

# A Clinical Diagnostic Test for Calcium Release Deficiency Syndrome

Mingke Ni, MD; Ziv Dadon, MD; Julian O. M. Ormerod, MD, PhD; Johan Saenen, MD, PhD; Wiert F. Hoeksema, MD; Pavel Antiperovitch, MD; Rafik Tadros, MD, PhD; Morten K. Christiansen, MD, PhD; Christian Steinberg, MD; Marine Arnaud, MD; Shanshan Tian, PhD; Bo Sun, PhD; John Paul Estillore, MD; Ruiwu Wang, PhD; Habib R. Khan, MD, PhD; Thomas M. Roston, MD, PhD; Andrea Mazzanti, MD, PhD; John R. Giudicessi, MD, PhD; Konstantinos C. Siontis, MD; Aiman Alak, MD; J. Gabriel Acosta, MD; Syamkumar M. Divakara Menon, MBBS, MSc; Nigel S. Tan, MD; Christian van der Werf, MD, PhD; Babak Nazer, MD; Hari Vivekanantham, MD; