mapping and its application to twelve-lead electrocardiograms. *J Cardiovasc Electrophysiol*. 2000;11:396–404.
45. Sangwatanaroj S, Prechawat S, Sunsaneewitayakul B, Sitthisook S, Tosukhowong P, Tungsanga K. New electrocardiographic leads and the procainamide test for the detection of the Brugada sign in sudden unexplained death syndrome survivors and their relatives. *Eur Heart J*. 2001;22:2290–2296.
46. Alings M, Wilde A. "Brugada" syndrome: clinical data and suggested pathophysiological mechanism. *Circulation*. 1999;99:666–673.
47. Bezzina C, Veldkamp MW, van Den Berg MP, Postma AV, Rook MB, Viersma JW, Van Langen IM, Tan-Sindhunata G, Bink-Boelkens MT, van Der Hout AH, Mannens MM, Wilde AA. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. *Circ Res*. 1999;85:1206–1213.
48. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Brignole M, Giordano U, Giovannini T, Menozzi C, Bloise R, Crotti L, Terreni L, Schwartz PJ. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: a prospective evaluation of 52 families. *Circulation*. 2000;102:2509–2515.
49. Pitzalis MV, Anaclerio M, Iacoviello M, Forleo C, Guida P, Troccoli R, Massari F, Mastropasqua F, Sorrentino S, Manghisi A, Rizzon P. QT-interval prolongation in right precordial leads: an additional electrocardiographic hallmark of Brugada syndrome. *J Am Coll Cardiol*. 2003;42:1632–1637.
50. Smits JP, Eckardt L, Probst V, Bezzina CR, Schott JJ, Remme CA, Haverkamp W, Breithardt G, Escande D, Schulze-Bahr E, LeMarec H, Wilde AA. Genotype-phenotype relationship in Brugada syndrome:

electrocardiographic features differentiate SCN5A-related patients from non-SCN5A-related patients. *J Am Coll Cardiol.* 2002;40:350–356.
51. Shimizu W, Antzelevitch C, Suyama K, Kurita T, Taguchi A, Aihara N, Takaki H, Sunagawa K, Kamakura S. Effect of sodium channel blockers on ST segment, QRS duration, and corrected QT interval in patients with Brugada syndrome. *J Cardiovasc Electrophysiol.* 2000;11:1320–1329.
52. Ayerza MR, de Zutter M, Goethals M, Wellens F, Geelen P, Brugada P. Heart transplantation as last resort against Brugada syndrome. *J Cardiovasc Electrophysiol.* 2002;13:943–944.
53. Remme CA, Wever EF, Wilde AA, Derksen R, Hauer RN. Diagnosis and long-term follow-up of the Brugada syndrome in patients with idiopathic ventricular fibrillation. *Eur Heart J.* 2001;22:400–409.
54. Bezzina CR, Rook MB, Groenewegen WA, Herfst LJ, van der Wal AC, Lam J, Jongsma HJ, Wilde AA, Mannens MM. Compound heterozygosity for mutations (W156X and R225W) in SCN5A associated with severe cardiac conduction disturbances and degenerative changes in the conduction system. *Circ Res.* 2003;92:159–168.
55. Ahmad F. The molecular genetics of arrhythmogenic right ventricular dysplasia-cardiomyopathy. *Clin Invest Med.* 2003;26:167–178.
56. Antzelevitch C. Molecular genetics of arrhythmias and cardiovascular conditions associated with arrhythmias. *J Cardiovasc Electrophysiol.* 2003;14:1259–1272.
57. Weiss R, Barmada MM, Nguyen T, Seibel JS, Cavlovich D, Kornblit CA, Angelilli A, Villanueva F, McNamara DM, London B. Clinical and molecular heterogeneity in the Brugada syndrome: a novel gene locus on chromosome 3. *Circulation.* 2002;105:707–713.
58. Ahmad F, Li D, Karibe A, Gonzalez O, Tapscott T, Hill R, Weilbaecher D, Blackie P, Furey M, Gardner MJ, Bachinski LL, Roberts R. Localization of a gene responsible for arrhythmogenic right ventricular dysplasia to chromosome 3p23. *Circulation.* 1998;98:2791–2795.
59. Corrado D, Basso C, Rizzoli G, Schiavon M, Thiene G. Does sports activity enhance the risk of sudden death in adolescents and young adults? *J Am Coll Cardiol.* 2003;42:1959–1963.
60. Matsuo K, Kurita T, Inagaki M, Kakishita M, Aihara N, Shimizu W, Taguchi A, Suyama K, Kamakura S, Shimomura K. The circadian pattern of the development of ventricular fibrillation in patients with Brugada syndrome. *Eur Heart J.* 1999;20:465–470.
61. Takagi M, Aihara N, Kuribayashi S, Taguchi A, Shimizu W, Kurita T, Suyama K, Kamakura S, Hamada S, Takamiya M. Localized right ventricular morphological abnormalities detected by electron-beam computed tomography represent arrhythmogenic substrates in patients with the Brugada syndrome. *Eur Heart J.* 2001;22:1032–1041.
62. Antzelevitch C. Brugada syndrome: historical perspectives and observations. *Eur Heart J.* 2002;23:676–678.
63. Antzelevitch C. Late potentials and the Brugada syndrome. *J Am Coll Cardiol.* 2002;39:1996–1999.
64. Futterman LG, Lemberg L. Brugada. *Am J Crit Care.* 2001;10:360–364.
65. Fujiki A, Usui M, Nagasawa H, Mizumaki K, Hayashi H, Inoue H. ST segment elevation in the right precordial leads induced with class IC antiarrhythmic drugs: insight into the mechanism of Brugada syndrome. *J Cardiovasc Electrophysiol.* 1999;10:214–218.
66. Nagase S, Kusano KF, Morita H, Fujimoto Y, Kakishita M, Nakamura K, Emori T, Matsubara H, Ohe T. Epicardial electrogram at the right ventricular outflow tract in patients with the Brugada syndrome: using the epicardial lead. *J Am Coll Cardiol.* 2002;39:1992–1995.
67. Tukkie R, Sogaard P, Vleugels J, de Groot IK, Wilde AA, Tan HL. Delay in right ventricular activation contributes to Brugada syndrome. *Circulation.* 2004;109:1272–1277.
68. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, Potenza D, Moya A, Borggrefe M, Breithardt G, Ortiz-Lopez R, Wang Z, Antzelevitch C, O'Brien RE, Schultze-Bahr E, Keating MT, Towbin JA, Wang Q. Genetic basis and molecular mechanisms for idiopathic ventricular fibrillation. *Nature.* 1998;392:293–296.
69. Antzelevitch C. The Brugada syndrome: ionic basis and arrhythmia mechanisms. *J Cardiovasc Electrophysiol.* 2001;12:268–272.
70. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Giordano U, Bloise R, Giustetto C, De Nardis R, Grillo M, Ronchetti E, Faggiano G, Nastoli J. Natural history of Brugada syndrome: insights for risk stratification and management. *Circulation.* 2002;105:1342–1347.
71. Balser JR. The cardiac sodium channel: gating function and molecular pharmacology. *J Mol Cell Cardiol.* 2001;33:599–613.
72. Tan HL, Bezzina CR, Smits JP, Verkerk AO, Wilde AA. Genetic control of sodium channel function. *Cardiovasc Res.* 2003;57:961–973.
73. Fondazione Salvatore Maugeri, Istituto di Ricovero e Cura a Carattere Scientifico. Brugada syndrome (BrS) mutations. Gene: SCN5A. Available at: http://pc4.fsm.it:81/cardmoc/SCN5A_bruada_mut.htm. Accessed November 30, 2004.
74. Schulze-Bahr E, Eckardt L, Breithardt G, Seidl K, Wichter T, Wolpert C, Borggrefe M, Haverkamp W. Sodium channel gene (SCN5A) mutations in 44 index patients with Brugada syndrome: different incidences in familial and sporadic disease. *Hum Mutat.* 2003;21:651–652.
75. Babaliaros VC, Hurst JW, Tricyclic antidepressants and the Brugada syndrome: an example of Brugada waves appearing after the administration of desipramine. *Clin Cardiol.* 2002;25:395–398.
76. Goldgran-Toledano D, Sideris G, Kevorkian JP. Overdose of cyclic antidepressants and the Brugada syndrome. *N Engl J Med.* 2002;346:1591–1592.
77. Tada H, Sticherling C, Oral H, Morady F. Brugada syndrome mimicked by tricyclic antidepressant overdose. *J Cardiovasc Electrophysiol.* 2001;12:275.
78. Pastor A, Nunez A, Cantale C, Cosio FG. Asymptomatic Brugada syndrome case unmasked during dimenhydrinate infusion. *J Cardiovasc Electrophysiol.* 2001;12:1192–1194.
79. Ortega-Carnicer J, Bertos-Polo J, Gutierrez-Tirado C. Aborted sudden death, transient Brugada pattern, and wide QRS dysrrhythmias after massive cocaine ingestion. *J Electrocardiol.* 2001;34:345–349.
80. Nogami A, Nakao M, Kubota S, Sugiyasu A, Doi H, Yokoyama K, Yumoto K, Tamaki T, Kato K, Hosokawa N, Sagai H, Nakamura H, Nitta J, Yamauchi Y, Aonuma K. Enhancement of J-ST-segment elevation by the glucose and insulin test in Brugada syndrome. *Pacing Clin Electrophysiol.* 2003;26:332–337.
81. Araki T, Konno T, Itoh H, Ino H, Shimizu M. Brugada syndrome with ventricular tachycardia and fibrillation related to hypokalemia. *Circ J.* 2003;67:93–95.
82. Noda T, Shimizu W, Taguchi A, Satomi K, Suyama K, Kurita T, Aihara N, Kamakura S. ST-segment elevation and ventricular fibrillation without coronary spasm by intracoronary injection of acetylcholine and/or ergonovine maleate in patients with Brugada syndrome. *J Am Coll Cardiol.* 2002;40:1841–1847.
83. Kasanuki H, Ohnishi S, Ohtuka M, Matsuda N, Nirei T, Isogai R, Shoda M, Toyoshima Y, Hosoda S. Idiopathic ventricular fibrillation induced with vagal activity in patients without obvious heart disease. *Circulation.* 1997;95:2277–2285.
84. Proclemer A, Facchin D, Feruglio GA, Nucifora R. Recurrent ventricular fibrillation, right bundle-branch block and persistent ST segment elevation in V1-V3: a new arrhythmia syndrome? A clinical case report [in Italian]. *G Ital Cardiol.* 1993;23:1211–1218.
85. Mizumaki K, Fujiki A, Tsuneda T, Sakabe M, Nishida K, Sugao M, Inoue H. Vagal activity modulates spontaneous augmentation of ST elevation in the daily life of patients with Brugada syndrome. *J Cardiovasc Electrophysiol.* 2004;15:667–673.
86. Wichter T, Matheja P, Eckardt L, Kies P, Schafers K, Schulze-Bahr E, Haverkamp W, Borggrefe M, Schober O, Breithardt G, Schafers M. Cardiac autonomic dysfunction in Brugada syndrome. *Circulation.* 2002;105:702–706.
87. Litovsky SH, Antzelevitch C. Differences in the electrophysiological response of canine ventricular subendocardium and subepicardium to acetylcholine and isoproterenol. A direct effect of acetylcholine in ventricular myocardium. *Circ Res.* 1990;67:615–627.
88. Yan GX, Antzelevitch C. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. *Circulation.* 1999;100:1660–1666.
89. Nimmannit S, Malasit P, Chaovakul V, Susaengrat W, Vasuvattakul S, Nilwarangkur S. Pathogenesis of sudden unexplained nocturnal death (lai tai) and endemic distal renal tubular acidosis. *Lancet.* 1991;338:930–932.
90. Dumaine R, Towbin JA, Brugada P, Vatta M, Nesterenko DV, Nesterenko VV, Brugada J, Brugada R, Antzelevitch C. Ionic mechanisms responsible for the electrocardiographic phenotype of the Brugada syndrome are temperature dependent. *Circ Res.* 1999;85:803–809.
91. Gonzalez Rebollo JM, Hernandez Madrid A, Garcia A, Garcia de Castro A, Mejias A, Moro C. Recurrent ventricular fibrillation during a febrile illness in a patient with the Brugada syndrome [in Spanish]. *Rev Esp Cardiol.* 2000;53:755–757.
92. Madle A, Kratochvil Z, Polivkova A. The Brugada syndrome [in Czech]. *Vnitr Lek.* 2002;48:255–258.
93. Saura D, Garcia-Alberola A, Carrillo P, Pascual D, Martinez-Sanchez J, Valdes M. Brugada-like electrocardiographic pattern induced by fever. *Pacing Clin Electrophysiol.* 2002;25:856–859.

94. Porres JM, Brugada J, Urbistondo V, García F, Reviejo K, Marco P. Fever unmasking the Brugada syndrome. *Pacing Clin Electrophysiol.* 2002;25:1646–1648.
95. Kum LC, Fung JW, Sanderson JE. Brugada syndrome unmasked by febrile illness. *Pacing Clin Electrophysiol.* 2002;25:1660–1661.
96. Brugada J, Brugada R, Antzelevitch C, Towbin J, Nademanee K, Brugada P. Long-term follow-up of individuals with the electrocardiographic pattern of right bundle-branch block and ST-segment elevation in precordial leads V1 to V3. *Circulation.* 2002;105:73–78.
97. Kanda M, Shimizu W, Matsuo K, Nagaya N, Taguchi A, Suyama K, Kurita T, Aihara N, Kamakura S. Electrophysiologic characteristics and implications of induced ventricular fibrillation in symptomatic patients with Brugada syndrome. *J Am Coll Cardiol.* 2002;39:1799–1805.
98. Eckardt L, Kirchhof P, Schulze-Bahr E, Rolf S, Ribbing M, Loh P, Bruns HJ, Witte A, Milberg P, Borggrefe M, Breithardt G, Wichter T, Haverkamp W. Electrophysiologic investigation in Brugada syndrome: yield of programmed ventricular stimulation at two ventricular sites with up to three premature beats. *Eur Heart J.* 2002;23:1394–1401.
99. Atarashi H, Ogawa S; Idiopathic Ventricular Fibrillation Investigators. New ECG criteria for high-risk Brugada syndrome. *Circ J.* 2003; 67:8–10.
100. Morita H, Takenaka-Morita S, Fukushima-Kusano K, Kobayashi M, Nagase S, Kakishita M, Nakamura K, Emori T, Matsubara H, Ohe T. Risk stratification for asymptomatic patients with Brugada syndrome. *Circ J.* 2003;67:312–316.
101. Viskin S. Inducible ventricular fibrillation in the Brugada syndrome: diagnostic and prognostic implications. *J Cardiovasc Electrophysiol.* 2003;14:458–460.
102. Fish JM, Antzelevitch C. Role of sodium and calcium channel block in unmasking the Brugada syndrome. *Heart Rhythm.* 2004;1:210–217.
103. Carlsson J, Erdogan A, Schulte B, Neuzner J, Pitschner HF. Possible role of epicardial left ventricular programmed stimulation in Brugada syndrome. *Pacing Clin Electrophysiol.* 2001;24:247–249.
104. Brugada J, Brugada R, Brugada P. Determinants of sudden cardiac death in individuals with the electrocardiographic pattern of Brugada syndrome and no previous cardiac arrest. *Circulation.* 2003;108: 3092–3096.
105. Brugada J, Brugada R, Brugada P. Pharmacological and device approach to therapy of inherited cardiac diseases associated with cardiac arrhythmias and sudden death. *J Electrocardiol.* 2000;33:41–47.
106. Brugada P, Brugada R, Brugada J, Geelen P. Use of the prophylactic implantable cardioverter defibrillator for patients with normal hearts. *Am J Cardiol.* 1999;83:98D–100D.
107. Haissaguerre M, Extramiana F, Hocini M, Cauchemez B, Jais P, Cabrera JA, Farre G, Leenhardt A, Sanders P, Scavee C, Hsu LF, Weerasooriya R, Shah DC, Frank R, Maury P, Delay M, Garrigue S, Clementy J. Mapping and ablation of ventricular fibrillation associated with long-QT and Brugada syndromes. *Circulation.* 2003;108:925–928.
108. Brugada J, Brugada R, Brugada P. Right bundle-branch block and ST-segment elevation in leads V1 through V3: a marker for sudden death in patients without demonstrable structural heart disease. *Circulation.* 1998;97:457–460.
109. Antzelevitch C, Brugada P, Brugada J, Brugada R, Nademanee K, Towbin JA. *Clinical Approaches to Tachyarrhythmias. The Brugada Syndrome.* Armonk, NY: Futura Publishing Co, 1999.
110. Belhassen B, Viskin S, Antzelevitch C. The Brugada syndrome: is an implantable cardioverter defibrillator the only therapeutic option? *Pacing Clin Electrophysiol.* 2002;25:1634–1640.
111. Alings M, Dekker L, Sadee A, Wilde A. Quinidine induced electrocardiographic normalization in two patients with Brugada syndrome. *Pacing Clin Electrophysiol.* 2001;24:1420–1422.
112. Hermida JS, Denjoy I, Clerc J, Extramiana F, Jarry G, Milliez P, Guicheney P, Di Fusco S, Rey JL, Cauchemez B, Leenhardt A. Hydroquinidine therapy in Brugada syndrome. *J Am Coll Cardiol.* 2004;43:1853–1860.
113. Suzuki H, Torigoe K, Numata O, Yazaki S. Infant case with a malignant form of Brugada syndrome. *J Cardiovasc Electrophysiol.* 2000;11: 1277–1280.
114. Tanaka H, Kinoshita O, Uchikawa S, Kasai H, Nakamura M, Izawa A, Yokoseki O, Kitabayashi H, Takahashi W, Yazaki Y, Watanabe N, Imamura H, Kubo K. Successful prevention of recurrent ventricular fibrillation by intravenous isoproterenol in a patient with Brugada syndrome. *Pacing Clin Electrophysiol.* 2001;24:1293–1294.
115. Belhassen B, Viskin S, Fish R, Glick A, Setbon I, Eldar M. Effects of electrophysiologic-guided therapy with Class IA antiarrhythmic drugs on the long-term outcome of patients with idiopathic ventricular fibrillation with or without the Brugada syndrome. *J Cardiovasc Electrophysiol.* 1999;10:1301–1312.
116. Tsuchiya T, Ashikaga K, Honda T, Arita M. Prevention of ventricular fibrillation by cilostazol, an oral phosphodiesterase inhibitor, in a patient with Brugada syndrome. *J Cardiovasc Electrophysiol.* 2002;13: 698–701.
117. Krishnan SC, Josephson ME. ST segment elevation induced by class IC antiarrhythmic agents: underlying electrophysiologic mechanisms and insights into drug-induced proarrhythmia. *J Cardiovasc Electrophysiol.* 1998;9:1167–1172.
118. Gasparini M, Priori SG, Mantica M, Napolitano C, Galimberti P, Ceriotti C, Simonini S. Flecainide test in Brugada syndrome: a reproducible but risky tool. *Pacing Clin Electrophysiol.* 2003;26:338–341.
119. Takenaka S, Emori T, Koyama S, Morita H, Fukushima K, Ohe T. Asymptomatic form of Brugada syndrome. *Pacing Clin Electrophysiol.* 1999;22:1261–1263.
120. Shimizu W, Aiba T, Kurita T, Kamakura S. Paradoxic abbreviation of repolarization in epicardium of the right ventricular outflow tract during augmentation of Brugada-type ST segment elevation. *J Cardiovasc Electrophysiol.* 2001;12:1418–1421.
121. Matana A, Goldner V, Stanic K, Mavric Z, Zaputovic L, Matana Z. Unmasking effect of propafenone on the concealed form of the Brugada phenomenon. *Pacing Clin Electrophysiol.* 2000;23:416–418.
122. Rolf S, Bruns HJ, Wichter T, Kirchhof P, Ribbing M, Wasmer K, Paul M, Breithardt G, Haverkamp W, Eckardt L. The ajmaline challenge in Brugada syndrome: diagnostic impact, safety, and recommended protocol. *Eur Heart J.* 2003;24:1104–1112.
123. Tada H, Nogami A, Shimizu W, Naito S, Nakatsugawa M, Oshima S, Taniguchi K. ST segment and T wave alternans in a patient with Brugada syndrome. *Pacing Clin Electrophysiol.* 2000;23:413–415.
124. Matsuo K, Shimizu W, Kurita T, Inagaki M, Aihara N, Kamakura S. Dynamic changes of 12-lead electrocardiograms in a patient with Brugada syndrome. *J Cardiovasc Electrophysiol.* 1998;9:508–512.
125. Bolognesi R, Tsialtas D, Vasini P, Conti M, Manca C. Abnormal ventricular repolarization mimicking myocardial infarction after heterocyclic antidepressant overdose. *Am J Cardiol.* 1997;79:242–245.
126. Rouleau F, Asfar P, Boulet S, Dube L, Dupuis JM, Alquier P, Victor J. Transient ST segment elevation in right precordial leads induced by psychotropic drugs: relationship to the Brugada syndrome. *J Cardiovasc Electrophysiol.* 2001;12:61–65.
127. Antzelevitch C. The Brugada syndrome. *J Cardiovasc Electrophysiol.* 1998;9:513–516.
128. Littmann L, Monroe MH, Svenson RH. Brugada-type electrocardiographic pattern induced by cocaine. *Mayo Clin Proc.* 2000;75:845–849.
129. Chinushi M, Aizawa Y, Ogawa Y, Shiba M, Takahashi K. Discrepant drug action of disopyramide on ECG abnormalities and induction of ventricular arrhythmias in a patient with Brugada syndrome. *J Electrocardiol.* 1997;30:133–136.
130. Belhassen B, Viskin S, Fish R, Glick A, Setbon I, Eldar M. Effects of electrophysiologic-guided therapy with Class IA antiarrhythmic drugs on the long-term outcome of patients with idiopathic ventricular fibrillation with or without the Brugada syndrome. *J Cardiovasc Electrophysiol.* 1999;10:1301–1312.

Journal of the American College of Cardiology
© 2005 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 45, No. 2, 2005
ISSN 0735-1097/05/$30.00
doi:10.1016/j.jacc.2004.09.069

Cellular and Ionic Mechanism for Drug-Induced Long QT Syndrome and Effectiveness of Verapamil

Takeshi Aiba, MD, PHD,* Wataru Shimizu, MD, PHD,† Masashi Inagaki, MD,* Takashi Noda, MD, PHD,* Shunichiro Miyoshi, MD, PHD,‡ Wei-Guang Ding, MD, PHD,§ Dimitar P. Zankov, MD,§‖ Futoshi Toyoda, PHD,§ Hiroshi Matsuura, MD, PHD,§ Minoru Horie, MD, PHD,‖ Kenji Sunagawa, MD, PHD*

Osaka, Tokyo, and Shiga, Japan

OBJECTIVES We examined the cellular and ionic mechanism for QT prolongation and subsequent Torsade de Pointes (TdP) and the effect of verapamil under conditions mimicking *KCNQ1* (I_{Ks} gene) defect linked to acquired long QT syndrome (LQTS).

BACKGROUND Agents with an I_{Kr}-blocking effect often induce marked QT prolongation in patients with acquired LQTS. Previous reports demonstrated a relationship between subclinical mutations in cardiac K^+ channel genes and a risk of drug-induced TdP.

METHODS Transmembrane action potentials from epicardial (EPI), midmyocardial (M), and endocardial (ENDO) cells were simultaneously recorded, together with a transmural electrocardiogram, at a basic cycle length of 2,000 ms in arterially perfused feline left ventricular preparations.

RESULTS The I_{Kr} block (E-4031: 1 μmol/l) under control conditions (n = 5) prolonged the QT interval but neither increased transmural dispersion of repolarization (TDR) nor induced arrhythmias. However, the I_{Kr} blocker under conditions with I_{Ks} suppression by chromanol 293B 10 μmol/l mimicking the *KCNQ1* defect (n = 10) preferentially prolonged action potential duration (APD) in EPI rather than M or ENDO, thereby dramatically increasing the QT interval and TDR. Spontaneous or epinephrine-induced early afterdepolarizations (EADs) were observed in EPI, and subsequent TdP occurred only under both I_{Ks} and I_{Kr} suppression. Verapamil (0.1 to 5.0 μmol/l) dose-dependently abbreviated APD in EPI more than in M and ENDO, thereby significantly decreasing the QT interval, TDR, and suppressing EADs and TdP.

CONCLUSIONS Subclinical I_{Ks} dysfunction could be a risk of drug-induced TdP. Verapamil is effective in decreasing the QT interval and TDR and in suppressing EADs, thus preventing TdP in the model of acquired LQTS. (J Am Coll Cardiol 2005;45:300–7) © 2005 by the American College of Cardiology Foundation

The long QT syndrome (LQTS) is characterized by a prolongation of ventricular repolarization and recurrent episodes of atypical polymorphic ventricular tachycardia known as Torsade de Pointes (TdP) leading to sudden cardiac death (1–3). The molecular basis of congenital LQTS is attributed to defects in several ion channel genes encoding delayed rectifier K^+ or Na^+ currents. On the other hand, agents that block rapidly activating delayed rectifier potassium current (I_{Kr}) often induce marked QT prolongation with an inverted T wave in patients with acquired LQTS. Recent studies indicate that some cases of drug-induced LQTS can be associated with silent mutations and common polymorphism in genes responsible for the congenital LQTS (4), such as *KCNQ1* encoding slowly activating delayed rectifier potassium currents (I_{Ks}) (5–7). However, it remains unclear why subclinical I_{Ks} dysfunction is a risk of drug-induced LQTS.

Both early afterdepolarization (EAD)-induced triggered activity and increased dispersion of repolarization have been suggested as important in the genesis of ventricular arrhythmias in congenital and acquired LQTS. Moreover, verapamil, an L-type Ca^{2+} channel blocker, suppressed EADs and TdP in patients with LQTS (8,9). In the present study, we hypothesized that: 1) addition of I_{Kr} block to I_{Ks} dysfunction markedly prolongs action potential duration (APD) and induces TdP by producing EADs and/or increases transmural dispersion of repolarization (TDR); and 2) verapamil suppresses TdP by preventing EADs and decreasing TDR. In arterially perfused feline left ventricular wedge preparations, we demonstrated that subclinical I_{Ks} dysfunction, mimicking *KCNQ1* defect, could be a risk of drug-induced TdP, and verapamil successfully suppressed TdP in the model of acquired LQTS.

METHODS

Arterially perfused wedge preparations and electrophysiologic recordings. All animal care procedures were in accordance with the position of the American Heart Association research animal use (November 11, 1984). The

From the *Department of Cardiovascular Dynamics, Research Institute, and †Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, Suita, Osaka, Japan; the ‡Department of Physiology, Keio University School of Medicine, Tokyo, Japan; §Department of Physiology and ‖Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu, Shiga, Japan. This study was supported by the Program for Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research (of Japan) (to Dr. Sunagawa), a grant from the Japan Cardiovascular Research Foundation (to Dr. Aiba), Fukuda Foundation for Medical Technology (to Dr. Inagaki), Vehicle Racing Commemorative Foundation (to Dr. Shimizu), Health Sciences Research Grants from the Ministry of Health, Labour and Welfare (to Dr. Shimizu), and the Research grant for Cardiovascular Disease (15C-6) from the Ministry of Health, Labour and Welfare (to Dr. Shimizu).

Manuscript received August 24, 2004; revised manuscript received September 21, 2004, accepted September 28, 2004.

Abbreviations and Acronyms

APD_{90} = action potential duration measured at 90% repolarization
BCL = basic cycle length
EAD = early afterdepolarization
I_K = delayed rectifier potassium current
I_{Kr} = rapidly activating delayed rectifier potassium current
I_{Ks} = slowly activating delayed rectifier potassium current
LQTS = long QT syndrome
TdP = Torsade de Pointes
TDR = transmural dispersion of repolarization

methods used for isolation, perfusion, and recording of transmembrane activity from the arterially perfused feline left ventricle have been detailed in a previous study (10) and are similar to methods reported using canine or rabbit wedge preparations (11–15). Briefly, a transmural wedge was dissected from the anterior wall of the left ventricle, cannulated via the left descending coronary artery (or the first branch of the left circumflex), and placed in a small tissue bath arterially perfused with Tyrode's solution. The temperature was maintained at 37 ± 1°C and perfusion pressure maintained between 40 and 60 mm Hg. Ventricular wedges were stimulated with bipolar electrodes applied to the endocardial surface. We recorded a transmural electrocardiogram (ECG) (epicardial, positive pole) using Ag-AgCl electrodes, and transmembrane action potentials (APs) simultaneously from the epicardium, midmyocardium (M), and endomyocardium using three separate intracellular floating microelectrodes. The epicardial and endocardial APs were recorded from the epicardial and endocardial surfaces, respectively, at positions approximating the transmural axis of the ECG. The M-cell's AP was recorded from the transmural surface, mainly at the subendocardium, along the same axis.

An I_{Kr} blocker, E-4031 1 μmol/l, was used in control condition (n = 5) or under condition with I_{Ks} suppression by chromanol 293B 10 μmol/l, mimicking *KCNQ1* defect (n = 10). The effects of an L-type Ca^{2+} channel blocker, verapamil, were evaluated at 0.1, 1, 2.5, and 5 μmol/l under the I_{Ks} and I_{Kr} suppression (acquired LQTS condition). Epinephrine 0.5 μmol/l was used to mimic increased sympathetic activity in the absence and presence of verapamil under the acquired LQTS condition. The spontaneous or epinephrine-induced EADs and subsequent TdP were evaluated under each set of conditions.

Data using E-4031, 293B, 293B + E-4031, and additional verapamil on top of 293B + E-4031 were collected for a period of 30 min starting 30 min after applying the above compounds to the perfusion. The APD was measured at 90% repolarization (APD_{90}). The TDR was defined as the difference between the longest and shortest repolarization times (activation time + APD_{90}) of the APs recorded across the wall. The QT interval was defined as the time interval between the QRS onset and the point at which the line of maximal downslope of the positive T wave and the line of the maximal upslope of the negative T wave crossed the baseline.

Whole-cell patch-clamp experiments. Epicardial, M, and endocardial cells isolated from the feline left ventricle were voltage-clamped using whole-cell configuration of the patch-clamp technique (16). Patch electrodes were pulled from borosilicate glass capillaries, heat-polished, and had a tip resistance of 2.0 to 3.0 MΩ when filled with standard pipette solution containing (mmol/l): 70 potassium aspartate, 50 KCl, 10 KH_2PO_4, 1 $MgSO_4$, 3 Na_2-ATP, 0.1 Li_2-GTP, 5 EGTA, and 5 HEPES (pH adjusted to 7.2 with KOH). Membrane currents were recorded from the epicardial, M, and endocardial cells superfused at 34 to 36°C with normal Tyrode's solution containing (mmol/l): 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 0.33 NaH_2PO_4, 5.5 glucose, and 5.0 HEPES (pH adjusted to 7.4 with NaOH). In all current measurements, nisoldipine (0.4 μmol/l) was added to normal Tyrode's solution to abolish $I_{Ca,L}$. The cell membrane capacitance (C_m) was calculated for each cell by fitting the single exponential function to the decay of the capacitive transient elicited by a 5-mV step hyperpolarization applied from a holding potential of −50 mV (17).

Simulation study. Isolated epicardial, M, and endocardial cells were simulated using a Luo-Rudy dynamic cell model modified by varying the maximum conductance (density) of I_{Kr} and I_{Ks} (G_{Kr} and G_{Ks}) as described previously (18), in which the G_{Ks}/G_{Kr} in the epicardial, M, and endocardial cells were 23, 17, and 19, respectively. The transient outward potassium current (I_{to}) was incorporated into the model using the formulation of Dumaine et al. (19), in which the maximum conductance of I_{to} (G_{to}) was set to 0.5, 0.25, and 0.05 mS/μF in the epicardial, M, and endocardial cells, respectively.

Statistics. Statistical analysis of the data was performed with a Student *t* test for paired data or analysis of variance coupled with Bonferroni's test, as appropriate. Data are expressed as mean values ± SD except for those shown in the figures, which are expressed as mean ± SEM. Significance was defined as a value of $p < 0.05$.

RESULTS

The QT interval, APD, and TDR under an acquired LQTS condition with or without epinephrine. Figure 1 shows transmembrane activity recorded simultaneously from the epicardium, M, and endocardium together with a transmural ECG at a basic cycle length (BCL) of 2,000 ms. E-4031 (1 μmol/l) alone significantly, but homogenously, prolonged APD of the three regions, causing no major change in TDR (Fig. 1B). Chromanol 293B (10 μmol/l) alone did not significantly increase the QT interval, APD of the three regions, and TDR (Fig. 1C). The additional E-4031 to 293B, mimicking acquired LQTS, preferentially prolonged epicardial APD, thus dramatically increased QT

JACC Vol. 45, No. 2, 2005
January 18, 2005:300–7

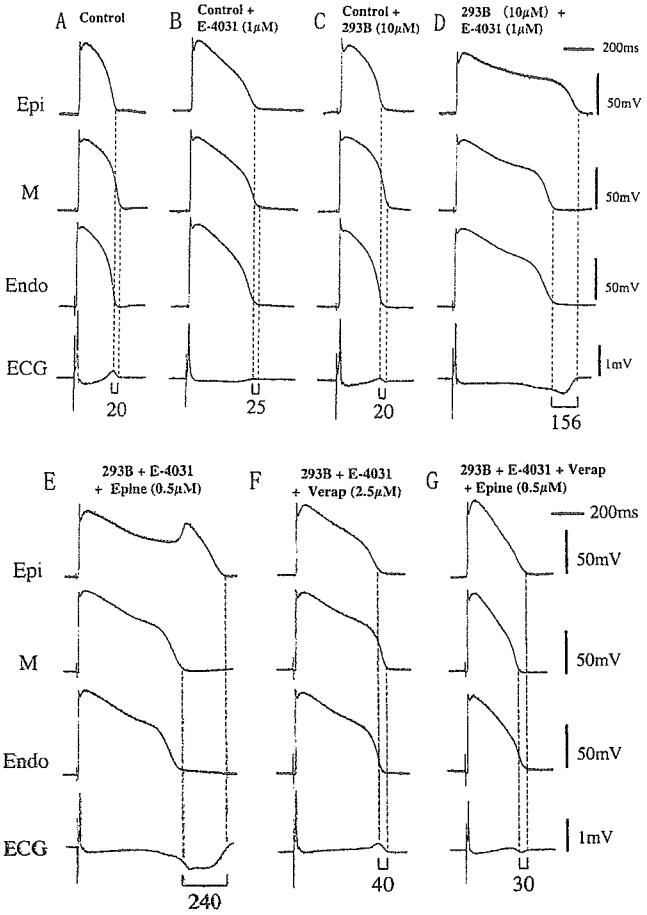

Figure 1. Transmembrane action potentials simultaneously recorded from the epicardial (Epi), midmyocardial (M), and endocardial (Endo) regions and a transmural electrocardiogram (ECG) at basic cycle length of 2,000 ms under each study condition. **(A)** Control. **(B)** E-4031 (1 μmol/l). **(C)** Chromanol 293B (10 μmol/l). **(D)** 293B + E-4031 (acquired long QT syndrome [LQTS] condition). **(E)** Epinephrine infusion (Epine: 0.5 μmol/l) under acquired LQTS condition. **(F)** Addition of verapamil (Verap) 2.5 μmol/l under acquired LQTS condition. **(G)** Further addition of Epine in the continued presence of Verap under acquired LQTS condition. Numbers at bottom of each ECG denote transmural dispersion of repolarization (ms).

interval and TDR (Fig. 1D). Epinephrine infusion (0.5 μmol/l) further prolonged epicardial APD associated with induction of EADs, but did not prolong M or endocardial

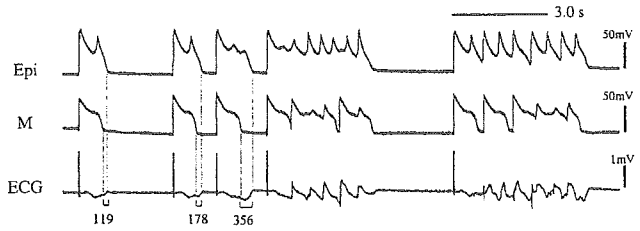

Figure 2. Spontaneous early afterdepolarization and subsequent Torsade de Pointes under the acquired long QT syndrome condition (293B 10 μmol/l + E-4031 1 μmol/l). Basic cycle length = 3,000 ms. Recordings and abbreviations as in Figure 1.

APD, resulting in further QT prolongation and increasing TDR (Fig. 1E).

The composite data of the QT interval, APD_{90} of the epicardium, M, and endocardium, and TDR at a BCL of 2,000 ms are shown in Table 1. E-4031 under control significantly, but homogeneously, prolonged APD_{90}, resulting in neither change of TDR nor induction of arrhythmia. Chromanol 293B under control did not significantly increase APD_{90} of the three regions, resulting in no major change in QT interval and TDR. Whereas additional E-4031 to 293B markedly prolonged QT interval as evidenced by preferential prolongation of the epicardial APD_{90} compared with M and endocardial APD_{90}, thus dramatically increased TDR. Epinephrine further prolonged the epicardial APD_{90}, but shortened the M region APD_{90}, resulting in further prolongation of the QT interval and increasing TDR.

Neither E-4031 alone nor 293B alone produced any EADs or TdP. However, additional E-4031 to 293B (acquired LQTS condition) induced spontaneous EADs from the epicardium in 5 of 10 preparations, including two preparations with spontaneous TdP (Fig. 2), but not from the M or endocardium. Further epinephrine infusion (n = 8) induced EADs from the epicardium in all preparations, including four preparations with subsequent TdP, but EADs from the M region were seen in only one preparation.

Effect of verapamil on the QT interval, APD, TDR, and induction of arrhythmias under an acquired LQTS condition. Under the acquired LQTS condition, verapamil dose-dependently (0.1 to 5 μmol/l) abbreviated APD of

Table 1. Effect of I_{Kr} Block With or Without Pretreated I_{Ks} Block on the QT Interval, APD_{90}, and Transmural Dispersion of Repolarization

	QT	APD_{90}			TDR
		Epi	M	Endo	
Control (n = 5)	283 ± 15	227 ± 16	259 ± 8	246 ± 13	31 ± 10
E-4031 (1 μM) (n = 5)	446 ± 42*	373 ± 30*	408 ± 28*	374 ± 25*	34 ± 4
Control (n = 10)	279 ± 12	230 ± 16	253 ± 14	237 ± 19	24 ± 5
293B (10 μM) (n = 10)	298 ± 34	252 ± 26	275 ± 33	253 ± 16	24 ± 9
293B (10 μM) + E-4031 (1 μM) (n = 10)	793 ± 183*	723 ± 164*	596 ± 131*	545 ± 78*	175 ± 68*
293B + E-4031 + Epine (0.5 μM) (n = 8)	866 ± 251	801 ± 217	506 ± 123	525 ± 118	191 ± 75
293B + E-4031 + Verap (2.5 μM) (n = 7)	557 ± 178‡	503 ± 171‡	483 ± 135†	516 ± 154	35 ± 37‡
293B + E-4031 + Verap + Epine (n = 6)	445 ± 113‡	403 ± 117‡	399 ± 93‡	411 ± 98†	30 ± 12‡

*$p < 0.001$ vs. control, †$p < 0.05$ vs. 293B + E-4031; ‡$p < 0.01$ vs. 293B + E-4031 by analysis of variance with Bonferroni's test.

APD_{90} = action potential duration at 90% repolarization; Endo = endocardium; Epi = epicardium; Epine = epinephrine; I_{Ks} = slowly activating delayed rectifier potassium current; I_{Kr} = rapidly activating delayed rectifier potassium current; M = mid-myocardium; QT = QT interval; TDR = transmural dispersion of repolarization; Verap = verapamil.

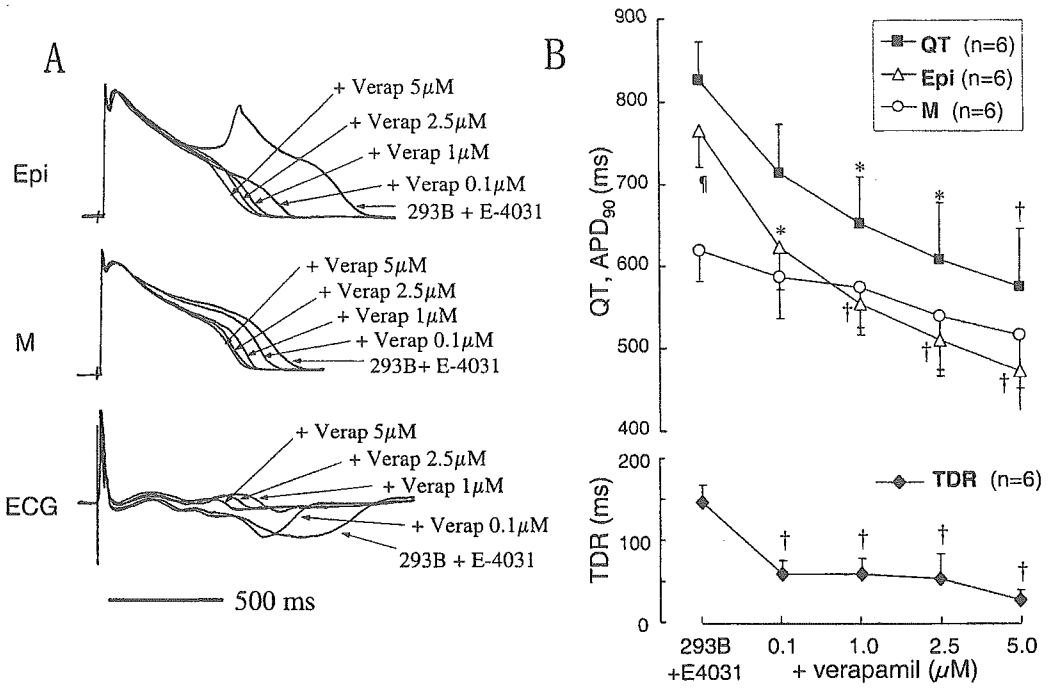

Figure 3. Dose-dependent effect of Verap (0.1 to 5 μmol/l) on transmembrane and ECG activity under acquired LQTS condition (293B 10 μmol/l + E-4031 1 μmol/l). **(A)** Superimposed action potentials recorded simultaneously from the epicardial and M regions together with a transmural ECG. **(B)** Composite data of the effect of Verap on QT interval **(solid squares)**, action potential duration measured at 90% repolarization (APD_{90}) of Epi **(open triangles)** and M **(open circles)** regions and transmural dispersion of repolarization (TDR) **(solid diamonds)**. Basic cycle length = 2,000 ms. *$p < 0.05$ vs. 293B + E-4031; †$p < 0.01$ vs. 293B + E-4031; ¶$p < 0.05$ vs. M region by analysis of variance with Bonferroni's test. Abbreviations as in Figure 1.

the epicardial and M regions as well as the QT interval (Fig. 3A). Figure 3B shows composite data of the dose-dependent effect of verapamil on the QT interval, APD_{90} of the epicardial and M regions, and TDR under the acquired LQTS condition (n = 6). A 5-μmol/l dose of verapamil under the acquired LQTS condition preferentially abbreviated the epicardial APD_{90} (761 ± 99 ms to 469 ± 95 ms; $p < 0.001$) compared with the M region APD_{90} (615 ± 83 ms to 512 ± 146 ms; p = NS), resulting in a significant decrease in TDR (146 ± 46 ms to 26 ± 28 ms; $p < 0.01$). The change in QT interval paralleled the decrease in the epicardial APD_{90}.

As shown in Figure 1F, 2.5-μmol/l verapamil preferentially abbreviated the epicardial APD_{90} rather than the M or endocardium, thus significantly abbreviated QT interval and TDR. Moreover, verapamil completely prevented the influence of epinephrine in inducing EADs and TdP as well as increasing the epicardial APD_{90}, QT interval, and TDR (Fig. 1G). The composite data of the effect of verapamil on the QT interval, APD, and TDR with or without epinephrine are shown in Table 1. Thus, verapamil totally suppressed EADs and TdP under the acquired LQTS condition with or without epinephrine.

Measurement of I_{Kr} and I_{Ks} in epicardial, M, and endocardial cells. Figure 4A represents the dose-dependent inhibition of I_{Ks} by 293B in an epicardial cell. Figure 4B illustrates the concentration-response relationships for the inhibition of I_{Ks} tail current. The data points were reasonably well described by a Hill equation with the following parameters: IC_{50} = 6.39 ± 1.17 μmol/l, n_H = 1.23 ± 0.05 (epicardial cells: n = 5); IC_{50} = 5.71 ± 1.32 μmol/l, n_H = 1.25 ± 0.12 (M cells: n = 5); IC_{50} = 5.73 ± 0.94 μmol/l, n_H = 1.07 ± 0.19 (endocardial cells: n = 5). There are no significant differences in IC_{50} and n_H values among the epicardial, M, and endocardial cells (analysis of variance with Bonferroni's test), thus indicating that I_{Ks} in these three cell types represents a similar sensitivity to inhibition by chromanol 293B.

Figure 5 represents the sensitivity of I_K to blockers of I_{Kr} and I_{Ks} (E-4031 and 293B, respectively). After the I_K reached a practically steady level (control, trace 1), application of E-4031 (3 μmol/l) markedly reduced the amplitude of I_K tail current (trace 2), and further addition of 293B (30 μmol/l) almost completely abolished the I_K tail current (trace 3). Table 2 summarizes densities of I_{Kr} and I_{Ks} in the epicardial, M, and endocardial cells, determined as E-4031- and 293B-sensitive tail currents normalized with reference to C_m. In each cell type, the density of I_{Ks} was significantly smaller than that of I_{Kr}. The density of I_{Kr} was almost equivalent among the three cell types, whereas I_{Ks} density was significantly smaller in M cells compared with that in the epicardial and endocardial cells.

Computer simulations. To understand why EAD developed from the epicardium under the acquired LQTS

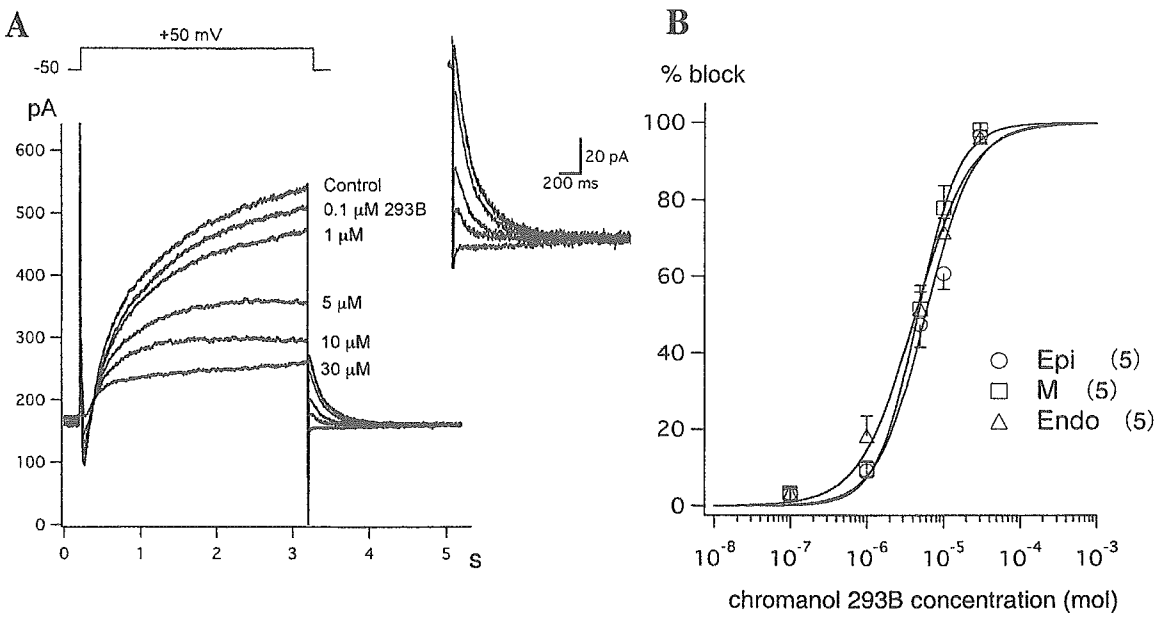

Figure 4. Sensitivity of I_{Ks} in the epicardial (Epi), midmyocardial (M), and endocardial (Endo) cells to inhibition by chromanol 293B. **(A)** Representative superimposed current traces elicited by 3-s depolarizing voltage-clamp steps applied from a holding potential of −50 mV to +50 mV in an epicardial cell, before (control) and during exposure to 293B at a concentration of 0.1, 1, 5, 10, and 30 μmol/l. The I_{Kr} inhibitor E-4031 (3 μmol/l) was present throughout. Tail currents were demonstrated on an expanded scale. **(B)** The percent block of I_{Ks} in the Epi **(open circles)**, M **(open squares)**, and Endo **(open triangles)** cells. The degree of I_{Ks} inhibition was measured as the fraction of the tail current reduced by each concentration of 293B with reference to the control amplitude of the tail current. **Smooth curves** through the data points represent a least-squares fit of a Hill equation: percent block = $100/(1 + (IC_{50}/[293B])^{nH})$, yielding the concentration required for the half-maximal block (IC_{50}) and the Hill coefficient (n_H). pA = pico ($\times 10^{-12}$) Ampere.

