Clinical Impact of Genetic Studies in Lethal Inherited Cardiac Arrhythmias

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Over the past decade, molecular genetic studies have established a link between a number of inherited cardiac arrhythmias, including congenital long QT syndrome (LQTS) and Brugada syndrome (BrS), and mutations in genes encoding for ion channels or other membrane components. Twelve forms of LQTS have been identified in 50–70% of clinically affected patients. Genotype–phenotype correlations have been rigorously investigated in LQT1, LQT2 and LQT3 syndromes, which constitute more than 90% of genotyped LQTS patients, enabling stratification of risk and effective treatment of genotyped patients. Genotype-specific triggers for both the cardiac events and the clinical course have been reported, and genotype-specific therapy has been already introduced. More recently, mutation site-specific differences in the clinical phenotype have been reported in LQT1 and LQT2 patients, indicating the possibility of mutation site-specific management or treatment. In contrast, only one-third of BrS patients can be genotyped, and data on genotype–phenotype relationships in clinical studies are limited. A Haplotype B consisting of 6 individual DNA polymorphisms within the proximal promoter region of the *SCN5A* gene was recently identified only in Asians (frequency 22%). Individuals with Haplotype B show significantly longer duration of both PQ and QRS than those without Haplotype B, indicating that Haplotype B likely contributes to the higher incidence of BrS in Asian populations. (Circ J 2008; 72: 1926–1936)

Key Words: Brugada syndrome; Genotype; Ion channel; Long QT syndrome; Sudden death

Advances in molecular genetic studies since the late 1990s have established a link between a number of lethal inherited cardiac arrhythmias and mutations in genes encoding for ion channels or other membrane components!^{1–11} Most inherited cardiac arrhythmias have been linked to ion channelopathies giving rise to primary electrical diseases, including congenital and acquired long QT syndrome (LQTS),^{1,2} Brugada syndrome (BrS),³ progressive cardiac conduction defect (Lenegre disease),⁴ catecholaminergic polymorphic ventricular tachycardia (CPVT),^{5,6} arrhythmogenic right ventricular cardiomyopathy,⁷ familial atrial fibrillation,⁸ familial sick sinus syndrome,^{9,10} and short QT syndrome¹¹ (Table 1). Among these primary electrical diseases, congenital LQTS is the Rosetta stone for understanding the genetic basis of inherited cardiac arrhythmias,¹² because the responsible mutations can be identified in multiple genes encoding different ion channels or membrane adaptor in approximately 50–70% of clinically affected patients. Similarly, causative mutations can be detected in the ryanodine receptor (*RyR2*) gene or calsequestrine gene in more than 60% of clinically diagnosed CPVT patients.¹³ BrS is another common inherited cardiac arrhythmia syndrome, and responsible mutations have been identified in 6 genes; however, only one-third of patients with BrS can be genotyped. Responsible mutations have been identified much less

in other inherited cardiac arrhythmias, so genetic screening is much more challenging. This review focuses on the recent progress in molecular genetic studies and their clinical impact on the inherited cardiac arrhythmias, congenital LQTS and BrS.

Congenital LQTS

Prolonged QT interval and polymorphic ventricular tachycardia, known as torsades de pointes (TdP), recorded on an electrocardiogram (ECG) are trademarks of congenital LQTS (Fig 1).^{12,14} The clinical diagnosis of congenital LQTS is mainly based on the corrected (QTc) interval at rest, and cardiac events such as syncope, aborted cardiac arrest and sudden cardiac death because of TdP.¹⁴ However, the ECG diagnosis at rest has long been reported to miss some patients affected by congenital LQTS, as evidenced

Table 1 Genotype of Inherited Cardiac Arrhythmias

Congenital LQTS	Romano-Ward	(1995-) CLQT 1-12
	JLN	(1997-) JLN 1, 2
Acquired LQTS		(1997-) ALQTS 1-2
BrS		(1998-) BrS 1-2
PCCD		(1999-) PCCD 1
CPVT		(2001-) CPVT 1, 2
Familial SSS		(2003-) SSS 1, 2
Familial AF		(2003-) AF 1-5
ARVC		(2004-) ARVC 1-5
SQTS		(2004-) SQTS 1-5

LQTS, long QT syndrome; JLN, Jervell & Lange-Nielsen; BrS, Brugada syndrome; PCCD, progressive cardiac conduction defect; CPVT, catecholaminergic polymorphic ventricular tachycardia; SSS, sick sinus syndrome; AF, atrial fibrillation; ARVC, arrhythmogenic right ventricular cardiomyopathy; SQTS, short QT syndrome.

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Fig 1. Twelve-lead electrocardiogram and torsades de pointes (TdP) in a patient with LQT2 syndrome. (A) Remarkable QT prolongation (corrected QT (QTc) interval=548ms) and a low amplitude T wave with a notched configuration are seen. (B) TdP was induced following the typical short–long–short initiating sequence.

