Phenotypic Overlap of Cardiac Sodium Channelopathies

— Individual-Specific or Mutation-Specific? —

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Mutations in the cardiac sodium channel gene *SCN5A* are responsible for a spectrum of hereditary arrhythmias, including type-3 long QT syndrome (LQT3), Brugada syndrome (BrS), conduction disturbance and sinus node dysfunction. These syndromes were originally regarded as independent entities with distinct clinical manifestations and biophysical properties, but recent evidence shows considerable clinical overlap, implying a new disease entity known as an overlap syndrome of cardiac sodium channelopathy. Class IC sodium-channel blockers often induced the BrS phenotype in some patients with LQT3, confirming the clinical overlap of LQT3 and BrS. It also raises a concern about the safety of the class IC drug and questions about the determinants of overlap. Here, an overview is given of current knowledge on the clinical features, prevalence, and molecular and biophysical mechanisms underlying overlap syndrome to gain more insight into this complex issue and generate better therapeutic strategies for patient management. (Circ J 2009; 73: 810–817)

Key Words: Brugada syndrome; Flecainide; Long QT syndrome; Overlap syndrome; *SCN5A*

Structure–Function of Cardiac Sodium Channels

The voltage-gated sodium (Na) channel is responsible for the rapid upstroke of the action potential in most of the excitable tissues, and plays a pivotal role in the initiation, propagation, and maintenance of normal cardiac rhythm. The cardiac Na channel comprises the most prevalent pore-forming α -subunit (Nav1.5) encoded by the gene *SCN5A* located on chromosome 3p21 and auxiliary β subunits (Nav β 1–Nav β 4) encoded by the genes *SCN1B–SCN4B*, respectively. The α -subunits have a 4-fold symmetry macromolecule consisting of structurally homologous domains (D1–D4) each containing 6 membrane-spanning segments (S1–S6) and a region (S5–S6 loop) controlling ion selectivity and permeation. The positively charged S4 segment of each domain functions as a voltage sensor.^{1,2}

The Na channels switch between 3 functional states (closed, open, inactivated), depending on the membrane potential. Membrane depolarization causes a rapid rise in local Na permeability because of opening (activation) of the Na channels from their resting closed state. Normally, activation of the Na channels is transient, because of inactivation, another gating process mediated by structures located on the cytoplasmic face of the channel protein (mainly the D3–D4 linker). The Na channels cannot reopen until the membrane is repolarized and they recover from inactivation. Membrane repolarization is achieved by fast inactivation of the Na channels and is augmented by activation of

voltage-gated K channels. Activation, inactivation, and recovery from inactivation occur within a few milliseconds. In addition to these rapid gating transitions, the Na channels are also susceptible to slower inactivating processes (slow inactivation) if the membrane remains depolarized for a longer time. These slower events may contribute to the availability of active channels under various physiological conditions.

Genetics of Cardiac Sodium Channelopathies

Mutations of *SCN5A* are responsible for a spectrum of hereditary arrhythmias, including type-3 congenital long QT syndrome (LQTS; LQT3);^{3,4} acquired LQTS;⁵ Brugada syndrome (BrS; BrS1);^{4,6} cardiac conduction disturbance (CCD);⁷ congenital sick sinus syndrome (SSS);⁸ atrial standstill;^{9–11} AV block;¹² sudden infant death syndrome;^{13–15} and familial atrial fibrillation^{16–18} (Table 1). In addition to these primary electrical diseases, which usually do not have structural abnormalities, *SCN5A* mutations have also been reported in patients with dilated cardiomyopathy.^{16,19} Moreover, recent genetic studies have indicated that mutations

Table 1. Inherited Cardiac Sodium Channelopathies

1. Cardiac Na channel α -subunit (*SCN5A*)
Congenital long QT syndrome (LQT3)
Acquired long QT syndrome
Brugada syndrome (BrS1)
Cardiac conduction disturbance (CCD)
Congenital sick sinus syndrome (SSS1)
Atrial standstill
AV block
Sudden infant death syndrome (SIDS)
Familial atrial fibrillation (FAF)
Dilated cardiomyopathy (DCM)
2. Sodium channel β 1-subunit (*SCN1B*)
Brugada syndrome with CCD (BrS5)
3. Sodium channel β 4-subunit (*SCN4B*)
Congenital long QT syndrome (LQT10)

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Table 2. Mutations in Overlapping Cardiac Sodium Channelopathies

Clinical manifestation	<i>SCN5A</i> and <i>SCN1B</i> (*) mutations	References
BrS+LQT3 (±CCD)	V411M, D1114N, W1191X, I1350T, ΔK1500, ΔKPQ, R1612P, P2006A	22, 35, 37, 42–46
BrS+LQT3+SSS (±CCD)	G1262S, ΔF1617, E1784K, 1795insD	8, 32, 33, 35, 40, 41, 47, 48
BrS+SSS	E161K, T187I, E1225K, ΔK1479, K1578fs, R1623X	31, 49, 50
BrS+AS	R367H	11
BrS+CCD	P336L, D356N, R376H, N406S, G752R, F861fs951X, E867X, G1319V	50–54
DCM (+/- CCD+SSS+AF)	G1406R, I1660V, S1710L, S1812X, E87Q*, Y179X*	20, 30, 50, 51, 55–57
CCD+SSS/AS	T220I, R814W, F851fs, D1275N, D1595H	9, 16, 19
AS+AF	G1408R	8
LQT3+CCD	L212P	10
	P1332L, V1763M, M1766L, V1777M, P2005A	58–65

BrS, Brugada syndrome; LQT3, type-3 long QT syndrome; SSS, sick sinus syndrome; CCD, cardiac conduction disturbance; AS, atrial standstill; DCM, dilated cardiomyopathy; AF, atrial fibrillation.

Figure 1. Pedigrees of 15 LQT3 families (A–O) carrying E1784K are shown. Proband is indicated by an arrow; 10 symptomatic mutation carriers, shown by the filled symbols, had episodes of syncope (n=9) and unexplained palpitations (n=1; B, II:2). Asymptomatic mutation carriers (n=31) are shown as symbols with a dot, and shaded symbols are the individuals with QT prolongation who declined genetic testing or sudden cardiac death victims (SCD; A, II:6 and C, III:1). *Individuals exhibiting ST elevation in the right precordial leads. Values for QTc intervals are given beneath each symbol. The Na-channel provocation test was positive in individuals with + (A, II:1; A, III:8; A, III:9; E, II:3; K, II:1), and negative in the individuals with – (A, II:3; E, I:2; E, II:2; N, II:1). Individuals with a positive and negative Na-channel blocker provocation test are shown as + and –, respectively. (Modified with permission from Makita et al. *J Clin Invest* 2008; **118**: 2219–2229⁴¹)

in the Na channel β -subunit genes, *SCN1B* and *SCN4B*, are associated with type-5 BrS complicated with CCD (BrS5)²⁰ and type-10 LQTS (LQT10), respectively.

Biophysical Properties of Cardiac Sodium Channelopathies

Congenital LQTS is characterized by prolongation of the QT interval on surface ECGs and an increased risk of potentially fatal ventricular arrhythmias, especially torsade de pointes.²¹ The QT interval is determined by the cardiac

action potential, which is orchestrated by a fine balance between inward and outward currents expressed in myocardial cells. After the first identification in 1995 of the *SCN5A* mutation ΔKPQ, comprising a deletion of 3 conserved amino acids, 1505–1507, in the cytoplasmic D3–D4 linker;²² more than 100 distinct *SCN5A* mutations responsible for LQT3 have been reported. The common in vitro consequence of most of these mutations is a persistent Na current during the action potential plateau because of destabilized fast Na channel inactivation.³ This failure of fast inactivation shifts the ionic balance during the plateau phase toward the