condition, we simulated APs of the three cell types using a Luo-Rudy model at a BCL of 2,000 ms. As shown in Figure 6A, the epicardial APD was shorter than the M cells under the control condition (dotted line). However, suppression of both I_{Kr} and I_{Ks} (70% and 80%, respectively) (solid line), simulating the condition of acquired LQTS, developed EAD (arrow) from the epicardial cell but not from M or endocardial cells. Moreover, Figure 6B shows that the reactivation of Ca^{2+} current through the L-type channel ($I_{Ca,L}$) was responsible for the development of epicardial EAD under the acquired LQTS condition. Furthermore, a decrease in I_{to} density changed by G_{to} from 0.5 to 0.05

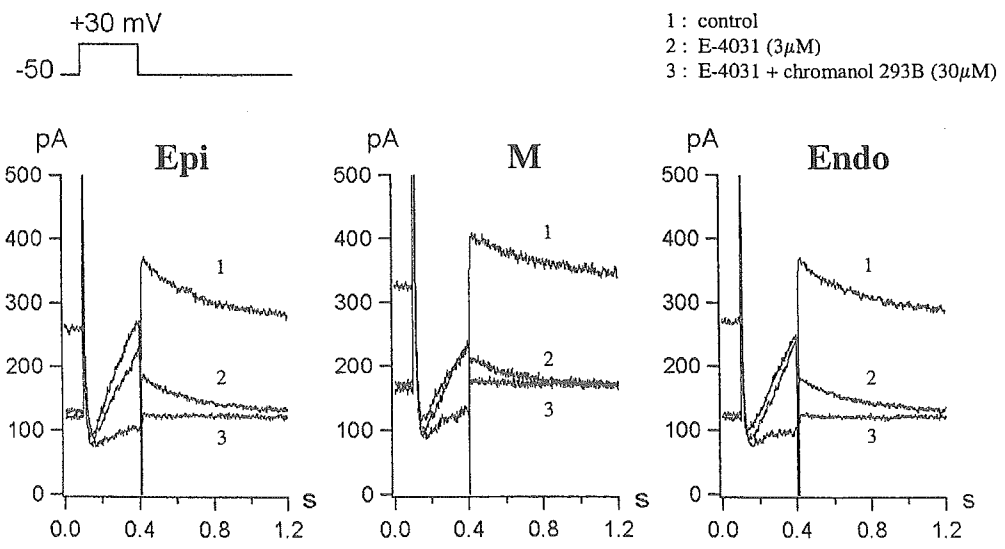

Figure 5. Detection of I_{Kr} and I_{Ks} in the epicardial (Epi), midmyocardial (M), and endocardial (Endo) cells. Depolarizing test pulses (to +30 mV for 300 ms) were repetitively applied (every 2 s) from a holding potential of −50 mV to activate I_K, and membrane currents were recorded from the Epi, M, and Endo cells, before (trace 1), and ~2 min after exposure to 3 μmol/l E-4031 (trace 2), and ~2 min after further addition of 30 μmol/l 293B in conjunction with 3 μmol/l E-4031 (trace 3). pA = pico ($\times 10^{-12}$) Ampere.

Table 2. Transmural Heterogeneity of I_{Ks} and I_{Kr} in Feline Left Ventricle

	Epi (n = 10)	M (n = 9)	Endo (n = 7)
I_{Ks}	0.35 ± 0.26*	0.13 ± 0.09*†	0.30 ± 0.09*
I_{Kr}	1.34 ± 0.51	1.10 ± 0.38	1.17 ± 0.30

*$p < 0.05$ vs. I_{Kr}; †$p < 0.05$ vs. Epi and Endo by analysis of variance with Bonferroni's test. Mean ± SD, (pA/pF). Current densities of I_{Kr} and I_{Ks} measured as E-4031- and chromanol 293B-sensitive tail currents at −50 mV.
Abbreviations as in Table 1.

mS/μF decreased the net charge entry carried by the $I_{Ca,L}$ during the AP, resulted in suppressing EAD as well as abbreviating APD.

DISCUSSION

Genetic and ionic substrates of acquired LQTS. Acquired QT prolongation and TdP arrhythmias usually require multiple risk factors, such as bradycardia, hypokalemia, female gender, and mostly agents with an I_{Kr}-blocking effect. Recent genetic studies suggest some forms of acquired LQTS can be associated with silent mutations in the LQTS-related genes (4), such as *KCNQ1* encoding I_{Ks} (so-called forme fruste type of congenital LQTS) (5–7). Roden (20) hypothesized "reduced repolarization reserve" as a potential mechanism underlying susceptibility to drug-induced LQTS. According to his hypothesis, I_{Ks} dysfunction could be potentially compensated by other K^+ currents, mainly I_{Kr}, thereby the repolarization defect is tolerated, and agents with I_{Kr} block could induce acquired QT prolongation and TdP.

Vos et al. (21–23) suggested a high incidence of EADs and TdP by *d*-sotalol in dogs with chronic complete atrioventricular block as a result of a significant down-regulation of I_{Ks} and I_{Kr}. Moreover, other experimental studies using canine and rabbit wedge showed combined I_{Ks} and I_{Kr} block caused a high incidence of EADs most likely arising from the epicardium (14,15). Burashnikov and Antzelevitch (24) suggested that the abundant I_{Ks} in the epicardium and endocardium compared with the M region under normal conditions contributed to the increase in TDR but protected against development of EADs in the epicardium and endocardium in dogs. Thus, I_{Ks} is critically important for the repolarization reserve in the epicardium and endocardium.

Although I_{Ks} in the feline heart is far smaller than that in other species (25,26), our result from a whole-cell patch-clamp study suggested that a 10-μmol/l 293B used in the wedge preparation reduced about 70% of I_{Ks} in the three cell types, which is consistent with degree of I_{Ks} blockade caused by a silent mutation or common polymorphism in human *KCNQ1* gene (6,7). We also showed that I_{Kr} block with E-4031 in control conditions prolonged the QT interval but did not increase TDR and developed neither EADs nor TdP. However, combined I_{Kr} block with 293B further prolonged the QT interval and inverted T wave, which, in turn, increased TDR and induced EADs and TdP. There-

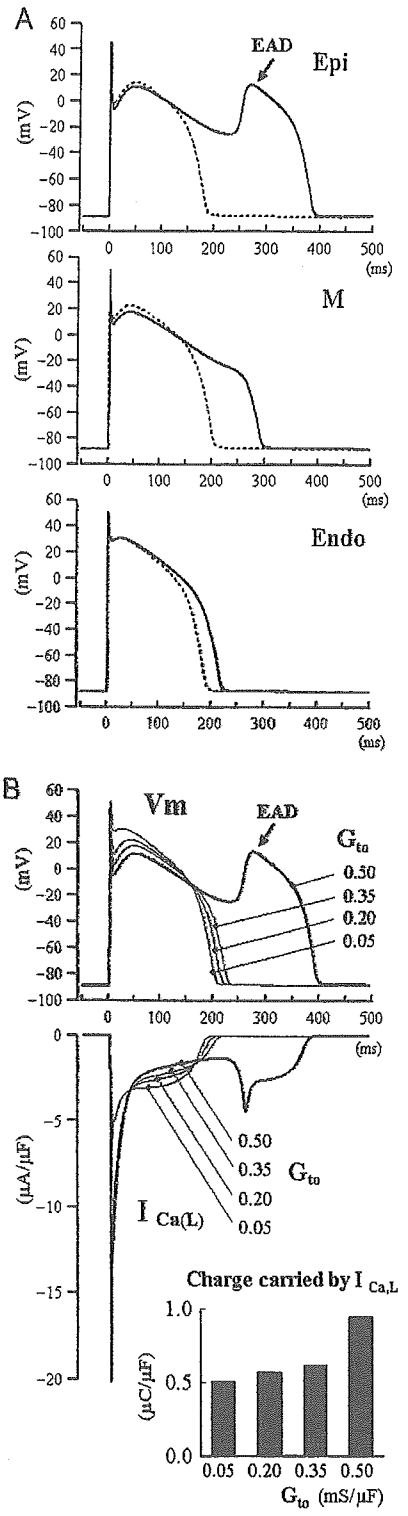

Figure 6. Effect of both I_{Kr} and I_{Ks} suppression on the simulated action potentials from the epicardial (Epi), midmyocardial (M), and endocardial (Endo) cells. **(A)** Superimposed action potentials simulated under baseline condition **(dotted lines)** and after both I_{Kr} and I_{Ks} suppression (70% and 80%, respectively) **(solid lines)**. **(B)** Effect of maximum conductance of I_{to} (G_{to}) on the simulated epicardial action potential (Vm), $I_{Ca,L}$ magnitude, and the net charge entry calculated by integration of the $I_{Ca,L}$ under the condition of both I_{Kr} and I_{Ks} suppression. Basic cycle length = 2,000 ms. EAD = early afterdepolarization.

fore, the feline heart is appropriate for a model of forme fruste LQTS. Our data also suggested that subclinical I_{Ks} dysfunction may become a genetic substrate, and additional I_{Kr} suppression may unmask marked QT prolongation and TdP in acquired form of LQTS.

Role of $I_{Ca,L}$ in increasing TDR and inducing EADs and TdP in acquired LQTS. Several clinical and experimental studies have suggested that EADs and triggered activity were important in the genesis of QT prolongation and TdP in LQTS (8,9,11–15,22–24). Induction of EADs generally requires an initiation or conditioning phase controlled by the sum of membrane currents present at the plateau AP (inward depolarization current and outward repolarization current). January and Riddle (27) suggested that the time- and voltage-dependent $I_{Ca,L}$ within its "window" was important in the induction and block of EADs. Luo and Rudy (28) suggested that EADs resulted from a secondary activation of the $I_{Ca,L}$ during the plateau of AP. However, the mechanism responsible for a high incidence of EADs (especially from the epicardium) and subsequent TdP under conditions of severely eliminated outward K^+ current, mimicking acquired LQTS, has not been mechanistically defined.

Our data indicate that accentuation of $I_{Ca,L}$ during the AP plateau preferentially prolonged APD and triggered EADs in the epicardium. This was based on the effect of verapamil on the epicardium. However, it is still unclear whether a larger $I_{Ca,L}$ in the epicardial cell compared with the M or endocardial cells contributed to the development of EADs. Recently, Bányász et al. (29) reported in their AP voltage clamp experiments that the epicardial cell had a pool of Ca^{2+} channels sufficient for a second activation, whereas the endocardial cells did not. Cordeiro et al. (30) also noted that the presence of spike-and-dome AP waveform in the epicardial cells resulted in a greater magnitude of $I_{Ca,L}$. Moreover, several simulation studies demonstrated a strong coupling between $I_{Ca,L}$ and I_{to} (31,32). Our simulation study also suggested that larger I_{to} in the epicardial cell caused larger $I_{Ca,L}$, developing EADs under the acquired LQTS condition. In the feline left ventricle, it has been reported that I_{to} is larger in the epicardium compared with the endocardium (33). Therefore, larger $I_{Ca,L}$ secondary to I_{to}-mediated spike-and-dome AP configuration in the epicardial cell might be responsible for the high incidence of EADs from the epicardium. This does not necessarily exclude the possible mechanisms of other ionic currents such as I_{NaCa} and Ca^{2+} release from sarcoplasmic reticulum, which may contribute to the prolonged AP as well as to the development of EADs under calcium-loading conditions (34).

Effects of catecholamines and verapamil in acquired LQTS. Treatment of drug-induced TdP begins with immediate withdrawal of any potential drugs and risk factors. Sanguinetti et al. (35) suggested that an increase of heart rate by isoproterenol was an effective therapeutic strategy in patients with acquired LQTS, because beta-adrenergic stimulation with isoproterenol abbreviates repolarization not only by increasing heart rate, but also by directly increasing the magnitude of I_{Ks}. However, our experimental data shows that epinephrine further prolonged APD in the epicardium and induced EADs and TdP probably due to augmentation of $I_{Ca,L}$ in the acquired LQTS condition. Thus, beta-adrenergic stimulation could be arrhythmogenic even in conditions of acquired LQTS when subclinical I_{Ks} dysfunction is present and heart rate is not fully increased.

Cosio et al. (8) used intravenous verapamil to treat three patients with TdP during an atrioventricular block. Shimizu et al. (9) reported that verapamil suppressed spontaneous or epinephrine-induced EADs and TdP in patients with congenital LQTS. Experimentally, Kimura et al. (36) reported that verapamil (2 μmol/l) suppressed cocaine-induced EADs in the myocytes isolated from feline left ventricle. Taken together with the data in the present study, $I_{Ca,L}$ block with verapamil may be a therapeutic choice for TdP in patients with acquired LQTS as well as congenital LQTS.

Study limitations. We assumed the activity recorded from the cut surface of the perfused wedge preparation represented cells within the respective layers of the wall throughout the wedge. Such validation was provided in previous studies that used the wedge preparation (10–15).

Pharmacologic block of I_{Ks} with 293B is not a complete surrogate for *KCNQ1* defect. However, our feline model closely mimicked the degree of I_{Ks} inhibition and pharmacologic features of acquired LQTS. Therefore, we believe these qualitative similarities validate 293B as a surrogate for forme fruste LQTS.

We simulated APs of the three cell types using a Luo-Rudy model, but it does not completely represent feline ventricular APs. However, the phenomenon that EAD frequently developed from the epicardium under the acquired LQTS condition was observed not only in cats but also in dogs and rabbits (14,15); thus, this simulation may support our speculation about the mechanism of this phenomenon.

Finally, the concentration of verapamil mainly used in this study (2.5 μmol/l = 1,250 ng/ml) was considerably higher than a typical clinical dose. However, verapamil was effective in suppressing EADs and decreasing TDR even at the lowest dose used in this study (0.1 μmol/l = 50 ng/ml), which is close to plasma concentration of verapamil after a 5-mg bolus injection (below 200 ng/ml).

Acknowledgments

The authors thank Drs. Charles Antzelevitch and Masahiko Kondo for their helpful suggestions and technical instructions, and Drs. Hans-J. Lang and Jürgen Pünter (Aventis Pharma Deutschland GmbH, Frankfurt, Germany) for kindly providing the chromanol 293B.

Reprint requests and correspondence: Dr. Wataru Shimizu, Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka, 565-8565 Japan. E-mail: wshimizu@hsp.ncvc.go.jp.

REFERENCES

1. Schwartz PJ, Periti M, Malliani A. The long Q-T syndrome. Am Heart J 1975;89:378–90.
2. Moss AJ, Schwartz PJ, Crampton RS, et al. The long QT syndrome: prospective longitudinal study of 328 families. Circulation 1991;84: 1136–44.
3. Roden DM, Lazzara R, Rosen M, et al. Multiple mechanisms in the long-QT syndrome: current knowledge, gaps and future directions. Circulation 1996;94:1996–2012.
4. Yang P, Kanki H, Drolet B, et al. Allelic variants in long-QT disease genes in patients with drug-associated Torsades de Pointes. Circulation 2002;105:1943–8.
5. Donger C, Denjoy I, Berthet M, et al. KVLQT1 c-terminal missense mutation causes a forme fruste long-QT syndrome. Circulation 1997;96:2778–81.
6. Napolitano C, Schwartz PJ, Brown AM, et al. Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. J Cardiovasc Electrophysiol 2000;11: 691–6.
7. Kubota T, Shimizu W, Kamakura S, et al. Hypokalemia-induced long QT syndrome with underlying novel missense mutation in S4-S5 linker of KCNQ1. J Cardiovasc Electrophysiol 2000;11:1048–54.
8. Cosio FG, Goicolea A, López-Gil L, Kallmeter C, Barroso L. Suppression of Torsades de Pointes with verapamil in patients with atrio-ventricular block. Eur Heart J 1991;12:635–8.
9. Shimizu W, Ohe T, Kurita T, et al. Effects of verapamil and propranolol on early afterdepolarizations and ventricular arrhythmias induced by epinephrine in congenital long QT syndrome. J Am Coll Cardiol 1995;26:1299–309.
10. Aiba T, Shimizu W, Inagaki M, et al. Transmural heterogeneity of the action potential configuration in the feline left ventricle. Circ J 2003;67:449–54.
11. Antzelevitch C, Sun ZQ, Zhang ZQ, Yan GX. Cellular and ionic mechanisms underlying erythromycin-induced long QT intervals and Torsade de Pointes. J Am Coll Cardiol 1996;28:1836–48.
12. Shimizu W, Antzelevitch C. Sodium channel block with mexiletine is effective in reducing transmural dispersion of repolarization and preventing Torsade de Pointes in LQT2 and LQT3 models of the long-QT syndrome. Circulation 1997;96:2038–47.
13. Shimizu W, Antzelevitch C. Effects of a K^+ channel opener to reduce transmural dispersion of repolarization and prevent Torsade de Pointes in LQT1, LQT2, and LQT3 models of the long-QT syndrome. Circulation 2000;102:706–12.
14. Emori T, Antzelevitch C. Cellular basis for complex T wave and arrhythmic activity following combined I_{Kr} and I_{Ks} block. J Cardiovasc Electrophysiol 2001;12:1369–78.
15. Yan GX, Wu Y, Liu T, et al. Phase 2 early afterdepolarization as a trigger of polymorphic ventricular tachycardia in acquired long-QT syndrome, direct evidence from intracellular recordings in the intact left ventricular wall. Circulation 2001;103:2851–6.
16. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 1981;391:85–100.
17. Benitah JP, Gomez AM, Bailly P, et al. Heterogeneity of the early outward current in ventricular cells isolated from normal and hypertrophied rat hearts. J Physiol 1993;469:111–38.
18. Clancy CE, Rudy Y. Na^+ channel mutation that causes both Brugada and long-QT syndrome phenotypes, a simulation study of mechanism. Circulation 2002;105:1208–13.
19. Dumaine R, Towbin JA, Brugada P, et al. Ionic mechanisms responsible for the electrocardiographic phenotype of the Brugada syndrome are temperature dependent. Circ Res 1999;85:803–9.
20. Roden DM. Taking the "idio" out of "idiosyncratic": predicting Torsades de Pointes. Pacing Clin Electrophysiol 1998;21:1029–34.
21. Vos MA, Verduyn SC, Gorgels APM, et al. Reproducible induction of early afterdepolarizations and Torsade de Pointes arrhythmias by d-sotalol and pacing in dogs with chronic atrioventricular block. Circulation 1995;91:864–72.
22. Vos MA, Groot SHM, Verduyn SC, et al. Enhanced susceptibility for acquired Torsade de Pointes arrhythmias in the dog with chronic, complete AV block is related to cardiac hypertrophy and electrical remodeling. Circulation 1998;98:1125–35.
23. Volders PGA, Sipido KR, Vos MA, et al. Downregulation of delayed rectifier K^+ currents in dogs with chronic complete atrioventricular block and acquired Torsades de Pointes. Circulation 1999;100:2455–61.
24. Burashnikov A, Antzelevitch C. Prominent I_{Ks} in epicardium and endocardium contributes to development of transmural dispersion of repolarization but protects against development of early afterdepolarizations. J Cardiovasc Electrophysiol 2002;13:172–7.
25. Follmer CH, Colatsky TJ. Block of delayed rectifier potassium current, I_K, by flecainide and E-4031 in cat ventricular myocytes. Circulation 1990;82:289–93.
26. Martínez HB, Elizalde A, Chapula JS. Developmental differences in delayed rectifying outward current in feline ventricular myocytes. Am J Physiol 2000;278:H484–92.
27. January CT, Riddle JM. Early afterdepolarizations: mechanism of induction and block, a role for L-type Ca^{2+} current. Circ Res 1989;64:977–90.
28. Luo CH, Rudy Y. A dynamic model of the cardiac ventricular action potential II afterdepolarizations, triggered activity, and potentiation. Circ Res 1994;74:1097–113.
29. Bányász T, Fülöp L, Magyar J, et al. Endocardial versus epicardial differences in L-type calcium current in canine ventricular myocytes studied by action potential voltage clamp. Cardiovasc Res 2003;58: 66–75.
30. Cordeiro JM, Greene L, Heilmann C, Antzelevitch D, Antzelevitch C. Transmural heterogeneity of calcium activity and mechanical function in the canine left ventricle. Am J Physiol 2004;286:H1471–9.
31. Greenstein JL, Wu R, Po S, et al. Role of the calcium-independent transient outward current Ito1 in shaping action potential morphology and duration. Cir Res 2000;87:1026–33.
32. Miyoshi S, Mitamura H, Fujikawa K, et al. A mathematical model of phase 2 reentry: role of L-type Ca current. Am J Physiol 2003;284: H1285–94.
33. Furukawa T, Myerburg RJ, Furukawa N, et al. Differences in transient outward currents of feline endocardial and epicardial myocytes. Circ Res 1990;67:1287–91.
34. Zygmunt AC, Goodrow RJ, Antzelevitch C. I_{NaCa} contributes to electrical heterogeneity within the canine ventricle. Am J Physiol 2000;278:H1671–8.
35. Sanguinetti MC, Jurkiewicz NK, Scott A, Siegl PKS. Isoproterenol antagonizes prolongation of refractory period by class III antiarrhythmic agent E-4031 in guinea pig myocytes, mechanism of action. Circ Res 1991;68:77–84.
36. Kimura S, Bassett AL, Xi H, Myerburg RJ. Early afterdepolarizations and triggered activity induced by cocaine, a possible mechanism of cocaine arrhythmogenesis. Circulation 1992;85:2227–35.

