KCNE3 T4A as the Genetic Basis of Brugada-Pattern Electrocardiogram

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Background: Brugada syndrome (BrS) is genetically heterogeneous. In Japanese BrS patients, except for *SCN5A* and *KCNE5*, mutations in the responsible genes have not yet been identified, and therefore the genetic heterogeneity remains poorly elucidated.

Methods and Results: Forty consecutive patients with Brugada-pattern electrocardiogram (ECG) underwent comprehensive genetic analysis of BrS-causing genes including *SCN5A*, *SCN1B*, *SCN3B*, *CACNA1C*, *CACNB2*, *KCNE3* and *KCNE5*. Besides identifying 8 *SCN5A* mutations in the present cohort, a *KCNE3* T4A mutation was found in a 55-year-old male patient who had experienced several episodes of syncope. A head-up tilt test during passive tilt provoked both hypotension and bradycardia, followed by syncope. He was therefore diagnosed with neurally mediated syncope (NMS). To characterize the functional consequence of the mutant, electrophysiological experiments using whole-cell patch-clamp methods and computer simulations using human right ventricular wall model were carried out. It was found that *KCNE3* T4A increased I_{to} recapitulated by heterologously coexpressing Kv4.3+KChIP2b+*KCNE3*-wild type or *KCNE3*-T4A in CHO cells.

Conclusions: A *KCNE3* T4A mutation was identified in a Japanese patient presenting Brugada-pattern ECG and NMS. Its functional consequence was the gain of function of I_{to} , which could underlie the pathogenesis of Brugada-pattern ECG. The data provide novel insights into the genetic basis of Japanese BrS. (*Circ J* 2012; **76**: 2763–2772)

Key Words: Brugada syndrome; I_{to} ; *KCNE3*; Mutation; Neurally mediated syncope

Brugada syndrome (BrS) is a hereditary disorder characterized by ST-segment elevations in the right precordial electrocardiogram (ECG) leads, associated with a high incidence of syncope or sudden death due to ventricular tachyarrhythmias, which mostly affects men. BrS is genetically heterogeneous, and has been linked to mutations in genes that perturb cardiac ion currents (I_{Na} , I_{Ca} , I_{K-ATP} and I_{to}) contributing to the early phase of action potential (AP).^{1–12} Among the BrS-causing genes, mutations in *SCN5A* (encoding the pore-forming α -subunit of the cardiac voltage-gated sodium channel) have accounted for the major form of BrS in approximately 20% of cases, and other gene mutations for approximately 10%,^{1–12} thus around 70% of BrS cases remain to be genetically

elucidated.

The transient outward potassium current (I_{to}) in the heart functions mainly during the early phase of AP because it activates and inactivates rapidly on membrane depolarization. Predominant expression of I_{to} in ventricular epimyocardium compared to endomyocardium, especially in the right ventricle, contributes to the rapid repolarization and the initial plateau formation of the AP (AP notch).^{13–15} Experimental studies suggested that the gain of function of I_{to} leads to augmentation of the AP notch in epimyocardium but not in endomyocardium, thus resulting in the enhancement of transmural voltage gradient during the ventricular repolarization, which is thought to be responsible for the ST-segment elevation in the right pre-

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cordial ECG leads (Brugada-pattern ECG) in BrS.¹⁴ Further augmentation of the AP notch in epimyocardium causes the loss of AP plateau phase (dome), which consequently leads to arrhythmogenesis in BrS defined as phase 2 reentry.¹⁴ I_{to} in human ventricle is thought to be consist of α -subunit Kv4.3 (encoded by *KCND3*) and β -subunits including Kv channel interacting protein (KChIP), KCNE, diaminopeptidyl transferase-like protein (DPP) and Kv β families.^{16–24} Accordingly, mutations in the related genes that increase I_{to} may underlie the pathogenesis of BrS.

KCNE3, 1 member of the *KCNE* gene family (*KCNE1–5*), modulates the function of Kv4.3 as an inhibitory β -subunit.^{19,20} Recently, Delpón et al identified a *KCNE3* R99H mutation in 1 BrS family.⁶ Functional analysis using the heterologous expression system that recapitulates I_{to} by coexpression of Kv4.3 with *KCNE3*-wild type (WT)/R99H showed that *KCNE3* R99H causes a gain of function of I_{to} by a dominant-positive effect, thus precipitating the development of BrS.⁶ Moreover, mutations in Kv4.3 and *KCNE5*, which also functions as an inhibitory β -subunit of I_{to} , were identified in BrS patients.^{9,10} Functional analysis of these mutations showed that they increase I_{to} .^{9,10} Therefore, it was established that the gain of function of I_{to} by mutations in genes that encode I_{to} could be one of the causes of BrS.

We have previously reported the identification of 8 mutations in *SCN5A* among 30 consecutive Japanese patients with Brugada-pattern ECG.²⁵ Considering that the genetic heterogeneity of BrS is poorly elucidated in Japan,^{10,26,27} we further conducted genetic screening of BrS-causing genes among the 30 consecutive patients and another 10 new patients with Brugada-pattern ECG, and identified a *KCNE3* T4A mutation in a patient presenting with neurally mediated syncope (NMS). In the present study, we describe the clinical phenotype of the *KCNE3* T4A carrier, and characterize the functional consequence of the I_{to} recapitulated by heterologously coexpressing Kv4.3 + KChIP2b + *KCNE3*-T4A in CHO cells. Furthermore, we performed computer simulations based on the I_{to} obtained in electrophysiological recordings, and showed that *KCNE3*-T4A recapitulated the ECG phenotype.

Methods

Subjects

The present subjects were 40 consecutive patients (probands; 35 male, 47±16 years of age) with Brugada-pattern ECG who were referred to Gunma University Hospital between April 2002 and September 2010. All patients, except for patient 27, presented with coved-type ST-elevation in the right precordial ECG leads with or without provocation of Na channel blocker (pilsicainide: 1 mg/kg, or procainamide 5 mg/kg), although it is still under debate whether patients with drug-induced Brugada-pattern ECG have poor prognosis.^{28,29} Echocardiography and conventional left catheterization, if performed, indicated no structural heart disease in all the patients. Thirty-two patients underwent electrophysiological assessment. Up to 3 extra stimuli (minimum coupling interval: 180 ms) were delivered from 2 ventricular sites (right ventricular apex and right ventricular outflow tract). A head-up tilt (HUT) test was performed using the same protocol as described previously.³⁰ Clinical features of the subjects are listed in Table 1.

Genetic Analysis

After obtaining appropriate approval from the institution review board and written informed consent from the patient, genomic DNA was extracted from peripheral blood lymphocytes

using the standard protocol of the QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany). All coding exons of *SCN5A*, *SCN1B*, *KCNE3*, *SCN3B* and *KCNE5*, and their splice sites were amplified on polymerase chain reaction (PCR) using primers flanking the intronic sequences as reported previously.^{1,5–7,25,31} The PCR products were purified and directly sequenced using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Regarding patient 18, *CACNA1C* and *CACNB2* were also analyzed.⁴ The mutation was analyzed twice on independent PCR amplification and sequencing. *KCNE3* T4A was not identified in 528 control alleles.

Heterologous Expression of hKv4.3 and β -Subunits in CHO Cells

Full-length cDNA fragment of WT *KCNE3* in pCR3.1 vector was subcloned into pIRES-CD8 vector that is useful in cell selection. The *KCNE3* mutant (T4A) was constructed using a Quick Change II XL site-directed mutagenesis kit according to the manufacturer's instructions (Stratagene, La Jolla, CA, USA) and subcloned to the same vector. The *KCNE3* mutant was fully sequenced (ABI PRISM 3130 Genetic analyzer) to ensure fidelity. Full-length cDNA encoding the short isoform of human Kv4.3 (hKv4.3) subcloned into the pIRES-GFP (Clontech, Palo Alto, CA, USA) expression vector was kindly provided by Dr GF Tomaselli (Johns Hopkins University). Full-length cDNA encoding Kv channel-interacting protein 2b (KChIP2b) subcloned into the PCMV-IRS expression vector was a kind gift from Dr GN Tseng (Virginia Commonwealth University). Kv4.3 was transiently transfected into CHO cells together with KChIP2b and *KCNE3*-WT (or T4A) cDNA at equimolar ratio (Kv4.3, 1.0 μ g; KChIP2b, 1.0 μ g; *KCNE3*, 1.0 μ g) using Lipofectamine (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. In a subset of experiments, 0.5 μ g *KCNE3*-WT and 0.5 μ g *KCNE3*-T4A were co-transfected into cells with 1.0 μ g Kv4.3 and 1.0 μ g KChIP2b. The transfected cells were then cultured in Ham's F-12 medium (Nakalai Tesque, Kyoto, Japan) as described previously.²⁴

Electrophysiologic Recording and Data Analysis

After 48 h of transfection, a coverslip with cells was transferred to a 0.5-ml bath chamber at 25°C on an inverted microscope stage and perfused at 1–2 ml/min with extracellular solution containing the following (in mmol/L): 140 NaCl, 5.4 KCl, 1.8 CaCl₂, 0.5 MgCl₂, 0.33 NaH₂PO₄, 5.5 glucose, and 5.0 HEPES; pH 7.4 with NaOH. Cells that emitted green fluorescence were chosen for patch-clamp experiments. If coexpressed with *KCNE3* (or its mutant), the cells were incubated with polystyrene microbeads precoated with anti-CD8 antibody (Dynabeads M450, Dynal, Norway) for 15 min. In these cases, cells that emitted green fluorescence and had attached beads were chosen for electrophysiologic recording. Whole-cell membrane currents were recorded with an EPC-8 patch-clamp amplifier (HEKA, Lambrecht, Germany), and data were low-pass filtered at 1 kHz, acquired at 5 kHz through an LIH-1600 analog-to-digital converter (HEKA), and stored on hard disk using PulseFit software (HEKA). Patch pipettes had a resistance of 2.5–5.0 mol/L Ω when filled with the following pipette solution (in mmol/L): 70.0 potassium aspartate, 50.0 KCl, 10.0 KH₂PO₄, 1.0 MgSO₄, 3.0 Na₂-ATP (Sigma, Japan, Tokyo), 0.1 Li₂-GTP (Roche Diagnostics, Mannheim, Germany), 5.0 EGTA, and 5.0 HEPES (pH 7.2).

Whole cell currents were elicited in a series of depolarizing voltage steps from a holding potential of –80 mV. The time

Table 1. Clinical Features and Genetic Analysis in Brugada-Pattern ECG Patients

Patient no.	Sex	Age (years)	ECG	Symptom	FH	EPS	ICD	SCN5A	SCN1B	SCN3B	KCNE3	KCNE5
1	M	49	Coved (P)	Sy	No	VF	Yes	c.393-1 c>t**				
2	F	58	Coved	Asy	No	N/A	No	R1193Q				
3	M	36	Coved (P)	Asy	No	VF	No	A586T**				
4	M	29	Coved (P)	Sy	No	VF	Yes		L210P, S248R, R250T			
5	M	56	Coved	CPA	No	VF	Yes		L210P			
6	M	30	Coved	CPA	No	VF	Yes					
7	M	42	Coved (P)	Sy	No	No VF	Yes					
8	M	53	Coved (P)	CPA	No	No VF	Yes		R187H, L210P			
9	M	45	Coved	CPA	No	VF	Yes	P1090L	L210P, S248R, R250T			
10	M	47	Coved (P)	CPA	No	VF	Yes		L210P			
11	M	39	Coved (P)	Asy	No	NSVT	No		L210P			
12	M	32	Coved	Asy	Yes	N/A	Yes		L210P, S248R, R250T			
13	M	42	Coved (Pro)	Sy	No	VF	Yes	P1090L, R1232W				
14	M	59	Coved (P)	Asy	No	No VF	No	c.4437+5 g>a				
15	M	64	Coved	Asy	No	N/A	No		L210P*, S248R*, R250T*			
16	M	39	Coved (P)	Asy	No	NSVT	No					
17	M	61	Coved (P)	Asy	Yes	NSVT	No	R689H**				
18	M	55	Coved (P)	Sy	No	NSVT	No				T4A**	
19	M	37	Coved (P)	Sy	Yes	VF	Yes		L210P, S248R, R250T			
20	M	49	Coved (P)	Asy	No	No VF	No	H558R*, S1553R**				
21	F	38	Coved (P)	Asy	No	N/A	No					
22	M	64	Coved (P)	Asy	Yes	VF	Yes	H558R, R1193Q	L210P, S248R, R250T			
23	M	71	Coved (P)	CPA	No	No VF	Yes	P1090L	L210P*, S248R*, R250T*			
24	M	44	Coved (P)	Sy	Yes	VF	Yes	E1784K**				
25	M	56	Coved (P)	Sy	No	VF	Yes		L210P, S248R, R250T			
26	M	15	Coved	Sy	No	N/A	No		L210P*, S248R, R250T			
27	M	25	Saddle-back	Sy	Yes	No VF	Yes	V1951M**				
28	F	67	Coved (P)	Asy	No	N/A	No					
29	F	28	Coved	Asy	No	N/A	No	Q1706H**				
30	M	38	Coved	Sy	No	NSVT	No					
31	M	30	Coved (P)	Asy	No	No VF	No					
32	M	63	Coved (P)	Asy	No	NSVT	No		L210P, S248R, R250T			
33	M	43	Coved	Asy	No	VF	No					
34	M	57	Coved	Asy	No	No VF	No		L210P, S248R, R250T			
35	M	40	Coved (P)	Sy	No	VF	Yes		L210P, S248R, R250T			
36	M	66	Coved (P)	Sy	No	VF	Yes		L210P			
37	M	72	Coved (P)	Asy	No	VF	No		L210P, S248R, R250T			
38	M	33	Coved	Asy	No	NSVT	No					
39	M	77	Coved	Asy	No	VF	No		L210P, S248R, R250T			
40	F	15	Coved (P)	CPA	No	N/A	Yes	A735E**				