Figure 2. ECG characteristics of E1784K mutation carriers. QT prolongation (QTc=470ms) and spontaneous saddle-back type ST elevation observed in the right precordial leads in a carrier (**Figure 1A**, II:1). (**A**) ECG recordings before and after the Na-channel blocker provocation test. Pilsicainide (**Left**, patient K, II:1) induced a coved-type ST elevation in V₁ and the QTc was concomitantly shortened (QTc: control 495 ms, pilsicainide 459 ms). Ajmaline (**Right**, patient A, III:9) also induced a coved-type ST elevation in V₁ and V₂ and QTc shortening (control 501 ms, ajmaline 490 ms). (**B**) SND demonstrated by a 3.9 s sinus arrest in a carrier (A, I:1). (**C**) Venn diagram representing electrophysiological manifestation of 41 *SCN5A*-E1784K mutation carriers: 38 carriers had an abnormally long QTc; 3 individuals had a normal QTc, and 1 exhibited sinus node dysfunction (SND) only. SND and Brugada syndrome were observed in 15 and 9 individuals, respectively, with 4 displaying both phenotypes. (Modified with permission from Makita et al. *J Clin Invest* 2008; **118**: 2219–2229⁴¹)

inward current (gain-of-function) and delays repolarization, thus increasing the action potential duration and the corresponding QT interval. Na channel blockers, such as mexiletine (class IB) or flecainide (class IC), shorten the QT in patients with LQT3 by blocking of this persistent current^{23–25} therefore are theoretically useful in the management of affected patients.

BrS is another primary electrical disorder without underlying structural heart diseases characterized by the coved-type ST elevation in the right precordial leads.^{26,27} It predisposes affected individuals to ventricular fibrillation (VF). Mutations in *SCN5A* are identified in 20–30% patients with BrS, and most of the heterologously expressed mutant Na channels exhibit biophysical abnormalities resulting in reduced cardiac Na current (loss-of-function)²⁸ Reduced Na current is thought to exaggerate differences in the action potential duration between the inner (endocardium) and outer (epicardium) layers of ventricular muscle, thereby favoring substrate promoting reentrant arrhythmias. Loss-of-function of the cardiac Na channels is either owing to (1) haplo-insufficiency because of non-functional mutations, (2) impaired altered channel-gating properties, including enhanced inactivation, disruption of activation and impaired recovery from inactivation, or (3) impaired intracellular trafficking and decreased membrane surface expression of the channel molecules.

Clinical Overlap of Cardiac Sodium Channelopathies

SCN5A mutations with loss-of-function properties have also been identified in patients with CCD^{7,29} SSS⁸ and atrial standstill⁹ and numbers of reports have shown that the mutation carriers tend to exhibit overlapping clinical properties of these syndromes^{30,31} (**Table 2**). Importantly, such loss-of-function properties are apparently opposite to those described in LQT3 (gain-of-function), and different *SCN5A* mutations were initially linked to separate arrhythmias syndromes. Surprisingly, some LQT3 patients display ECG findings characteristic of BrS, suggesting that a single mutation can be associated with a wide spectrum of disease phenotypes. Such phenotypic overlap between LQT3 and BrS was first reported in a large multigenerational Dutch family with an insertion mutation 1795insD, in which the mutation carriers showed ECG features of both LQT3 and BrS, and sinus node dysfunction (SND)^{32,33} Importantly, Na channel block in the overlap phenotype shortens QT, but exacerbated the ST segment elevation BrS phenotype, and thus enhances arrhythmia risk³³ Biophysical studies demonstrated that the mutant channels displayed enhancement of both closed-state inactivation and slow inactivation, which was thought to sensitize carriers to the BrS phenotype during flecainide therapy³⁴ in addition to the persistent Na current, a hallmark Na channel property of LQT3.