doi: 10.1046/j.1529-8817.2005.00167.x

Genetic Polymorphisms and Haplotypes of the Human Cardiac Sodium Channel α Subunit Gene (*SCN5A*) in Japanese and their Association with Arrhythmia

K. Maekawa[1,2,*], Y. Saito[1,2], S. Ozawa[1,3], S. Adachi-Akahane[4], M. Kawamoto[5], K. Komamura[6,7], W. Shimizu[6], K. Ueno[8], S. Kamakura[6], N. Kamatani[5], M. Kitakaze[6] and J. Sawada[1,2]

[1]*Project Team for Pharmacogenetics* [2]*Division of Biochemistry and Immunochemistry* [3]*Division of Pharmacology, National Institute of Health Sciences, Setagaya-ku, Tokyo, Japan*

[4]*Laboratory of Cell Signaling, Graduate school of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan*

[5]*Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japanv*

[6]*Department of Cardiology* [7]*Department of Cardiovascular Dynamics Research Institute* [8]*Department of Pharmacy, National Cardiovascular Center, Suita, Japan*

Summary

Genetic variations in cardiac ion channels have been implicated not only as the causes of inherited arrhythmic syndromes, but also as genetic risk factors for some acquired arrhythmias. To elucidate the potential roles of genetic polymorphisms of the α subunit of the voltage-gated sodium channel type V (*SCN5A*) in cardiac rhythm disturbance, the entire *SCN5A* coding exons and their flanking introns were sequenced in 166 Japanese arrhythmic patients and 232 healthy controls. We detected 69 genetic variations, including 54 novel ones. Out of the 12 novel nonsynonymous single nucleotide polymorphisms (SNPs), p.Leu1988Arg was found at a frequency of 0.015. The other 11 SNPs were rare (0.001), with 6 found in arrhythmic patients and 5 in healthy controls. The frequency of a novel intronic SNP, c.703+130G>A, was significantly higher in the patients than in the controls, suggesting this SNP is associated with an unknown risk factor for arrhythmia. Following linkage disequilibrium analysis, the haplotype structure of *SCN5A* was inferred using high-frequency SNPs. The frequency of the haplotype harbouring both p.Leu1988Arg and the common SNP p.His558Arg (haplotype GG) was significantly lower in the patients than in the controls. This finding suggests that this haplotype (GG) might have been positively selected in the controls because of its protective effect against arrhythmias. This study provides fundamental information necessary to elucidate the effect of genetic variations in *SCN5A* on channel function and cardiac rhythm in Japanese, and probably in the Asian population.

Keywords: Cardiac arrhythmia, *SCN5A*, SNP, haplotype, Japanese

Introduction

Voltage-gated cardiac sodium (Na^+) channels produce cationic currents that are responsible for the rapid upstroke of the cardiac action potential, and play a central role in the excitability of myocardial cells (Balser, 1999).

*Correspondence: Keiko Maekawa, Ph.D., Division of Biochemistry and Immunochemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel: 81-3-3700-9453. Fax: 81-3-5717-3832. E-mail: maekawa@nihs.go.jp

The channels are heteromeric assemblies composed of a pore forming α-subunit and a regulatory β-subunit. The gene encoding the α-subunit of the human cardiac Na^+ channel, *SCN5A*, consisting of 28 exons spanning approximately 80-kb, is located on chromosome 3p21 (Gellens *et al.* 1992; Wang *et al.* 1996). The α subunit is composed of 4 homologous domains (DI to DIV). Each domain consists of 6 transmembrane segments (S1 to S6) connected by linker segments.

Some inherited variations in *SCN5A* have been shown to cause severe disorders in cardiac rhythm that

require pacemaker or defibrillator implantation (Bezzina *et al.* 2001). These sodium channelopathies include the long QT syndrome type III (LQT-3) (Wang *et al.* 1995), Brugada syndrome (BrS) (Chen *et al.* 1998), idiopathic ventricular fibrillation (IVF) (Akai *et al.* 2000), sudden infant death syndrome (SIDS) (Ackerman *et al.* 2001) and cardiac conduction defects (CCD) (Schott *et al.* 1999). Electrophysiological studies on mutant Na^+ channels using heterologous expression systems have shown that the distinct effects of the mutant channels on the gating functions, and/or the difference in their availability, may result in these various clinical outcomes (Balser, 2001; Tan *et al.* 2003). Moreover, these genetic variations seem to modify responses to antiarrhythmic drug therapies, and in some cases to sensitize patients to the proarrhythmic effects of Na^+ channel-blocking antiarrhythmic drugs (Schwartz *et al.* 1995; Fujiki *et al.* 1999; Makita *et al.* 2002). As for the common single-nucleotide polymorphisms (SNPs) in *SCN5A*, they have also been shown to cause phenotypic variability of these channelopathies. For example, a common SNP, p.His558Arg, was reported to restore Na^+ channel function by counteracting the gating or trafficking defects caused by other variations (p.Thr512Ile and p.Met1766Leu) (Viswanathan *et al.* 2003; Ye *et al.* 2003). In contrast another SNP, p.Ser1102Tyr, which is frequently found in Africans, was reported to be a risk factor for arrhythmia (Splawski *et al.* 2002). Thus genetic variations, including common polymorphisms of *SCN5A*, affect cardiac electrophysiological properties in a wide range of arrhythmias, from the inherited sodium channelopathies to the common acquired rhythm disorders associated with coronary occlusion and structural heart disease.

In this study to elucidate the role of *SCN5A* variations in common cardiac rhythm disturbances in the Japanese population, all *SCN5A* coding exons and their flanking introns were sequenced in 166 Japanese arrhythmic patients who were not diagnosed with LQT or BrS, and in 232 healthy controls. We identified 69 genetic variations in *SCN5A*, including 12 novel missense variations, and then compared the frequencies of the SNPs and the haplotypes between the arrhythmic patients and the healthy controls.

Materials and Methods

Populations Studied and their Features

Unrelated Japanese arrhythmic patients (166) employed in this study were administered antiarrhythmic drugs (mexiletine, amiodarone, flecainide, or pilsicainide) at the National Cardiovascular Center (Suita, Japan). Informed consent was obtained from all patients. Genomic DNA was extracted from patient blood samples by standard protocols. Among the 166 patients, 126 were male with a mean age of 58 ± 11 years, and 40 were female with a mean age of 58 ± 13 years. Ventricular tachycardia was detected in 96 patients, premature ventricular contraction in 47 patients, atrial fibrillation and flutter in 36 patients, ventricular fibrillation in 16 patients, supraventricular tachycardia in 6 patients, and supraventricular premature contraction in 4 patients. As for the original causes of the arrhythmias, cardiomyopathy was observed in 78 patients, congestive heart failure in 28, myocardial infarction in 24, cornary artery disease (angina) in 18, valvular heart disease in 15, sarcoidosis in 7, and sick sinus syndrome in 4 patients. Arrhythmias were triggered by an unknown cause for 42 patients. Patients with LQT or BrS were not included in this study. Mexiletine, amiodarone, flecainide, and pilsicainide, were administered to 78 patients (100–450 mg/day), 89 patients (50–400 mg/day), 11 patients (100–200 mg/day), and 11 patients (50–150 mg/day), respectively. Twenty-two patients were administered both mexiletine and amiodarone. One patient was administered both flecainide and pilsicainide.

For the controls healthy subjects, with no history of syncope, ventricular tachycardia or ventricular fibrillation based on the medical examination, were recruited. Blood samples were collected from 232 healthy Japanese volunteers at the Tokyo Women's Medical University under the auspices of the Pharma SNP Consortium (Tokyo, Japan). Genomic DNA was extracted from Epstein-Barr virus-transformed lymphoblastoid cells. Informed consent was also obtained from all healthy subjects. Out of 232 healthy subjects, 135 were male with a mean age of 41±12 years, and 97 were female with a mean age of 37±13 years. The ethics committees of the National Cardiovascular Center, the