Table 2 Defect of Ion Channel or Membrane Adaptor Responsible for Congenital LQTS

Loci	Chromosome	Gene	Ion channel
<i>Romano-Ward syndrome</i>			
LQT1	11 (11p15.5)	KCNQ1	I _{Ks}
LQT2	7 (7q35-36)	KCNH2	I _{Kr}
LQT3	3 (3p21-23)	SCN5A	I _{Na}
LQT4	4 (4q25-27)	Ankyrin-B	Na-K ATPase, I _{Na-Ca}
LQT5	21 (21q22.1-q22.2)	KCNE1	I _{Ks}
LQT6	21 (21q22.1-q22.2)	KCNE2	I _{Kr}
LQT7	17 (17q23.1-24.2)	KCNJ2	I _{K1}
LQT8	12 (12p13.3)	CACNA1C	I _{Ca-L}
LQT9	3 (3p25)	CAV3	I _{Na}
LQT10	11 (11q23.3)	SCN4B	I _{Na}
LQT11	7 (7q21-q22)	AKAP-9	I _{Ks}
LQT12	20 (20q11.2)	SNTA1	I _{Na}
<i>JLN syndrome</i>			
JLN1	11 (11p15.5)	KCNQ1 (homozygous)	I _{Ks}
JLN2	21 (21q22.1-q22.2)	KCNE1 (homozygous)	I _{Ks}

Abbreviations see in Table 1.

by syncopal events occurring among family members with a “normal” QT interval;¹⁵ therefore, provocative testing using catecholamine infusion or exercise was developed to unmask concealed forms of congenital LQTS, before genetic screening became available.^{16–20}

Genotype in Congenital LQTS

Because familial forms of congenital LQTS have long been recognized, a genetic background (inheritance) has long been expected. Since the first 2 genes responsible for LQTS were identified in 1995^{21,22} molecular genetic studies have revealed 12 forms of Romano-Ward-type congenital LQTS caused by mutations in the genes of the potassium, sodium and calcium channels or the membrane adapter

located on chromosomes 3, 4, 7, 11, 12, 17, 20 and 21 (Table2).^{23–31} Mutations in *KCNQ1* and *KCNE1*, the α and β subunits of the potassium channel gene, are responsible for defects (loss of function) in the slowly activating component of the delayed rectifier potassium current (I_{Ks}) underlying the LQT1 and LQT5 forms of LQTS.^{2,33} Mutations in *KCNH2* and *KCNE2* cause defects in the rapidly activating component of the delayed rectifier potassium current (I_{Kr}), which is responsible for the LQT2 and LQT6 forms.^{21,34} Mutations in *SCN5A*, the gene that encodes the α subunit of the sodium channel, result in an increase (gain of function) in the late sodium current (I_{Na}), which is responsible for LQT3.²² Mutations in *KCNJ2* encoding the inward rectifier potassium current (I_{K1}) underlie Andersen’s

Fig 2. Ionic mechanism of QT prolongation in the LQT1 to LQT12 forms. Decreases in the outward potassium currents (I_{Ks} , I_{Kr} , I_{K1}) or increases in the inward sodium or calcium current (late I_{Na} , I_{Ca-L}) prolong the action potential duration, resulting in prolongation of the QT interval in all 12 genetic forms. ECG, electrocardiogram.

syndrome (LQT7), in which QT prolongation and ventricular arrhythmias are accompanied by periodic paralysis and dysmorphic features.²⁴ A mutation in *Ankyrin-B*, a member of a family of versatile membrane adapters, produces intracellular calcium overload, which underlies LQT4 syndrome and is associated with sinus bradycardia and paroxysmal atrial fibrillation, in addition to QT prolongation.²⁵ A mutation in *CACNA1C* is reported to be responsible for the defect in the L-type calcium current (I_{Ca-L}) underlying the LQT8 form, an arrhythmia disorder associated with dysfunction in multiple organ systems, including congenital heart disease, syndactyly, immune deficiency, and autism.²⁶ *CAV3* encoding caveolin-3 and *SCN4B* encoding *NavB4*, an auxiliary subunit of the cardiac sodium channel, are also reported to be associated with the LQT9 and LQT10 forms, respectively. Mutations in both genes result in a gain of function of late I_{Na} , thus causing an LQT3-like phenotype.^{27,28} *AKAP-9* encoding Yotiao, which assembles *KCNQ1*, is reported to be linked to the LQT11 form.²⁹ Most recently, we and others reported mutations in a cytoskeletal protein syntrophin- $\alpha 1$ (*SNTA1*), which interacts with the cardiac sodium channel, thus resulting in an LQT3-like phenotype (LQT12).^{30,31} At least some cases of sudden infant death syndrome (SIDS) are attributable to congenital LQTS.³⁵ Mutations in *SCN5A*,^{35,36} *CAV3*,³⁷ *KCNQ1*,³⁸ and *KCNH2*³⁸ are reported to be associated with SIDS. As a common mechanism, decreases in the outward potassium currents (I_{Ks} , I_{Kr} , I_{K1}) or increases in the inward sodium or calcium current (late I_{Na} , I_{Ca-L}) prolong the action potential duration (APD), resulting in prolongation of the QT interval, a common phenotype in LQTS in all 12 genetic forms (Fig 2). Among the 12 forms, the LQT1 and LQT2 syndromes are the most common genetic variants, and each accounts for approximately 40% of genotyped patients; LQT3 syndrome accounts for approximately 10% of genotyped patients.²³ Because the LQT1, LQT2 and LQT3 syndromes constitute more than 90% of genotyped patients with LQTS, the genotype-phenotype correlation has been rigorously investigated, and enables stratification of risk and effective treatment of genotyped patients with these 3 major forms.²³

Autosomal recessive forms (JLN1 and JLN2) of the Jervell & Lange-Nielsen syndrome are associated with neurosensory deafness and generally more prominent QT prolongation and more severe ventricular arrhythmias compared with the autosomal dominant forms of the Romano-Ward syndrome.³⁹ JLN1 and JLN2 are reported to be responsible

for homozygous or compound heterozygous mutations in the *KCNQ1* and/or *KCNE1* genes. Approximately 8% of LQTS patients carries homozygous or compound heterozygous mutations in 2 LQTS-causing genes, and are reported to have longer QTc and 3.5-fold more risk of cardiac arrest.⁴⁰ On the other hand, an autosomal recessive form of LQT1 syndrome without neurosensory deafness was reported by Priori et al.⁴¹ Some single mutations in the *SCN5A* gene are reported to cause multiple phenotypes, such as BrS, sick sinus syndrome, and conduction disease, in addition to the LQT3 phenotype.^{42,43}

Genotype-Phenotype Relationships in Congenital LQTS

T-Wave Morphology on the ECG A series of experimental studies using arterially-perfused canine wedge preparations have revealed that intrinsic transmural electrical heterogeneity of ventricular repolarization from the epicardial, mid-myocardial to the endocardial cells contributes to ST-T morphology and the QT interval on ECG, especially in the left precordial (V4-6) leads, which are thought to reflect the potentials of the left ventricular anterolateral wall.⁴⁴⁻⁴⁶ Under normal conditions, repolarization of the epicardial action potential occurs first, coinciding with the peak of the normal T wave, whereas repolarization of the longest action potential in the mid-myocardial layer coincides with the end of the T wave.⁴⁴ Repolarization of endocardial cells usually occurs between repolarization of the epicardial and mid-myocardial cells.⁴⁴ The amplified transmural electrical heterogeneity of ventricular repolarization associated with differential modification of ionic currents in each cell type, which is caused by mutations in each LQTS gene, results in genotype-specific T-wave morphology on the ECG.^{44,45} Moss et al first proposed genotype-specific T-wave morphology in genotyped patients with the LQT1, LQT2 and LQT3 forms in 1995.⁴⁷ Broad-based, prolonged T waves are more commonly observed in LQT1 syndrome; low-amplitude T waves with a notched or bifurcated configuration are more frequently observed in LQT2; and late-appearing T waves with a prolonged isoelectric ST-segment are more specific in LQT3 syndrome. The genotype-specific T-wave pattern was further evaluated by Zhang et al in 2000, and numerous exceptions are reported for all 3 genotypes.⁴⁸

Natural History Zareba et al suggested a higher cumulative probability of cardiac events in LQT1 and LQT2 patients than in LQT3 patients.⁴⁹ More than 50% of patients

Fig 3. Genotype-specific responses of the corrected QT (QTc) interval to epinephrine provocative testing in patients with LQT1, LQT2 and LQT3 syndromes. Shown are V₄ lead electrocardiogram recordings under baseline conditions, at peak and steady-state epinephrine effects in LQT1 (A), LQT2 (B), LQT3 (C) and Control (D) patients. The QTc was prominently prolonged from 576 to 711 ms at peak epinephrine effect, and remained prolonged at the steady-state (696 ms) in the LQT1 patient. In the LQT2 patient, the QTc was also markedly prolonged from 592 to 684 ms at peak, but returned to the baseline level at the steady-state (611 ms). It was much less prolonged (LQT3: 560 to 582 ms, Control: 389 to 450 ms) at the peak in LQT3 and Control patients than in either the LQT1 or LQT2 patients, and was shortened to the baseline level at the steady-state (532, 409 ms). Modified from Shimizu et al. *Heart Rhythm* 2004; 1: 276–283¹⁷ with permission.

experience cardiac events before age 40 years in the LQT1 and LQT2 syndromes, whereas less than 30% of patients do so in LQT3 syndrome; however, the lethality of the cardiac events is significantly higher in LQT3 patients than in LQT1 or LQT2 patients. Generally, male patients experience their first cardiac events at a younger age than female patients.⁵⁰ Approximately 90% of first cardiac events occur before the age of 15 years in male patients, particularly in LQT1 males, whereas female patients do not rarely experience their first cardiac events after the age of 20.⁵⁰ These tendencies were recently confirmed using the largest cohort of LQT1 syndrome patients.⁵¹ The data suggested that LQT1 males before age 13 years and LQT1 females after age 13 years had a significant and independent clinical risk associated with first cardiac events.⁵¹

Triggers for Cardiac Events Genotype-specific triggers for cardiac events have been reported in patients with LQT1, LQT2 and LQT3 syndromes.^{12,52,53} Cardiac events occur most frequently during exercise (62%), and swimming is a common trigger in LQT1 syndrome.⁵² LQT2 and LQT3 patients are less likely to have cardiac events during exercise (13% and 13%, respectively) and more likely to have cardiac events during rest/sleep (29% and 39%, respectively).⁵² In LQT2 syndrome, being startled by an auditory stimulus (telephone, alarm clock, ambulance siren etc) is a specific trigger.^{52,53} LQT2 women are reported to be most susceptible to cardiac events in the postpartum period.⁵⁴ The differential sensitivity in cardiac events to sympathetic (β -adrenergic) stimulation has been suggested to be caused by the differential response of ventricular repolarization to sympathetic

stimulation in both experimental studies employing arterially perfused wedge preparations^{45,46} and in clinical studies using catecholamine provocative testing or exercise testing.^{16–20}

Catecholamine Provocative Testing

Infusion of isoproterenol, a β -adrenergic agonist, or epinephrine, an α - + β -adrenergic agonist, has been used as a provocative test in patients with LQTS since the 1980s.⁵⁵ Before the discovery of distinct genetic subtypes of congenital LQTS, the responses to either epinephrine or isoproterenol were extremely heterogeneous, and deemed impossible to interpret. Now, however, the heterogeneous response is understood to stem from underlying genetic heterogeneity, and genotype-specific responses to epinephrine can be exploited to expose different genotypes of LQTS in its otherwise concealed state, particularly LQT1 syndrome. Although isoproterenol is still used occasionally, recent major insights have been gleaned from using epinephrine. The 2 major protocols developed for epinephrine provocative testing include the escalating-dose protocol by Ackerman's group (Mayo protocol)^{18,19} and bolus injection followed by brief continuous infusion by my group (Shimizu protocol).^{16,17,19}

The bolus (Shimizu) protocol was developed on the basis of a differential response of the APD and QT interval to sympathetic stimulation with isoproterenol between experimental models of LQT1, LQT2 and LQT3 using arterially-perfused canine left ventricular wedge preparations.⁴⁶ Clinical data from the use of the bolus protocol suggested that sympathetic stimulation produces genotype-specific

Fig 4. Genotype prediction by epinephrine provocative testing in patients with LQT1, LQT2 and LQT3 syndromes. Flow chart predicting genotype with epinephrine provocative testing. Modified from Shimizu et al. *Heart Rhythm* 2004; 1: 276–283¹⁷ with permission.

responses of the QTc interval in patients with LQT1, LQT2 and LQT3 syndromes (Fig 3).^{16,17,19} Epinephrine remarkably prolongs the QTc interval at peak effect when the heart rate is maximally increased (1–2 min after the bolus injection), and the QTc remains prolonged during the steady-state epinephrine effect (3–5 min) in patients with LQT1.^{16,17,19} In LQT1 patients, a paradoxical QT prolongation, defined as an absolute increase in the QT (not QTc) interval, despite a shortening of the RR interval, is often observed during epinephrine infusion. Ackerman et al reported that the paradoxical QT prolongation had a sensitivity of 92.5%, specificity of 86%, positive predictive value of 76%, and negative predictive value of 96% for LQT1 patients vs non-LQT1 patients.¹⁸ In the bolus protocol, QTc is also prolonged at peak epinephrine effect (during bolus) in patients with LQT2, but returns to close to the baseline level at steady-state epinephrine effect.^{17,19} In contrast, the QTc is less prolonged at peak epinephrine effect in LQT3 patients than in LQT1 or LQT2 patients, and is abbreviated below the baseline level at steady-state epinephrine effect.^{17,19} Using the steady-state epinephrine effect, an improvement of clinical ECG diagnosis (sensitivity) from 68% to 87% in 31 patients with LQT1 syndrome and from 83% to 91% in 23 patients with LQT2 syndrome, but not in 6 patients with LQT3 syndrome (from 83% to 83%), was reported.¹⁷ The bolus protocol of epinephrine effectively predicts the underlying genotype of LQT1, LQT2 and LQT3 (Fig 4).^{17,19} The prolongation of QTc ≥ 35 ms at steady-state epinephrine effect can differentiate LQT1 from LQT2, LQT3 or control patients with a predictive accuracy $\geq 90\%$. The prolongation of QTc ≥ 80 ms at peak epinephrine effect can differentiate LQT2 from LQT3 or control patients with predictive accuracy of 100%. Although induction of TdP or ventricular fibrillation (VF) is extremely uncommon, intravenous β -blockers and a cardioverter defibrillator need to be available during testing.

Because molecular diagnosis is still unavailable in many institutes and is time-consuming, a clinical diagnosis of patients with concealed LQTS by epinephrine provocative testing can direct appropriate counseling and facilitate the initiation of preventive measures such as avoidance of QT-prolonging drugs. Moreover, a presumptive, pre-genetic diagnosis of either LQT1, LQT2, or LQT3 based on the response to epinephrine can guide genotype-specific treatment strategies.

Table 3 Genotype-Specific Therapy Based on Clinical and Experimental Data in LQTS

	LQT1	LQT2	LQT3
Prevalence	40%	30–40%	10%
Exercise restriction	+++++	+++	–
β -blockers	+++++	+++	–
Potassium supply	++?	++++	++?
Class IB sodium channel blockers	+++	+++	+++++
Calcium channel blockers	+++	+++	++?
Potassium channel openers	++	++	–
Pacemaker	++	++++	+++++
ICD	++++	++++	+++++

ICD, implantable cardioverter-defibrillator; +++++, most effective. Other abbreviation see in Table 1.

Genotype-Specific Therapy

Based on the natural history and specific sensitivity to sympathetic stimulation or catecholamine in the LQT1 syndrome, stricter exercise restriction, in particular swimming or diving, is required, especially for LQT1 males.¹² Exercise restriction is also required in LQT2 syndrome.¹²

Although β -blockers are empirically believed to be the most effective therapy for patients with congenital LQTS, they are not protective in all LQTS patients. Since molecular genetic studies have become available, genotype-specific pharmacological and non-pharmacological therapies have been introduced clinically, based on data derived from both clinical and experimental studies (Table 3).

In LQT1 patients, β -blockers frequently suppress episodes of syncope and sudden cardiac death.⁵² Data from a recent international cohort of 600 LQT1 patients has suggested that time-dependent β -blocker use is associated with a significant 74% reduction in the risk of first cardiac events.⁵¹ Mexiletine, a class IB sodium channel blocker, which blocks late I_{Na} , or verapamil, an I_{Ca-L} blocker, may warrant consideration as adjunctive therapy to β -blockers in LQT1 patients, based on ECG changes with these agents or experimental data.^{44,45} An implantable cardioverter-defibrillator (ICD) is indicated for LQTS patients who have suffered an aborted cardiac arrest and/or who have repetitive episodes of syncope in the presence of β -blockers.

Beta-blockers are also the first choice as pharmacological therapy in LQT2 patients, but the recurrence rate is higher than in LQT1 patients.⁵² Increase in the extracellular potassium concentration by exogenously administered potassium or long-term oral potassium administration has been reported to shorten the QT interval in LQT2 patients.⁵⁶ The indication for an ICD is similar to that in LQT1 syndrome. My group recently reported that patients with LQT2 syndrome show a specific short–long–short initiating pattern of TdP more frequently than those with LQT1 syndrome,⁵⁷ so pacemaker therapy is expected to be more effective in LQT2 than in LQT1 patients by suppressing that specific pattern.⁵⁷

In LQT3 patients, β -blockers are less effective than in LQT1 or LQT2 patients.⁵² Mexiletine is more effective for abbreviating the QT interval in LQT3 than in LQT1 or LQT2 syndrome, and is therefore a promising therapeutic choice in LQT3 syndrome. Pacemaker therapy may be most beneficial in LQT3 patients with bradycardia, based on the experimental data.

Genotype-specific therapy is unknown for the other forms: LQT4, LQT5, LQT6, LQT7, LQT8, LQT9, LQT10, LQT11, and LQT12. Beta-blockade is the first-line therapy in patients with LQT4, LQT5, LQT6, LQT7, LQT8 and

Fig 5. Kaplan-Meier cumulative cardiac event curves from birth to age 50 for patients with *KCNQ1* mutations located in transmembrane regions (n=66, 19 mutations; closed circles) and the C-terminal regions (n=29, 8 mutations; gray circles) in LQT1 syndrome. The difference in the clinical course by mutation location was significant (log-rank, $p=0.005$), with a greater risk of first cardiac events in patients with transmembrane mutations than in those with C-terminal mutations. Modified from Shimizu et al. *J Am Coll Cardiol* 2004; 44: 117–125⁵⁹ with permission.

Fig 6. Twelve-lead electrocardiogram and ventricular fibrillation (VF) in a patient with Brugada syndrome. Spontaneous type 1 coved-type ST-segment elevation is recorded in leads V1 and V2 (arrows).

LQT12, and unknown LQTS genotypes. The class IB sodium channel blocker, mexiletine, may be theoretically effective in LQT9, LQT10, and LQT12 patients.

Possibility of Mutation Site-Specific Therapy

The structure of each cardiac ion channel, or correspondence between the mutation site and channel function, has been increasingly elucidated, suggesting mutation site-specific differences in the severity of the clinical phenotype or responses to therapy in each genotype. From data in the International LQTS Registry, Moss et al suggested that LQT2 patients with mutations in the pore region of *KCNH2* had a greater risk of arrhythmia-related cardiac events than patients with non-pore mutations;⁵⁸ thus indicating the possibility of mutation site-specific management or treatment of

LQT2 syndrome. With regard to LQT1 syndrome, in 2004 the arrhythmic risk and sensitivity to sympathetic stimulation with treadmill exercise testing was compared between Japanese LQT1 patients with transmembrane mutations and those with C-terminal mutations in *KCNQ1*, and the LQT1 patients with transmembrane mutations showed a longer QTc interval and more frequent LQTS-related cardiac events than those with C-terminal mutations (Fig 5).⁵⁹ Moreover, the QTc interval was more prominently increased with exercise in patients with transmembrane mutations.⁵⁹ The international cohort of 600 LQT1 patients recently confirmed the Japanese data,⁵¹ suggesting that transmembrane mutations and mutations with dominant-negative functional effect adversely influence the outcome of LQT1 patients, independent of traditional clinical risk factors and β -blocker

Table 4 Defect of Ion Channel or Membrane Adaptor Responsible for BrS

<i>Loci</i>	<i>Chromosome</i>	<i>Gene</i>	<i>Ion channel</i>
<i>BrS1</i>	3 (3p21-24)	<i>SCN5A</i>	<i>I_{Na}</i>
<i>BrS2</i>	12 (12p13.3)	<i>CACNA1C</i>	<i>I_{Ca-L}</i>
<i>BrS3</i>	10 (10p12.33)	<i>CACNB2</i>	<i>I_{Ca-L}</i>
<i>BrS4</i>	3 (3p21)	<i>GPD1-L</i>	<i>I_{Na}</i>
<i>BrS5</i>	19 (19q13.1)	<i>SCN1B</i>	<i>I_{Na}</i>
<i>BrS6</i>	11 (11q13-q14)	<i>KCNE3</i>	<i>I_{to}</i>

Abbreviation see in Table 1.

therapy.

Brugada Syndrome

In 1992, Brugada and Brugada first reported 8 patients with a history of aborted sudden cardiac death caused by VF and a characteristic ECG pattern, consisting of right bundle branch block (RBBB) and ST-segment elevation in the right precordial ECG leads (V₁₋₃) as a distinct clinical entity.⁶⁰⁻⁶⁵ The Brugada Consensus Report in 2002 suggested 3 patterns of ST-segment elevation:⁶³ the type 1 ST-segment elevation is characterized by a coved-type ST-segment elevation displaying J-wave amplitude or ST-segment elevation ≥ 0.2 mV with or without a terminal negative T wave (Fig 6); type 2 and type 3 ST-segment elevations show a saddle-back configuration, which has a high take-off ST-segment elevation (≥ 0.2 mV), followed by a gradually descending ST-segment elevation (type 2 ≥ 0.1 mV, type 3 < 0.1 mV above the baseline) and a positive or biphasic T wave. ST-segment elevation is often accentuated and the coved type ST-segment elevation is more frequently recognized just before and after episodes of VF.^{66,67} The second Consensus Report published in 2005 emphasized that type 1 ST-segment elevation is required to diagnose BrS⁶⁴ because the type 1 ECG is reported to relate to a higher incidence of VF and sudden cardiac death. Type 1 ST-segment elevation recorded only in the higher (3rd and 2nd intercostal spaces) V₁₋₂ leads is reported to show a similar prognostic value for subsequent cardiac events as that recorded in the standard V₁₋₂ leads.^{64,68-70} The prevalence of BrS is estimated to be up to 5 per 10,000 inhabitants, and is an important cause of sudden cardiac death of middle-aged males, particularly in Asian countries.^{64,65} BrS usually manifests during adulthood,⁶⁴ and more than 80–90% of patients clinically affected are men.

Genotype in BrS (Table 4)

In 1998, Chen et al identified the first mutation linked to BrS in *SCN5A*, the *I_{Na}* gene that is responsible for the LQT3 form of congenital LQTS.³ *SCN5A* mutations are reported to account for 18–30% of clinically diagnosed BrS patients at present.⁶⁴ Functional analysis using expression systems has shown that all *SCN5A* mutations so far identified result in decreased (loss of function) *I_{Na}* by several mechanisms⁶⁵ including (1) lack of expression of the sodium channel; (2) a shift in the voltage dependence and time dependence of *I_{Na}* activation, inactivation or reactivation; (3) entry of the sodium channel into an intermediate state of inactivation from which it recovers more slowly; (4) accelerated inactivation of the sodium channel; or (5) a trafficking defect. The 2nd and 3rd mutations linked to BrS were reported by Antzelevitch et al in 2007, when they identified mutations in *CACNA1C* or *CACNB2*, the gene encoding the $\alpha 1$ or $\beta 2$ b

subunit of the L-type calcium channel, in 3 probands with Brugada-like ST-segment elevation associated with a short QT interval.⁷¹ Heterologous expression studies for the mutations revealed loss of function of *I_{Ca-L}*. Thereafter, London et al identified a mutation in a conserved amino acid of the glycerol-3-phosphate dehydrogenase 1-like (*GPD1-L*) gene in affected individuals of a large Brugada family.⁷² The *GPD1-L* mutation decreases *SCN5A* surface membrane expression and reduces *I_{Na}*, thus causing BrS.⁷² Watanabe et al recently identified a nonsense mutation (W179X) in *SCN1B*, which encodes the function-modifying sodium channel $\beta 1$ subunit, in a family with BrS associated with cardiac conduction disease.⁷³ They reported that the *I_{Na}* current was decreased when α subunit (Nav1.5) of the sodium channel was coexpressed with the mutant $\beta 1$ subunit compared with when it was coexpressed with the wild-type $\beta 1$ subunit. More recently, Delpon, Antzelevitch et al reported a missense mutation (R99H) in *KCNE3*, which encodes the potassium channel β subunit and interacts with Kv4.3 (transient outward current: *I_{to}*) channel, in a proband with BrS.⁷⁴ Coexpression of the mutant *KCNE3* with *KCND3*, which encodes Kv4.3, increases the *I_{to}* intensity (gain of function) compared with that by the coexpression of wild-type *KCNE3* with *KCND3*.⁷⁴ Thus, decreases in the inward sodium or calcium current (late *I_{Na}*, *I_{Ca-L}*) or increases in the outward potassium currents (*I_{to}*) produce a Brugada phenotype in all 6 genotypes, as indicated by previous experimental studies; however, approximately two-thirds of Brugada patients have not yet been genotyped, suggesting the presence of genetic heterogeneity.⁶⁵

Genotype–Phenotype Correlations in BrS

The genotype–phenotype correlation in BrS has been less investigated than that in congenital LQTS, because more than two-thirds of patients clinically affected with BrS are not genotyped. Mild conduction abnormalities, such as widening of the P wave, prolongation of the QRS duration, PQ interval, and HV interval, and a higher incidence of RBBB have been described in patients with BrS, especially those with the *SCN5A* mutation.⁶⁴ Significantly longer PQ and HV intervals at baseline and a larger increase in the PQ and QRS intervals after sodium channel blockers have been reported by Smits et al in Brugada patients with *SCN5A* mutations than in those without *SCN5A* mutations.⁷⁵ Several ECG parameters were measured during long-term follow up and prospectively compared between Brugada patients with and without the *SCN5A* mutation by Yokokawa and co-workers, with the results suggesting that P wave, QRS, S wave durations, and PQ intervals were all significantly longer, and the S wave amplitude was significantly deeper in the *SCN5A*-positive group than in the *SCN5A*-negative group (Figs 7,8).⁷⁶ In addition, the PQ interval and QRS duration in lead V₂ were prolonged more markedly with aging in the

Brugada patient with SCN5A mutation

Fig 7. Twelve-lead electrocardiogram in the early and late periods during follow-up (7 years) in a Brugada patient with the *SCN5A* mutation. The P wave (lead II), QRS (lead V2), and S wave (lead V5) durations and PQ interval (lead II) are prolonged, even in the early period (47 years old) (A). The S wave amplitude (lead V5) is also deep, and the QRS axis is deviated to the left. The corrected QT (QTc) interval (lead V2) is borderline prolonged. In the late period (B), all these parameters are further increased. Modified from Yokokawa et al. *Am J Cardiol* 2007; **100**: 649–655⁷⁶ with permission.

Fig 8. Electrocardiographic parameters during long-term follow-up in 8 Brugada patients with the *SCN5A* mutation and in 36 Brugada patients without the mutation. Both the PQ interval in lead II (A) and the QRS duration in lead V2 (B) are significantly longer in the *SCN5A*-positive (+) group than in the *SCN5A*-negative (–) group in both the early and late periods. Both the PQ interval and QRS duration increased with aging during follow-up in both groups, but more prominently in the *SCN5A*(+) group than in the *SCN5A*(–) group. **p*<0.05 vs *SCN5A*(–), †*p*<0.05 vs early.

Fig 9. Haplotypes identified within the proximal promoter region of the *SCN5A*, a cardiac sodium channel gene. The 6 polymorphisms are in near-complete linkage disequilibrium. Haplotype A is designated as containing all common alleles, and Haplotype B as containing all minor alleles. The discordant haplotype is designated Haplotype C. *Frequency in the Japanese (control) population. Modified from Bezzina et al. *Circulation* 2006; **113**: 338–344⁷⁹ with permission.

Fig 10. *SCN5A* promoter haplotype (Hap) pair effects on QRS duration in lead V6 and PR duration in lead II in patients with Brugada syndrome and control subjects. In Brugada patients without the *SCN5A* mutation and in control subjects, both QRS and PR durations show a gene-dose effect, being longest in HapB homozygotes (BB), intermediate in HapA/HapB heterozygotes (AB) and shortest in HapA homozygotes (AA). Brugada patients with the *SCN5A* mutation show longer QRS and PR durations than those without the *SCN5A* mutations. Patient numbers are indicated in parentheses. Data are mean ± SD. Modified from Bezzina et al. *Circulation* 2006; **113**: 338–344⁷⁹ with permission. * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$ vs HapA/HapA. † $p < 0.05$; †† $p < 0.001$; ††† $p < 0.0001$ vs HapA/HapB. ‡ $p < 0.05$ vs HapB/HapB.

SCN5A-positive group than in the *SCN5A*-negative group during the follow-up period (Figs 7,8).⁷⁶ Frustaci et al reported significant myocyte apoptosis in both the right and left ventricular myocardium in a histological study in Brugada patients with *SCN5A* mutations, and suggested that abnormal function of the sodium channels may lead to a masked degree of cellular damage, contributing to arrhythmic events.⁷⁷ These electrocardiographic and histologic data indicate that progressive depolarization abnormalities (conduction slowing) with aging may contribute to the pathogenesis of BrS.

SCN5A Promoter Polymorphism

The incidence of BrS is significantly higher in Asian countries, including Japan, than in the USA and European countries.⁶⁴ It has been reported that common polymorphisms may modulate the activity of the primary disease-causing mutation in inherited cardiac arrhythmias, and/or influence the susceptibility to arrhythmia even in the general population.⁷⁸ The common polymorphisms are expected to relate to ethnic differences in the clinical phenotype in inherited cardiac arrhythmias, including BrS, because some common polymorphisms are ethnically dependent. A Haplotype B consisting of 6 individual DNA polymorphisms in near-complete linkage disequilibrium within the proximal promoter region of the *SCN5A* gene has been identified in only Asians (allele frequency of 22%), but not in Caucasians or African-Americans (Fig 9).⁷⁹ Luciferase reporter activity of the Haplotype B is reduced by 62% in cardiomyocytes compared with the wild type, Haplotype A.⁷⁹ The relationship between the *SCN5A* promoter haplotype and indices of conduction velocity, PR and QRS durations was further analyzed in a cohort of 71 Japanese BrS subjects without the *SCN5A* mutation and in 102 Japanese controls to examine the role of Haplotype B in cardiac conduction. PR and QRS durations were significantly longer in the Haplotype B individuals, with a gene-dose effect in both groups (Fig 10).⁷⁹ Moreover, the increases in both the PR and the QRS dura-

tion with sodium channel blockers were genotype-dependent and a gene-dose effect was also observed.⁷⁹ These data demonstrate that Haplotype B within the *SCN5A* promoter region alone does not give rise to BrS; however, it is possible that the *SCN5A* promoter Haplotype B contributes to the higher incidence of BrS in Asian populations, in combination with other as-yet-unknown factors.

Conclusions

Genetic studies and the genotype–phenotype correlation in lethal inherited cardiac arrhythmias have encouraged cardiologists to perform genotype-specific, so-called tailor-made, management and therapy, and possibly mutation site-specific therapy in patients with genotyped congenital LQTS. Genetic studies are now an important diagnostic tool for stratifying risk and effectively managing and treating genotyped patients. Reflecting the clinical impact of genetic studies in the real world of management and therapy for patients with congenital LQTS, genetic studies to screen for the LQTS gene have been reimbursed by National Insurance in Japan since April 1, 2008. On the other hand, genetic studies of other inherited arrhythmias, including BrS, are still experimental, and further investigations of the genotype–phenotype correlations are required.

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