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# A type 2 ryanodine receptor variant associated with reduced Ca<sup>2+</sup> release and short-coupled torsades de pointes ventricular arrhythmia



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**BACKGROUND** Ventricular fibrillation may be caused by premature ventricular contractions (PVCs) whose coupling intervals are < 300 ms, a characteristic of the short-coupled variant of torsades de pointes (scTdP).

**OBJECTIVE** The purpose of this study was to analyze the underlying cardiac ryanodine receptor (*RyR2*) variants in patients with scTdP.

**METHODS** Seven patients with scTdP (mean age 34 ± 12 years; 4 men and 3 women) were enrolled in this study. The *RyR2* gene was screened by targeted gene sequencing methods; variant minor allele frequency was confirmed in 3 databases; and the pathogenicity was investigated in silico analysis using multiple tools. The activity of wild-type and mutant *RyR2* channels was evaluated by monitoring Ca<sup>2+</sup> signals of HEK293 cells with a [<sup>3</sup>H]ryanodine binding assay.

**RESULTS** The mean coupling interval of PVCs was 282 ± 13 ms. The 12-lead electrocardiogram had no specific findings except PVCs with an extremely short-coupling interval. Genetic analysis revealed 3

novel *RyR2* variants and 1 polymorphism, all located in the cytoplasmic region. p.Ser4938Phe was not detected in 3 databases, and in silico analysis indicated its pathogenicity. In functional analysis, p.Ser4938Phe demonstrated loss of function and impaired *RyR2* channel Ca<sup>2+</sup> release, while 2 other variants, p.Val1024Ile and p.Ala2673Val, had mild gain-of-function effects but were similar to the polymorphism p.Asn1551Ser.

**CONCLUSION** We identified an *RyR2* variant associated with reduced Ca<sup>2+</sup> release and short-coupled torsades de pointes ventricular arrhythmia. The mechanisms of arrhythmogenesis remain unclear.

**KEYWORDS** Torsades de pointes; Catecholaminergic polymorphic ventricular tachycardia; Idiopathic ventricular fibrillation; *RyR2*; Long QT

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## Background

During the eighties and nineties, several case reports described a different form of polymorphic ventricular tachycardia (VT) triggered by premature ventricular contractions (PVCs) with an extremely short-coupling interval.<sup>1–3</sup> This specific form of ventricular arrhythmia was named the *short-coupled variant of torsades de pointes* (scTdP) and now constitutes another type of idiopathic ventricular fibrillation (IVF) without structural heart disease.<sup>4</sup>

The underlying molecular pathogenesis defining the arrhythmogenicity of this disorder has been partially elucidated. In 2011, we reported a case with a heterozygous *KCNE5* Y81H variant (case 5 in Table 1)<sup>5</sup> after the diagnosis of scTdP 2 years after receiving an implantable cardioverter-defibrillator (ICD) because it recorded the initiation of fatal arrhythmias. Functional assays revealed that the variant caused a gain-of-function effect on reconstituted transient outward currents when coexpressed with Kv4.3 and Kv channel interacting protein (KChIP2).<sup>5</sup> After treatment with oral verapamil, the patient remained free from ventricular fibrillation (VF) recurrence. Then 2 years later, Wilde's group<sup>6</sup> reported a mutation of *DPP6* in an IVF family with extremely short-coupling interval PVCs and demonstrated that *DPP6* plays an important role in IVF arrhythmogenicity by affecting transient outward currents in Purkinje fibers. These studies suggest that scTdP may be associated with Purkinje network and intracellular Ca<sup>2+</sup> handling.

scTdP is electrocardiographically defined by (1) PVCs with a short-coupling interval, (2) relatively narrow QRS durations, and (3) normal corrected QT intervals.<sup>7</sup> One protein that helps maintain healthy intracellular Ca<sup>2+</sup> dynamics is the cardiac ryanodine receptor (*RyR2*), encoded by the *RyR2* gene, which is Ca<sup>2+</sup>-releasing channel expressed at sarcoplasmic reticulum membranes.<sup>8</sup> Therefore, *RyR2* malfunction may be related to scTdP-type arrhythmias. *RyR2* mutations are generally known to cause catecholaminergic polymorphic ventricular tachycardia (CPVT),<sup>9</sup> which is also related to abnormal intracellular Ca<sup>2+</sup> handling but recently an *RyR2* mutation (p.His29Asp) was reported to be associated with scTdP.<sup>10</sup>

In the present study, we extensively examined the fashion of TdP or short-coupled PVC initiation in our IVF cohort and identified 7 cases that fulfilled the scTdP criteria (as listed in the Methods section) and then sequenced *RyR2*. We then conducted the functional analysis of wild-type (WT) or patient *RyR2* variants in the HEK293 cells to determine whether the intracellular Ca<sup>2+</sup> dynamics of mutant *RyR2* channels were associated with scTdP arrhythmogenicity.

## Methods

### Patients

Seven patients demonstrating PVCs with a short-coupling interval ( $\leq 300$  ms) were enrolled in this study (Table 1). Case 7 presented multiple VF episodes induced by PVCs with a short-coupling interval (230 ms) after ICD implantation while PVCs were not recorded on a regular 12-lead electrocardiogram (ECG). All patients displayed arrhythmic syncope, documented VF, or TdP, where TdP was defined as a polymorphic VT of  $\geq 3$  beats with a QRS axis that revolved around the baseline (the latter definition was not applied when only intracardiac tachycardia ECGs were available).