Mutations and non-synonymous variants are listed. *Homozygous; **mutations.

Asy, asymptomatic; CPA, cardiopulmonary arrest; ECG, electrocardiogram; EPS, up to 3 extrastimuli from right ventricular apex and right ventricular outflow tract; FH, family history of sudden death under age 45; N/A, not accessed; NSVT, non-sustained ventricular tachycardia; P, pilsicainide provocation; Pro, procainamide provocation; Sy, syncope; VF, ventricular fibrillation.

Figure 1. (A) Direct sequencing of the *KCNE3* gene in the index patient. Nucleotide and amino acid substitutions are indicated below. (B) Twelve-lead ECG recorded 2 months before admission. (C) V₁₋₃ lead electrocardiogram before (baseline) and after pilsicainide provocation (Pilsicainide). ics, intercostal space.

interval between each voltage pulse was 10 s. The difference between the peak current amplitude and the current at the end of a test pulse was referred to as the transient outward current. To control for cell size variability, currents are expressed as densities (pA/pF) as described previously.²⁴ Steady-state activation curves were obtained by plotting the normalized conductance as a function of peak outward potentials. Steady-state inactivation curves were generated by a standard 2-pulse protocol with a conditioning pulse of 500 ms and obtained by plotting the normalized current as a function of the test potential. Steady-state inactivation/activation kinetics were fitted to the following Boltzmann equation:

$$Y(V) = 1 / (1 + \exp[(V_{1/2} - V)/k]),$$

where Y = normalized conductance or current, $V_{1/2}$ = potential for half-maximum inactivation or activation, respectively, and k = slope factor.

Data relative to inactivation time constants, time to peak, and mean current levels were obtained using current data recorded at +50 mV. Recovery from inactivation was assessed using a standard paired-pulse protocol: a 1-s test pulse to +50 mV (P1) followed by a variable recovery interval at -80 mV and then a second test pulse to +50 mV (P2). Both the inactivation time constants and the time constant for recovery from inactivation were determined by fitting the data to a single exponential:

$$I(t) \text{ (or } P2/P1) = A + B \exp(-t/\tau),$$

where I(t) = current amplitude at time t, A and B = constants, and τ = inactivation time constant or time constant for recovery from inactivation. For measurement of recovery from inactivation, the plot of P2/P1 instead of I(t) was used.

All data are given as mean $\pm$ SEM. Statistical comparisons between 2 groups were analyzed using Student's unpaired t-test. Comparisons among multiple groups were analyzed using

analysis of variance followed by Dunnett test. $P < 0.05$ was considered significant.