The overlap between the LQT3 and BrS phenotypes was also reported in other *SCN5A* mutations such as Δ KPQ^{35,36}

Figure 3. Properties of E1784K whole-cell current. (A) Representative whole-cell current traces obtained from tsA-201 cells transfected with either wild-type (WT) or E1784K Na channels; all studies were conducted in cells co-transfected with human sodium channel $\beta 1$ -subunits. Currents were recorded from a holding potential of -120 mV and stepped from -90 mV to $+90$ mV for 20 ms in 10 mV increments. (B) Current-voltage relationship. Current was normalized to cell capacitance to give a measure of Na current density. (C) Na currents were recorded with a test pulse potential of -20 mV from a holding potential of -120 mV showed prominent tetrodotoxin-sensitive late Na current (shown with arrows) and the faster decay in E1784K. (D) Steady-state availability for fast inactivation and the conductance-voltage relationship were measured with standard pulse protocols shown in the inset. Curves were fit with the Boltzmann equation. The voltage-dependence of steady-state fast inactivation and activation were significantly shifted in the hyperpolarizing (-15.0 mV) and depolarizing ($+12.5$ mV) directions, respectively. (Modified with permission from Makita et al. *J Clin Invest* 2008; 118: 2219–2229⁴¹)

E1784K³⁵ and $\Delta K1500$ ³⁷ Priori et al showed the additional evidence for the elusive link between these 2 clinical syndromes by the fact that the class IC sodium channel blocker flecainide induced ST-segment elevation in the right precordial leads not only in patients with BrS, but also with LQT3³⁵. Of 13 patients in 7 LQT3 families (*SCN5A* mutations of V411M, T1304M, $\Delta K1500$, ΔKPQ , R1626P, E1784K, P2006A), 6 showed flecainide-induced ST elevation. However, they failed to identify the determinants of flecainide-induced ST elevation in patients with LQT3. In fact, they assumed that the clinical overlap appeared to be individual-specific, rather than gene-specific or mutation-specific, most likely because the size of their patients was rather small. Nonetheless, these observations raise a concern about the safety of class IC drug therapy in LQT3 patients and questions about the underlying mechanisms.

Phenotypic variability in LQT3 has thus far been reported sporadically or only within a single kindred^{32,33}. Therefore, it is unclear whether development of the BrS phenotype in a patient with LQT3 is solely determined by the biophysical properties of the mutant channel, or by co-inherited genetic variations, gender, ethnicity, or other environmental factors. One approach to dissecting such phenotypic variability is to perform a clinical assessment of individuals with multiple pedigrees from genetically heterogeneous populations with the same mutation.

Clinical Phenotypes in 15 LQT3 Families With *SCN5A*-E1784K

From 7 institutions in Japan, Italy, Germany, UK and the

US, 44 genotyped LQT3 families with different ethnic backgrounds were enrolled (Asian 20, Caucasian 24). E1784K was the most prevalent *SCN5A* mutation, identified in 15 families (34%). Two probands died suddenly, and 66 of the 93 surviving members underwent genetic testing. There were 41 mutation carriers and 25 non-carriers, and QTc was significantly prolonged in the carriers.

Spontaneous ST elevation in the right precordial leads occurred in 5/41 mutation carriers (Figure 1; coved-type: $n=1$, saddle-back type: $n=4$, Figure 2A). Nine mutation carriers without diagnostic ST elevation at baseline underwent provocation with flecainide, ajmaline, or pilsicainide, and the test was positive (coved-type ST elevation, Figure 2B) in 5 (shown with + in Figure 1). Thus, the diagnosis of BrS was established in 9/41 mutation carriers (1 individual showed spontaneous saddle-back ST elevation which was converted to coved-type by ajmaline).

SND was common in the cohort, presenting in 16/41 mutation carriers (Figure 2C), and 4 of these 16 carriers with SND also exhibited the BrS phenotype (Figures 2B, D). Moreover, 1 carrier showed SND without manifesting QT prolongation or ST elevation. Four patients received a permanent pacemaker and 3 received an implantable cardioverter defibrillator.

Biophysical Properties and Membrane Trafficking of E1784K

Whole-cell patch clamp recording showed that E1784K has the following biophysical abnormalities: (1) significantly smaller peak current density, (2) persistent Na current, (3)

Figure 4. Tonic block, use-dependent block, and the dose-dependence of flecainide. (A) Representative current traces of wild-type (WT), E1784K, and T1304M before and after 10 $\mu\text{mol/L}$ flecainide. A train of 100 pulses (to -20 mV for 20 ms) was applied at 2 Hz from a holding potential of -120 mV . Numbers indicate the 1st (1), 30th (30), and 100th (100) pulse of the train. (B) Time course of the peak current levels after application of 10 $\mu\text{mol/L}$ flecainide. Peak current levels recorded with each pulse were normalized to the baseline prior to flecainide. (C) Representative steady-state current traces of WT and E1784K before and after flecainide (10 and 100 $\mu\text{mol/L}$). Cells were depolarized by -20 mV from a holding potential of -150 mV . (D) Concentration-response curve for flecainide-induced tonic block in wild-type (WT) and E1784K. The normalized peak currents were fitted to the Hill equation. The IC_{50} values, representing dissociation constants for resting state were: WT, 150.3 $\mu\text{mol/L}$; E1784K, 20.4 $\mu\text{mol/L}$. Thus, the mutant channel was far more sensitive to tonic block by flecainide. (Modified with permission from Makita et al. *J Clin Invest* 2008; **118**: 2219–2229⁴¹.)

Table 3. Reported Biophysical Properties of LQT3 Mutations

	SCN5A mutations				
	1795insD	ΔKPQ	ΔK1500	E1784K	T1304M
Clinical features					
QT prolongation	+	+	+	+	+
ST elevation [†]	+	+	+	+	–
Sinus node dysfunction	+	+	+	+	–
Biophysical and pharmacological properties					
Persistent Na current	+	+	+	+	+
Shift of $V_{1/2}$ (inactivation)	Negative	Negative	Negative	Negative	Positive
Shift of $V_{1/2}$ (activation)	Positive	Positive	Positive	Positive	Positive
Current decay	Slower	Faster	$\leftrightarrow$	Faster	Faster
Recovery from inactivation	Slower	Faster	ND	$\leftrightarrow$	Faster
Slow inactivation	Enhanced	$\leftrightarrow$	ND	Enhanced	$\leftrightarrow$
Tonic block by flecainide	Enhanced	Enhanced	Enhanced	Enhanced	$\leftrightarrow$
UDB by flecainide	Enhanced	Enhanced	ND	Diminished	$\leftrightarrow$
References	32, 33, 38, 66	3, 41, 67	37	40, 41	13, 41

[†]Spontaneous or Na-channel blocker-induced ST elevation; $\leftrightarrow$ comparable to wild type.

ND, not determined.

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significantly faster macroscopic current decay, (4) hyperpolarizing shift of the steady-state inactivation, (5) significant depolarizing shift in the voltage-dependence of activation, and (6) normal recovery from inactivation (Figure 3). Furthermore, using a Na channel plasmid construct with an extracellular FLAG epitope, membrane trafficking of E1784K determined by a confocal laser scanning microscopy was comparable to the wild-type (WT). These observations provide strong evidence that the loss-of-function properties displayed by E1784K are most likely attributable

to the aforementioned changes in gating properties rather than a change in channel density.

Molecular Mechanisms of Enhanced Flecainide Sensitivity

Class IC drug challenge test was positive in 56% patients with E1784K. Tonic block and use-dependent block by flecainide in WT and E1784K channels have been investigated, and compared with those of T1304M, a mutation that did not

show ST elevation during flecainide challenge test³⁵ Cells transfected with WT, E1784K, or T1304M were depolarized by 2 Hz pulse trains in the absence or presence of 10 μ M/L flecainide. During exposure to flecainide, peak currents normalized to predrug baseline were progressively reduced by the repetitive pulses (**Figures 4A,B**). There was a remarkable difference in the extent of first pulse (tonic) block that was only $4.5 \pm 4.0\%$ for WT, and $7.1 \pm 2.7\%$ for T1304M, compared with substantial tonic block in E1784K ($43.7 \pm 8.0\%$, $P < 0.001$). Conversely, use-dependent block, determined by the difference in peak current values between the 1st and 100th test pulses relative to the 1st pulse, was slightly attenuated in E1784K. The net effect of flecainide after a train of 100 pulses was significantly greater in E1784K than in WT, but not in T1304M. Moreover, dose-response curves for flecainide block measured at a holding potential of -150 mV (thus representing drug affinity for the resting state) showed that the E1784K channels were 7.5-fold more sensitive to resting-state block by flecainide than were the WT channels (IC_{50} : WT = 150.3μ M/L, E1784K = 20.4μ M/L) (**Figures 4C,D**). These results indicate that the E1784K channels are much more sensitive to blocking by flecainide than are the WT and T1304M channels, and that this augmented sensitivity is attributable to enhanced tonic block rather than a change in use-dependent block.

Functional Determinants of LQT3 Associated With BrS and SND

To explore the functional determinants for the phenotypic overlap of BrS in LQT3 patients, the biophysical and pharmacological properties of reported LQT3 mutations were compared, and features commonly and specifically observed in those manifesting a BrS phenotype were sought (**Table 3**). The overlapping phenotype (LQT3 and BrS) has been previously reported for 1795insD,^{32,33} Δ KPQ,^{35,36} Δ K1500,³⁷ and E1784K.³⁵ In contrast, a carrier of T1304M did not show ST elevation during a flecainide test.³⁵ Similarly, SND has been reported in carriers of the same *SCN5A* mutations, 1795insD,³⁸ Δ KPQ,³⁹ Δ K1500,³⁷ E1784K,⁴⁰ and D1790G²⁴ but not in other *SCN5A* mutations, including T1304M. Thus, it is plausible to speculate that the biophysical characteristics common to these mutations, but not found in T1304M, are channel properties responsible for evoking the mixed phenotypes of BrS and SND in patients with LQT3. **Table 3** is a comparison of the functional properties of E1784K, and those reported for 1795insD, Δ KPQ, Δ K1500, E1784K, and T1304M.¹³ Among the biophysical properties listed in **Table 3**, both the negative-shift in steady-state inactivation, and the enhanced tonic block by flecainide are common to 1795insD, Δ KPQ, Δ K1500, and E1784K, but not to T1304M. This negative shift of inactivation will reduce the availability of the channels at the resting membrane potential, and increase the proportion of inactivated channels in both the open and closed state, reducing the Na current and increasing the sensitivity to Na-channel blockers. A positive shift in activation is another “loss-of-function” property evident in all the mutants, including T1304M, making it less likely that this specific channel property underlies the mixed clinical phenotypes in LQT3. Other channel properties, such as current decay, recovery from inactivation, slow inactivation or use-dependent block, were not common among 1795insD, Δ KPQ, Δ K1500, and E1784K.

A negative shift in inactivation is observed in E1784K, 1795insD, Δ KPQ, and Δ K1500, and may play a role in the

overlap of the LQT3 clinical phenotype with BrS and SND in the mutation carriers, although the number of LQT3 mutations that have been evaluated in this detail is still small, the biophysical and pharmacological properties presented in a cultured cell line may not necessarily reflect the situation in vivo, and the effects of the mutation may be different in ventricular myocytes vs sinus node cells. Further studies that combine clinical and in vitro phenotyping in LQT3 mutations with and without overlapping clinical phenotypes will be required to confirm these findings. Nevertheless, a negative shift in inactivation and enhanced tonic block are common biophysical properties observed among *SCN5A* mutations in the LQT3/BrS overlapping phenotype.

Conclusions

E1784K is the most common LQT3 mutation. In patients with this and other LQT3 mutations, overlap of BrS and SND is relatively common. In vitro studies with E1784K and previous reports of LQT3 mutations with and without this clinical overlap syndrome implicate a negative shift in inactivation and enhanced tonic block by drugs as the underlying mechanisms. These findings suggest that patients with LQT3 mutations displaying these characteristics in vitro should not receive class IC drugs. Furthermore, they reinforce the general concept that in vitro characterization of the function of ion channel variants is a key component in generating specific therapeutic strategies for patient management.

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