### Clinical scTdP data and genetic analysis

Detailed methods are available in the [Online Supplemental Material](#). Genetic analysis was performed after obtaining written informed consent in accordance with the study protocol approved by the Shiga University of Medical Science Review Board. Genomic DNA was extracted from peripheral blood lymphocytes. The *RyR2* gene underwent targeted gene sequencing method using the TruSeq Custom Amplicon kit (Illumina, San Diego, CA) and the MiSeq System (Illumina). Detected variants were confirmed using Sanger methods. We verified minor allele frequency (MAF) of the variants in 3 databases including Japanese Human Genetic Variation Database (<http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html>), ExAC Database (<http://exac.broadinstitute.org/>), and 1000 Genomes Project (<http://browser.1000genomes.org/index.html>), and we considered variants to be mutations when MAF is  $< 0.005$ .

### *RyR2* functional analysis

*RyR2* complementary DNA was obtained from mouse ventricles, and then stable inducible HEK293 cells were generated using the Flp-In T-REx system (Invitrogen, Carlsbad, CA). Cytoplasmic and endoplasmic reticulum (ER) luminal Ca<sup>2+</sup> signals were monitored with fluo-4 and R-CEPIA1er, respectively. [<sup>3</sup>H]Ryanodine binding assays were carried out with microsomes isolated from HEK293 cells. Detailed methods are available in the [Online Supplemental Material](#).

### Statistical analysis

Data are expressed as means  $\pm$  SD or SEM with a specific comment. Statistical analysis was performed using the unpaired Student *t* test, and the *P* value of  $< .05$  was considered significant.

## Results

### Clinical characteristics of scTdP

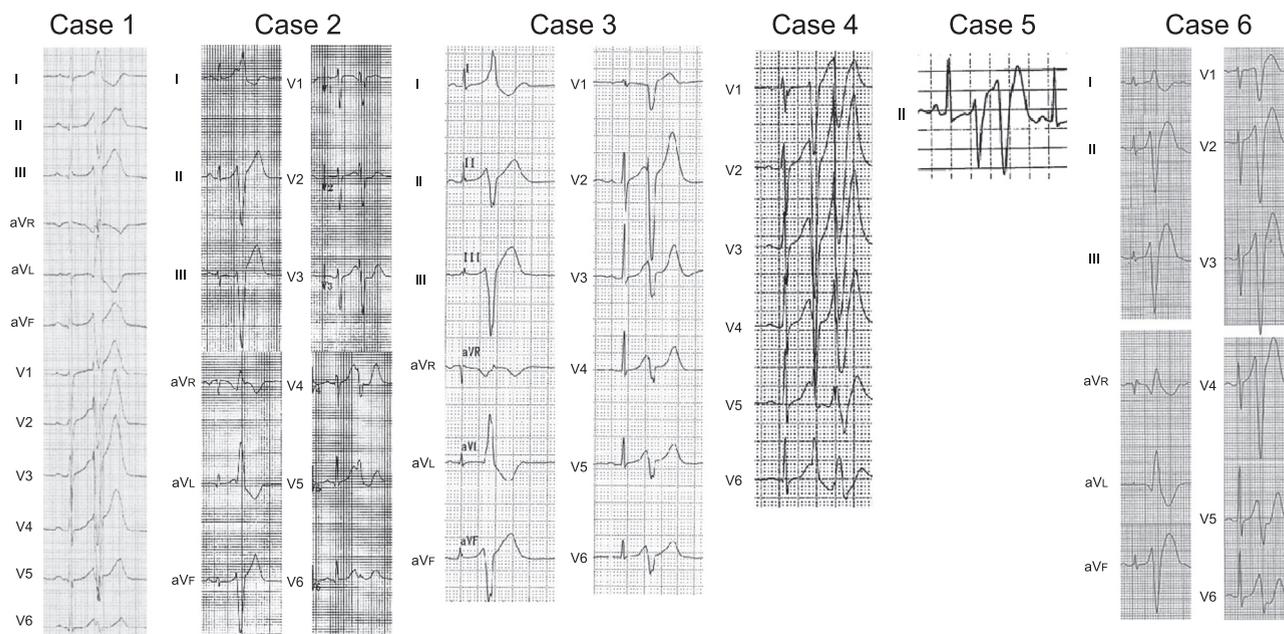
Table 1 summarizes the clinical characteristics of 7 cases (mean age of  $34 \pm 12$  years; 4 men and 3 women). None of the patients had a family history of sudden cardiac death. Figure 1 shows representative ECGs recorded from the first 6 cases. PVC coupling intervals were short with a mean of  $282 \pm 13$  ms. QRS intervals were prolonged, and their average width was  $138 \pm 16$  ms. PVC morphology indicated left bundle branch block with left axis deviation in 5 cases. In the remaining 1 case, this could not be determined because a 12-lead ECG was not available at the time of appearance of PVCs. The average corrected QT interval was normal ( $422 \pm 21$  ms), and Brugada-like ST-segment or J-point elevations were not observed at the onset of TdP. Echocardiography revealed no structural heart disease, and the cardiac function was judged to be normal (ejection fraction  $> 50\%$ ; mean 62%