Computer Simulation

To confirm the exact role of the *KCNE3* T4A mutation, we conducted simulations of paced propagation in a 0.5-cm 1-D bidomain myocardial model with transverse conductivity, mimicking transmural section of right ventricular wall. Membrane kinetics were represented by the Priebe-Beuckelmann human ventricular model,³² of which original I_{to} was replaced by the I_{to} with *KCNE3*-WT or *KCNE3*-T4A mutation obtained in electrophysiologic recording.

To obtain the transmural gradient in the right ventricular wall, we defined endocardial and epicardial tissues as each of length 0.25 cm, and we set the conductances of the slowly activating component of the delayed rectifier potassium channel (I_{Ks}), the inward rectifier potassium channel (I_{K1}), and I_{to} in the endocardial layers to 46%, 82%, and 29%, respectively, of those in the epicardial layers. Pacing stimuli of 2 ms and strength twice-diastolic threshold were applied transmurally to the endocardial end at a cycle length of 1,000 ms. The time and spatial discretization steps were 10 μ s and 50 μ m, respectively. Other model parameters and the numerical approach have been described elsewhere.³³

Results

Genetic Analysis

We conducted comprehensive genetic analysis of BrS-causing genes including *SCN5A*, *SCN1B*, *KCNE3*, *SCN3B* and *KCNE5*, among 40 consecutive patients with Brugada-pattern ECG. Besides identifying 8 *SCN5A* mutations in the present cohort, we also found a T4A mutation in *KCNE3* in the patient 18 who had no mutations in the other genes (including *CACNA1C* and *CACNB2*) associated with BrS (Figure 1A; Table 1).

Figure 2. KCNE3-T4A increased I_0 current reconstituted by coexpression of 1 μ g Kv4.3 + 1 μ g KChIP2b + 1 μ g KCNE3 in CHO cells. (A) Representative current traces recorded from cells expressing (Left) Kv4.3 + KChIP2b, (Middle) Kv4.3 + KChIP2b + KCNE3-WT and (Right) Kv4.3 + KChIP2b + KCNE3-T4A. Inset, depolarizing test pulses introduced from -80 mV holding potential to potentials ranging from -40 to $+50$ mV, with 300-ms duration, in 10-mV increments. (B) Peak current density of I_0 , (C) time to peak of activation course and (D) inactivation time constants recorded at $+50$ mV. (n), no. of experiments. * $P < 0.05$ vs. WT.

Clinical Presentation

A 55-year-old man (patient 18) was referred to hospital to examine the cause of syncope. He had experienced several episodes of syncope under specific conditions, such as when sitting at a funeral, and standing up after drinking alcohol, since his 30s. He had no previous history of illness except for syncope episodes, and no family history of sudden cardiac death. A physical examination, chest X-ray, and blood test showed no remarkable abnormalities. His 12-lead ECG, recorded 2 months before admission, showed saddle-back-type ST-segment elevation in the right precordial ECG leads (Figure 1B). The QTc interval was 414 ms. A coved-type ST-segment elevation in the right precordial ECG leads at the second intercostal space appeared after provocation with pilsicainide (Figure 1C). Signal-averaged ECG showed no late potentials. Transthoracic echocardiography showed no apparent structural heart disease.

The patient underwent electrophysiological assessment. Up to 3 extrastimuli induced non-sustained polymorphic ventricular tachycardia, but not ventricular fibrillation. An HUT test was performed because syncopal episodes had occurred under specific conditions that could evoke NMS. The HUT test during passive tilt provoked both hypotension and bradycardia, followed by syncope. Therefore, the patient was diagnosed as having NMS. The patient was not prescribed medication or implanted with an implantable cardioverter defibrillator.

KCNE3-T4A Mutation Increased the Current Amplitude of the Kv4.3 + KChIP2b + KCNE3 Channel

Because KCNE3 was shown to co-associate with Kv4.3 in the human heart, and I_0 is thought to underlie the development of Brugada phenotype,^{6,15} KCNE3-WT/T4A was coexpressed together with Kv4.3 and KChIP2b, which has been shown to serve as a principal β -subunit of I_0 .^{18,19,34,35} Figure 2A shows representative whole-cell current traces recorded from CHO cells expressing Kv4.3 + KChIP2b, Kv4.3 + KChIP2b + KCNE3-WT and Kv4.3 + KChIP2b + KCNE3-T4A.