**Table 1** Clinical characteristics of scTdP cases

Characteristic	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Age at onset (y)	30	48	24	38	47	41	13
Sex	M	F	F	M	F	M	M
Family history of SCD	No	No	No	No	No	No	No
Symptoms	VF, syncope	VF, syncope	VF, syncope	VF, syncope	VF, syncope	VF, syncope	VF, syncope
Conditions at the first onset	During talking at night	After waking in the morning	After lunch	During dinner	During working in the daytime	In the room at night	After exercise
PVC morphology							
PVC origin	LBBB, LAD	LBBB, LAD	LBBB, LAD	LBBB, LAD	NA	LBBB, LAD	NA
PVC coupling (ms)	300	290	280	280	260	280	NA
PVC duration (ms)	130	130	130	130	140	170	NA
ECG findings							
RR interval (ms)	857	800	800	909	811	937	720
PR interval (ms)	162	146	160	170	164	170	160
QRS interval (ms)	94	94	72	98	90	106	80
QRS axis	Normal	Normal	Normal	Normal	Normal	Normal	Normal
QTc duration (ms)	440	431	425	397	455	408	401
J wave	No	No	No	No	No	Yes*	No
Pilsicainide test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Electrophysiology study							
VF induced by ventricular stimuli	Induced VF by 2 extrastimuli from the apex of the right ventricle	Not induced	Not induced	Not induced	Induced VF by 2 extrastimuli from the apex of the right ventricle	Induced VF by 2 extra stimuli from the apex of the right ventricle	Not induced
Documented Purkinje origin	Yes	NA	Yes	Yes	NA	NA	NA
Ablation	Yes	No	Yes	Yes	No	No	No
Ejection fraction (%)	61	57	54	64	63	75	Normal
Treadmill exercise test	NA	NA	Not induced VT	NA	NA	NA	Not induced VTs
Coronary angiography	NA	No CAD	No CAD	NA	No CAD	No CAD	No CAD
Follow-up after the onset							
Follow-up period (mo)	69	90	25	37	80	68	7
Implanted ICD	Yes	Yes	Yes	Yes	Yes	Yes	Yes
ICD therapy	Appropriate shock	No	No	No	Appropriate shock	Appropriate shock	Appropriate shock
Present medication	No drug	No drug	Verapamil, 120 mg	No drug	Verapamil, 120 mg	Bepridil, 100 mg	Verapamil, 120 mg

CAD = coronary artery disease; ECG = electrocardiographic; F = female; ICD = implantable cardioverter-defibrillator; LAD = left axis deviation; LBBB = left bundle branch block; M = male; NA = not available; PVC = premature ventricular contraction; QTc = corrected QT; SCD = sudden cardiac death; scTdP = short-coupled variant of torsades de pointes; VF = ventricular fibrillation; VT = ventricular tachycardia.

\*J wave appeared regardless the onset of ventricular arrhythmias.



**Figure 1** PVC in 12-lead ECG for each scTdP case. PVCs with left bundle branch block and left axis deviation were representative in 5 cases. Case 5 had ECG monitoring during a TdP episode on admission. ECG = electrocardiogram; PVC = premature ventricular contraction; TdP = torsades de pointes.

$\pm 6\%$ ) with normal left ventricular size. Two cases (cases 3 and 7) did not have VT during exercise. Pilsicainide tests failed to provoke a typical Brugada ECG (covered type) in all cases displaying negative late potentials. T-wave alternans was positive in case 1, but not in case 5. Coronary angiography failed to reveal coronary disease in 5 cases. Electrophysiological investigations with the programmed ventricular stimulation (up to 3 stimuli) at the apex and outflow tract of the right ventricle were performed in all patients. Nonsustained VT was induced in 2 patients, and sustained VT was induced in 1 patient. VT was not induced in the remaining 4 cases. Frequent PVCs were present in cases 1, 3, and 4, and catheter ablation was performed.

### Novel RyR2 mutations in scTdP

As shown in [Figure 2A](#), genetic analysis revealed missense variants in 3 cases (cases 3, 6, and 7) and a polymorphism in 1 case (case 1). The p.Val1024Ile (V1024I, case 3) and p.Ala2673Val (A2673V, case 6) variants were located in the N-terminal cytoplasmic region and involved a guanine to adenine substitution in exon 27 (c.3070G>A) and a cytosine to thymine in exon 53 (c.8018C>T). p.Ser4938Phe (S4938F, case 7) was located in the C-terminal cytoplasmic region where a cysteine was changed to thymine in the last exon, exon 105 (c.14813C>T). Clinical information including familial analysis is shown in [Figures 2B–E](#) and identified 1 asymptomatic carrier and 3 noncarriers. Although A2673V and S4938F were not detected in 3 databases listed in the Methods section, V1024I was reported in

the Human Genetic Variation Browser with an MAF of 0.0035. As shown in [Table 2](#), in silico analysis using multiple tools suggested S4938F pathogenicity, while pathogenicity predictions were nonconclusive for V1024I and A2673V. The p.Asn1551Ser (c.4652A>G, N1551S) variant was a common variant in East Asians and listed as a polymorphism detected in >1% Japanese controls.

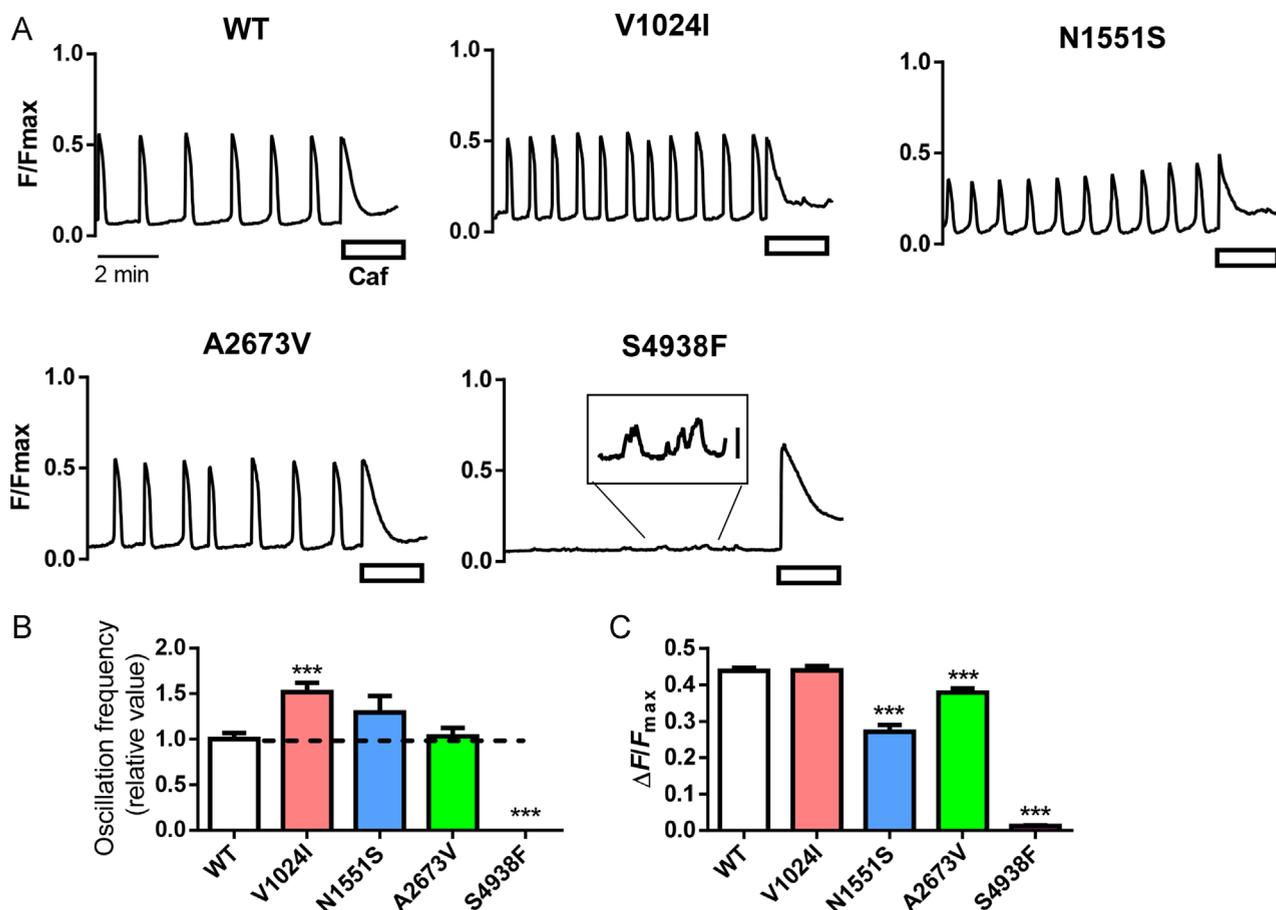
### Ca<sup>2+</sup> homeostasis in HEK cells expressing WT and mutant RyR2s

To determine the Ca<sup>2+</sup> releasing properties of scTdP-related RyR2 variants, we generated stable inducible HEK293 cell lines expressing WT and mutant murine RyR2s and monitored cytoplasmic Ca<sup>2+</sup> signals with fluo-4 ([Figure 3](#)). When WT RyR2 was expressed in HEK293 cells, they showed spontaneous Ca<sup>2+</sup> oscillations as reported<sup>11</sup> ([Online Supplemental Movie 1](#)). Among scTdP-related variants, N1551S, V1024I, and A2673V demonstrated similar profiles and exhibited only slightly modified Ca<sup>2+</sup> oscillations compared with WT. The oscillation frequency was slightly but significantly higher in V1024I cells (~1.5 fold) than in WT cells ([Online Supplemental Movie 2](#)), whereas oscillation amplitudes were moderately and significantly decreased in N1551S and A2673V cells ([Figures 3A and B](#)). These results suggest that the 3 variants may have slightly increased Ca<sup>2+</sup> releasing activity. In contrast, S4938F cells exhibited only occasional tiny fluctuations without oscillations ([Online Supplemental Movie 3](#)). The addition of caffeine—a known RyR enhancer—caused massive Ca<sup>2+</sup> release so that S4938F channels appeared functional but with low activity.

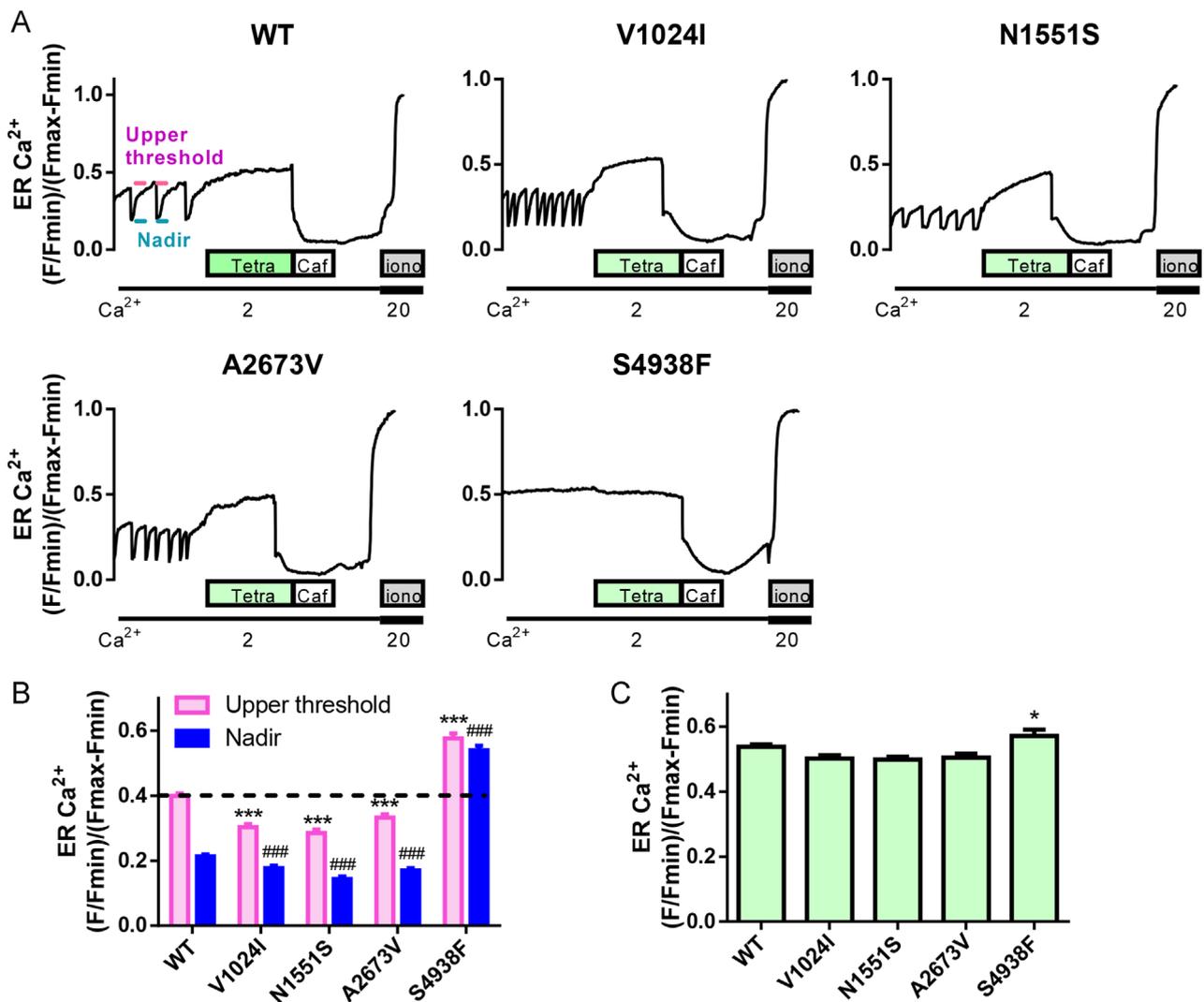


**Table 2** Characteristics of *RyR2* mutations

Characteristic	Case 1	Case 3	Case 6	Case 7
Nucleotide change	c.4652A>G	c.3070G>A	c.8018C>T	c.14813C>T
Amino acid change	p.Asn1551Ser	p.Val1024Ile	p.Ala2673Val	p.Ser4938Phe
Location	Cytosol	Cytosol	Cytosol	Cytosol
Allele frequency in normal controls				
1000 Genomes Project*	None	None	None	None
ExAC Browser†	0.0003	None	None	None
Human Genetic Variation Browser‡	0.005	0.0035	None	None
In silico data				
MutationAssessor§	Low	Neutral	Medium	Medium
SIFT	Tolerated	Tolerated	Damaging	Damaging
SNPs&GO¶	Disease	Disease	Neutral	Disease
MutationTaster#	Disease causing	Disease causing	Disease causing	Disease causing
PolyPhen-2**	Possibly damaging	Probably damaging	Probably damaging	Possibly damaging
PROVEAN††	Neutral	Neutral	Deleterious	Deleterious
CADD‡‡	23.9	15.75	34	24.7

\*<http://browser.1000genomes.org>.†<http://exac.broadinstitute.org/>.‡<http://www.genome.med.kyoto-u.ac.jp/SnpDB/>.§<http://mutationassessor.org>.||<http://sift.jcvi.org>.¶<http://snps-and-go.biocomp.unibo.it/snps-and-go>.#<http://www.mutationtaster.org>.\*\*<http://genetics.bwh.harvard.edu/pph2>.††<http://provean.jcvi.org/index.php>.‡‡<http://cadd.gs.washington.edu>.

**Figure 3** Cytoplasmic  $\text{Ca}^{2+}$  signals in HEK293 cells expressing WT and mutant *RyR2*s. **A:** Representative fluo-4 signals obtained in normal Krebs solution after the addition of 30 mM caffeine. Fluorescence signal values are normalized to  $F_{\text{max}}$  (see Online Supplemental Methods). The vertical bar in the S4938F inset indicates  $0.02F_{\text{max}}$ . **B:** Average oscillation frequency in WT and mutant cells. **C:** Average oscillation amplitude ( $\Delta F/F_{\text{max}}$ ) in WT and mutant cells. Data are presented as mean  $\pm$  SEM.  $n = 31\text{--}69$ . \*\*\* $P < .001$  vs WT. WT = wild type.



**Figure 4** ER Ca<sup>2+</sup> signals in HEK293 cells expressing WT and mutant RyR2s. **A:** Representative R-CEPIA1er signals obtained in normal Krebs solution followed by sequential treatment with 1 mM tetracaine, 30 mM caffeine, and high Ca<sup>2+</sup> Krebs solution with ionomycin. ER Ca<sup>2+</sup> signals were expressed as normalized values,  $(F - F_{\min}) / (F_{\max} - F_{\min})$  (see Online Supplemental Methods). **B:** ER upper threshold and nadir Ca<sup>2+</sup> levels in normal Krebs solution. Data are presented as mean  $\pm$  SEM.  $n = 57$ –112. \*\*\* $P < .001$  vs WT upper threshold. ### $P < .001$  vs WT nadir level. **C:** ER Ca<sup>2+</sup> levels in the presence of 1 mM tetracaine. \* $P < .05$  vs WT. ER = endoplasmic reticulum; WT = wild type.

potent RyR2 inhibitor. R-CEPIA1er ER signals clearly increased in WT, N1551S, V1024I, and A2673V cells after treatment, whereas S4938F cell ER Ca level remained unaffected (Figures 4A and B).

### Ca<sup>2+</sup>-dependent [<sup>3</sup>H]ryanodine binding activity

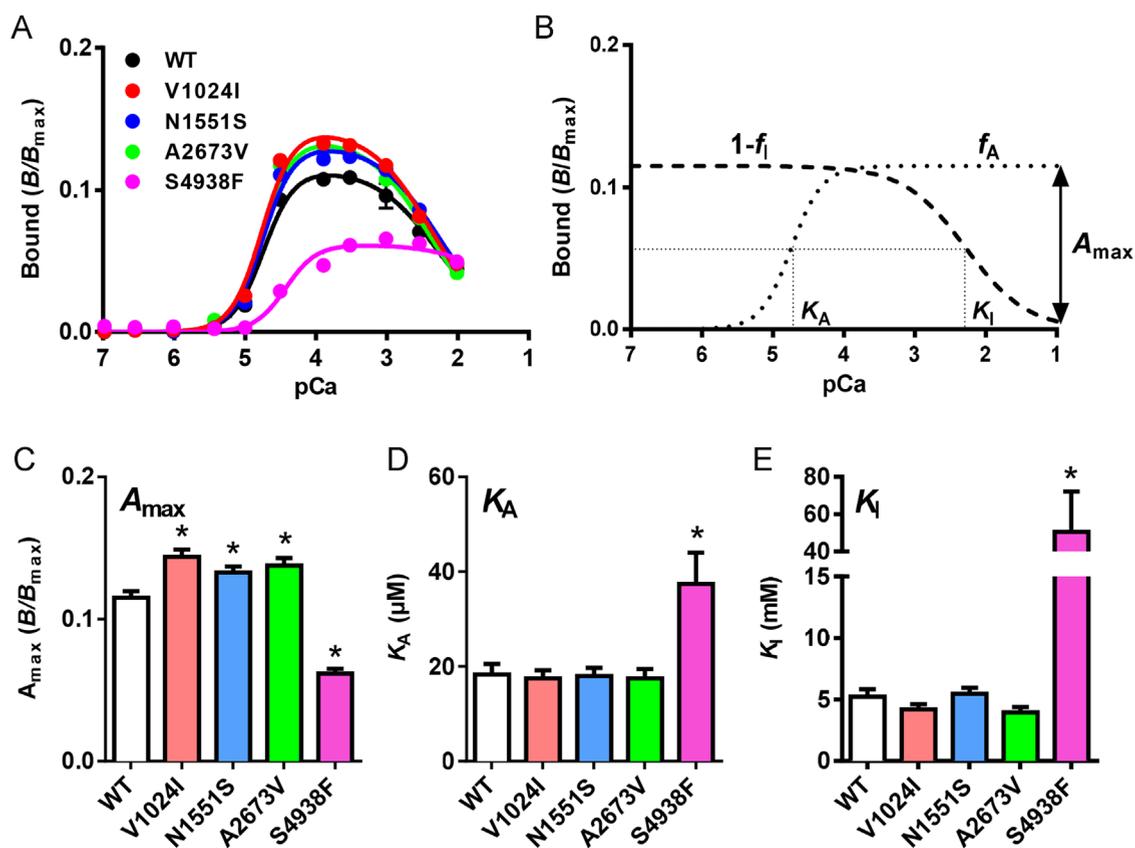
The properties of Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR) activity were evaluated by Ca<sup>2+</sup>-dependent [<sup>3</sup>H]ryanodine binding.<sup>12</sup> WT channels exhibited biphasic Ca<sup>2+</sup> dependence (Figure 5A). Three variants (V1024I, N1551S, and A2673V) showed slight increases in [<sup>3</sup>H]ryanodine binding, whereas it was greatly reduced by S4938F. The biphasic properties of CICR activity are explained by 3 parameters, that is, gain constant ( $A_{\max}$ ) and dissociation constants for activating Ca<sup>2+</sup> ( $K_A$ ) and inactivating Ca<sup>2+</sup> ( $K_I$ ) (Figure 5B).<sup>12</sup> The [<sup>3</sup>H]ryanodine binding data for the mutant channels were fitted to Equations (1)–(3) (see Online Supplemental Methods) to obtain the 3 parameters (Figures 5C–E). Three

variants (V1024I, N1551S, and A2673V) slightly but significantly increased  $A_{\max}$  with no alterations in  $K_A$  or  $K_I$ . In contrast, S4938F halved  $A_{\max}$  and doubled  $K_A$  compared with WT. S4938F exhibited no apparent Ca<sup>2+</sup>-dependent inactivation, resulting in a large  $K_I$  value. Taken altogether, these results suggest that the S4938F variant greatly decreases the CICR activity, whereas the other 3 variants slightly increase it.

### Discussion

In the present study, we examined representative scTdP cases in our patient cohort and their underlying genetics. We confirmed existing knowledge and provided novel insights via the functional analysis of an scTdP variant causing the loss of ER Ca<sup>2+</sup> release.

TdPs often observed in long QT syndrome are initiated by PVCs occurring on the downslope of T waves (R-on-



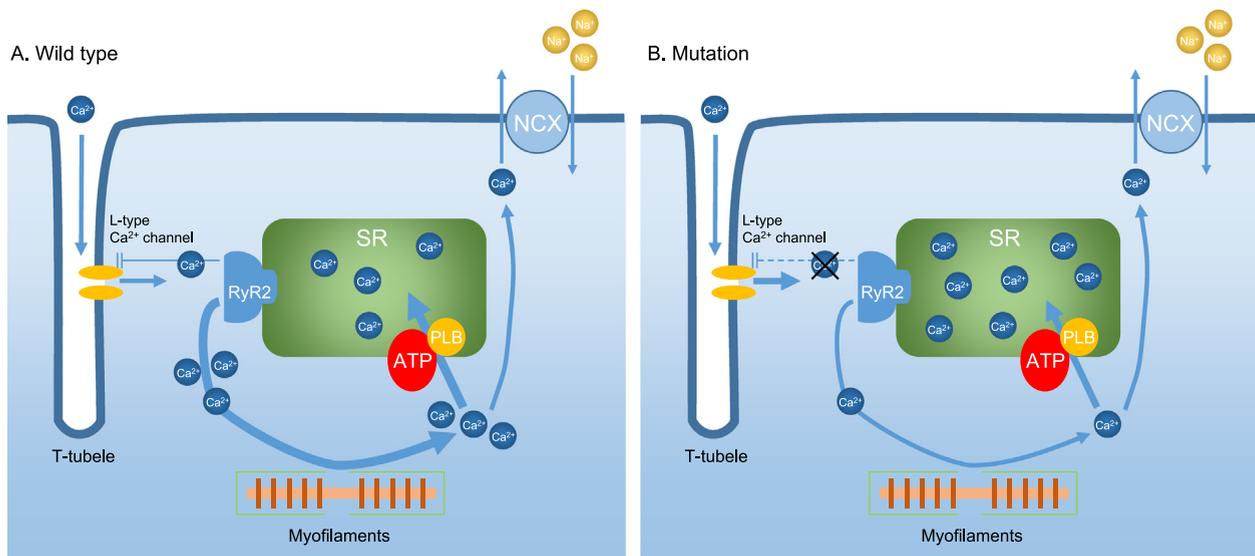
**Figure 5** A:  $\text{Ca}^{2+}$ -dependent [ $^3\text{H}$ ]ryanodine binding to microsomes from HEK293 cells expressing WT and mutant RyR2 channels. B: Three parameters for CICR ( $A_{max}$ ,  $K_A$ , and  $K_I$ ). CICR are expressed as  $A = A_{max} \times f_A \times (1 - f_i)$  (see Online Supplemental Methods). C–E: Calculated parameters for each mutant. Although 3 variants (V1024I, N1551S, and A2673V) exhibited a slight increase in gain, S4938F showed decreased gain with reduced sensitivities to activating and inactivating  $\text{Ca}^{2+}$ . \* $P < .05$  ( $n = 3$ –5). CICR =  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release; WT = wild type.