Consistent with the previous literature, coexpression of KCNE3-WT dramatically reduced the current amplitude of the Kv4.3 + KChIP2b channel.^{6,20,24} Further analysis of peak current showed that the current density of the coexpression with the KCNE3-T4A mutant was significantly larger than that for KCNE3-WT (Kv4.3 + KChIP2b + KCNE3-WT, 51.7 ± 7.3 pA/pF, $n=25$ vs. Kv4.3 + KChIP2b + KCNE3-T4A, 80.2 ± 9.1 pA/pF, $n=22$, $P < 0.05$; Figure 2A,B). Figure 2C shows mean time interval from the onset of the pulse to maximum current (time to peak), and Figure 2D shows the time constants (τ) of inactivation obtained using a single exponential at $+50$ mV. These data indicate that KCNE3-T4A mutation increased the current amplitude of the Kv4.3 + KChIP2b + KCNE3 channel, but did not affect time to peak and the inactivation time course.

Effect of KCNE3-T4A on Activation/inactivation Kinetics of Kv4.3 + KChIP2b + KCNE3 Channels

To further examine the effect of KCNE3-T4A on gating kinetics,

Table 2. Current Density and Biophysical Kinetics								
	Current density	Activation	Steady-state activation		Steady-state inactivation		Inactivation	Recovery from inactivation
	At +50 mV (pA/pF)	Time to peak (ms)	V _{1/2} (mV)	k (mV)	V _{1/2} (mV)	k (mV)	τ (ms)	τ (ms)
Kv4.3+KChIP2b+KCNE3-WT	51.7 $\pm$ 7.3 (n=25)	6.6 $\pm$ 0.7 (n=25)	–6.5 $\pm$ 2.0 (n=21)	17.2 $\pm$ 1.1 (n=21)	–52.7 $\pm$ 1.7 (n=22)	–9.9 $\pm$ 1.7 (n=22)	73.0 $\pm$ 4.2 (n=25)	65.7 $\pm$ 10.2 (n=9)
Kv4.3+KChIP2b+KCNE3-T4A	80.2 $\pm$ 9.1 [†] (n=22)	6.0 $\pm$ 0.6 (n=22)	–11.6 $\pm$ 1.2 [†] (n=19)	14.2 $\pm$ 0.8 (n=19)	–54.7 $\pm$ 1.6 (n=15)	–8.8 $\pm$ 2.1 (n=15)	67.1 $\pm$ 4.1 (n=22)	66.9 $\pm$ 10.7 (n=9)
Kv4.3+KChIP2b+KCNE3-(WT+T4A)	77.7 $\pm$ 8.6 [†] (n=11)	6.7 $\pm$ 0.8 (n=11)	–7.2 $\pm$ 1.1 (n=10)	15.4 $\pm$ 0.8 (n=10)	–52.2 $\pm$ 2.0 (n=10)	–6.1 $\pm$ 1.1 (n=10)	64.3 $\pm$ 9.8 (n=11)	72.8 $\pm$ 12.6 (n=6)

[†] P <0.05 vs. Kv4.3+KChIP2b+KCNE3-WT.

ics of Kv4.3 + KChIP2b + KCNE3 channel, we assessed the current-voltage (*I*-*V*) relationship of the Kv4.3 + KChIP2b + KCNE3-T4A and Kv4.3 + KChIP2b + KCNE3-WT + KCNE3-T4A channels. Coexpression of KCNE3-T4A (at –20 to +50 mV) or KCNE3-WT + KCNE3-T4A (at 0 to +50 mV) significantly increased peak current densities (**Figure 3A**; **Table 2**). Meanwhile, coexpression of KCNE3-T4A, but not KCNE3-(T4A + WT), also caused a negative shift (approximately –5 mV) of voltage dependence of steady-state activation as assessed by plotting the normalized conductance as a function of test potentials (**Figure 3B**; **Table 2**).

Figure 3C shows the representative current traces elicited by a double-step pulse method (inset) used to evaluate steady-state inactivation. Peak currents recorded at various levels of pre-pulse (**Figure 3C**) were normalized by that measured after a 500-ms pre-pulse at –90 mV and plotted as a function of pre-pulse test potentials (**Figure 3D**). Coexpression of KCNE3-T4A or KCNE3-(WT + T4A) with Kv4.3 + KChIP2b did not significantly modify the steady-state inactivation of Kv4.3 + KChIP2b + KCNE3 channels (**Figure 3D**; **Table 2**).

Because the changes in the time course of reactivation can also affect *I*_{to} current, a double-pulse protocol (**Figure 4A**) was used to test the effect of KCNE3-T4A or KCNE3-(WT + T4A) coexpression on the time course for recovery from inactivation. **Figures 4A,B** shows the representative current traces for coexpression of KCNE3-WT and KCNE3-T4A. **Figure 4C** shows the time courses of recovery of KCNE3-WT, KCNE3-T4A and KCNE3-(WT + T4A) coexpression together with

Kv4.3 + KChIP2b. Time constants (τ) of recovery from inactivation are listed in **Table 2**. Coexpression of KCNE3-T4A or KCNE3-(WT + T4A) did not affect the time course of recovery from inactivation.

Phenotype of KCNE3-T4A Mutation in a Simulated Human Right Ventricular Wall Model

To clarify whether the gain of function of *I*_{to} resulting from KCNE3-T4A mutation is indeed responsible for the Brugada-pattern ECG, we performed computer simulations using the 1-D myocardial model of human right ventricular wall. Based on the *I*_{to} obtained in electrophysiologic recording (**Figures 3A,B,D**), we numerically reproduced the current-voltage relationship curves (**Figure 5A**), the normalized conductance-voltage relationship curves (**Figure 5B**), and the steady-state inactivation curves (**Figure 5C**) of the KCNE3-WT and KCNE3-T4A channels. The numerically reproduced *I*_{to} was incorporated into the 1-D right ventricular wall model, consisting of endocardial and epicardial layers (**Figure 5D**). We found that the difference in the phase 2 AP between endocardial and epicardial layers in the KCNE3-T4A model was larger than that in the KCNE3-WT model, and therefore the simulated right precordial ECG showed ST-segment elevation in the case of KCNE3-T4A (**Figure 5E**).

To examine the adequacy of the KCNE3-T4A model, we additionally conducted simulations with sodium channel blockade. In the case of KCNE3-WT (**Figure 5F**), because sodium channel block (*I*_{Na} 50%) did not increase the difference in phase

Figure 5. (A–C) Numerically reproduced (A) current-voltage relationship curves, (B) normalized conductance-voltage relationship curves, and (C) steady-state inactivation curves of KCNE3-WT and KCNE3-T4A models. (D) One-dimensional bidomain myocardial computer model, representing the electrical behaviors of the right ventricular wall. The sites for pacing and recording action potential (AP) and electrocardiogram (ECG) are shown. (E) Influence of KCNE3-T4A mutation on both AP and ECG. Endo, endocardial layer; Epi, epicardial layer. (F) Effect of sodium channel blockade (I_{Na} 50%) on both AP and ECG in KCNE3-WT and KCNE3-T4A cases.

1 AP between endocardial and epicardial layers, no ST-segment elevation was observed in the simulated ECG. In contrast, in the case of KCNE3-T4A (Figure 5F), the same sodium channel block caused the loss of dome of AP in the epicardial layer rather than the endocardial layer, and therefore the simulated ECG showed marked ST-segment elevation, consistent with clinical observation (Figure 1C).

Discussion

We identified a *KCNE3* T4A mutation among 40 consecutive patients with Brugada-pattern ECG. The index patient had experienced several episodes of syncope, possibly due to NMS rather than BrS-related ventricular tachyarrhythmias.

The gain of function of I_{to} by mutations in genes that encode I_{to} had been thought to cause BrS.^{14,15} Indeed, an R99H mutation in *KCNE3* was recently identified in 1 BrS family.⁶ Functional analysis of I_{to} reconstituted by Kv4.3/KCNE3-R99H showed a gain of function of the channel with a dominant-positive effect. Moreover, the mutation was co-segregated

with ECG phenotype.⁶ Therefore, the mutation underlies the development of BrS. *KCNE3* T4A, the variant identified in the present cohort, could be a candidate for another *KCNE3* mutation that is associated with BrS.

We reconstituted I_{to} by coexpressing Kv4.3 with KCNE3 + KChIP2b in CHO cells, and examined the functional consequence of KCNE3-T4A, because KChIP2 associates with Kv4 and increases Kv4 current with the increase of surface expression of Kv4, by facilitating the trafficking of the channel and the kinetic changes that more resemble native I_{to} .³⁵ KCNE3-T4A also increased I_{to} with a dominant-positive effect. Moreover, computer simulations based on the I_{to} obtained in electrophysiological recordings demonstrated that KCNE3-T4A recapitulated the ECG phenotype in the present case. These results indicate that *KCNE3* T4A could underlie the pathogenesis of Brugada-pattern ECG. Although it may be required to demonstrate a co-segregation of *KCNE3* T4A with ECG phenotype to validate that the *KCNE3* T4A is associated with Brugada-pattern ECG, we unfortunately could not investigate the patient's family members because of lack of consent.

The precise molecular mechanisms of the reverse of KCNE3-induced suppression of Kv4.3 by KCNE3-T4A remain to be elucidated. Delpón et al demonstrated, using coimmunoprecipitation techniques, that KCNE3 coassociates with Kv4.3 in human atrial myocardium and rat ventricular myocardium.⁶ In addition, Lundby and Olesen reported that KCNE3 has an inhibitory effect on Kv4.3 independent of the presence of KChIP2 in a heterologous expression system.²⁰ Therefore, although we recapitulated I_{to} in the presence of KChIP2b, KCNE3-T4A might reverse the KCNE3-induced suppression of Kv4.3 in the absence of KChIP2b, as is the case with KCNE3-R99H reported by Delpón et al.⁶ Interestingly, Lundby and Olesen also reported that delayed injection of KCNE3 into *Xenopus* oocytes can almost completely inhibit Kv4.3 current, suggesting that KCNE3 transcription can act as a regulatory mechanism of the Kv4.3 current density.²⁰ Further studies of the transcription, trafficking and turnover rate of K4.3 channel and channel complexes in the presence or absence of KChIP2b and KCNE3-WT/T4A, would be required to elucidate the mechanisms of the reverse of KCNE3-induced suppression of I_{to} by KCNE3-T4A.

Yokokawa et al reported that 35% of patients with Brugada-pattern ECG had positive responses during the HUT test,³⁰ demonstrating a high prevalence of NMS among individuals with Brugada-pattern ECG. Although the autonomic nervous system may play an important role in the development of both BrS and NMS, the precise pathophysiological link between BrS and NMS remains to be elucidated. The identification of a novel *SCN5A* Q55X mutation in a patient with Brugada-pattern ECG presenting as NMS and the expression of *SCN5A* in both myocardial cells and intracardiac ganglia raise the possibility of a genetic association between BrS and NMS.^{36,37} *KCNE3* (MiRP2) is also expressed in not only the heart but also the central nervous system, and it modulates delayed rectifier currents in mammalian neurons by forming native channel complexes with Kv2.1 and Kv3.1b.³⁸ Taken together, *KCNE3* T4A may be associated with both phenotypes of BrS and NMS.

In contrast, we have previously reported that *KCNE3* mutations (R99H and T4A) are associated with long QT syndrome (LQTS).³⁹ A functional analysis of KCNQ1 + KCNE3-R99H coexpression demonstrated a reduction of the repolarizing potassium current, thus supporting the proposition that *KCNE3* R99H could be a cause of LQTS. A functional analysis of KCNQ1 + KCNE3-T4A, however, could not demonstrate significant functional abnormalities. Regarding the present case, the QTc interval was not prolonged. Therefore, further studies would be necessary to establish the association between *KCNE3* T4A and LQTS. Along with this line, 2 *KCNE3* T4A carriers we previously reported had no apparent spontaneous ST-segment elevation in the right precordial ECG leads.³⁹ One carrier was a 16-year-old boy, much younger than most affected patients, and another was an old woman. Pharmacological provocation tests were not performed to study whether they had Brugada-pattern ECG.

Conclusions

We identified a *KCNE3* T4A mutation in a Japanese patient with Brugada-pattern ECG presenting as NMS. Its functional consequence was the gain of function of I_{to}, which could underlie the pathogenesis of Brugada-pattern ECG. The data provide novel insight into the genetic basis of Japanese BrS. Further studies are required to clarify whether the *KCNE3* T4A mutation is also associated with NMS and/or LQTS.

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Disclosures

Conflict of interest to declare: None.

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