T).<sup>14</sup> In patients without QT interval prolongation, Leenhardt et al<sup>4</sup> reported 14 cases with TdP caused by PVCs whose coupling intervals were extremely short. Their ECGs displayed typical TdP, with an unusually short-coupling interval during the first TdP beat (always <300 ms). Most cycle lengths just before the start of episodes did not show short-long-short-coupling patterns, and episodes occurred during regular sinus rhythm. Only 1 patient with scTdP (case 6) presented J waves in the inferior leads, which were of the slurred type and were transiently observed in leads III and aVF throughout the clinical course. J waves appeared despite the presence of VT or short-coupled PVCs and disappeared with the onset of VF. J waves in this case may not be a critical finding, but rather a sign of clinical or genetic heterogeneity.

Thus, the ECG and clinical characteristics of scTdP are normal except for the presence of PVCs with a short-coupling interval. It is therefore often difficult for us to diagnose patients with scTdP after PVCs have disappeared. Although the mechanisms underlying scTdP remain unclear, the syndrome may have a genetic background in some cases, since ~30% of cases have a family history of sudden death of unknown etiology.<sup>4</sup> A recent study<sup>10</sup> revealed an RyR2

missense mutation in an scTdP case without any CPVT phenotypes.

The occurrence of PVCs during ventricular refractory periods may be linked to abnormalities of action potential duration during transmural repolarization. Although there are no unique ECG findings in scTdP other than the characteristics of PVCs, ion channel dysfunction may contribute to their occurrence or altered Purkinje network activity.<sup>6,7,15,16</sup> According to our experimental data, the intracellular  $\text{Ca}^{2+}$  dynamics can be associated with scTdP arrhythmogenicity. A mutation in the cytoplasmic C terminus, S4938F, produced RyR2 channels with severe loss of function. Candidate RyR2 mutations in CPVT causing gain-of-function RyR2 abnormalities<sup>17–20</sup> have been reported, while RyR2 p.Ala4860Gly reported by Priori et al<sup>21</sup> demonstrated loss of function similar to S4938F in our patient.<sup>22,23</sup> Although it remains unknown how loss-of-function type of RyR2 variants cause 2 phenotypes (CPVT and scTdP),<sup>24</sup> as schematically shown in Figure 6, the abnormal  $\text{Ca}^{2+}$  dynamics in the cytosol and ER lumen may induce triggered activity afterdepolarizations, especially via ER Ca overload,<sup>16</sup> and nonfunctioning RyR2 mutant channels with loss of function may cause early afterdepolarization but not



**Figure 6** Mechanism of short-coupled variant of torsades de pointes with a loss-of-function *RyR2* variant. *RyR2* mutant channels with loss of function may cause early afterdepolarization but not delayed afterdepolarization through the inhibition of  $\text{Ca}^{2+}$ -dependent Ca channel inactivation. Modified from Zhao et al.<sup>24</sup> ATP = adenosine triphosphate; NCX =  $\text{Na}^+/\text{Ca}^{2+}$  exchanger; PLB = phospholamban; SR = sarcoplasmic reticulum.

delayed afterdepolarization, inhibiting  $\text{Ca}^{2+}$ -dependent Ca channel inactivation.<sup>24</sup>

### Study limitations

Our scTdP cohort was limited in size and therefore results require confirmation in a larger number of cases. We would emphasize that 3 *RyR2* variants with mild gain of function are not pathogenic but “genetic noise” and are unlikely to play a disease-causing role as their functional changes were too mild to explain clinical phenotypes. Unlike CPVT, bidirectional VT or frequent PVCs failed to appear during exercise in this patient with scTdP with *RyR2* loss of function. The typical pattern of CPVT-associated arrhythmias is a bidirectional VT with long PVC coupling interval with mean values of 418 ms reported by Sy et al.<sup>25</sup> and 476 ms by Blich et al.<sup>26</sup> Thus, there may be distinct clinical features that distinguish between typical CPVT and scTdP even among *RyR2* mutation carriers. The potential dysfunction by respective mutation may be associated with the phenotype of *RyR2* mutation carriers.<sup>10</sup>

### Conclusion

scTdP should be considered as a diagnosis when the etiology of aborted sudden cardiac death is unknown. Despite unique ECG features at the onset of TdP, other ECG findings specific to Brugada, long QT, or short QT syndrome are all lacking. The diagnosis of scTdP is of clinical and therapeutic importance, since some drugs or even catheter ablation may be effective in reducing or suppressing arrhythmic episodes.<sup>27,28</sup> Among patients initially diagnosed as IVF and implanted with ICD, some may have scTdP; therefore, an in-depth analysis of arrhythmic events (especially their initiation) monitored by ICD may allow further clarification of this specific IVF

form and improve treatment. *RyR2* loss-of-function mutations may thus cause scTdP.

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### Appendix Supplementary data

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.hrthm.2016.10.015>.

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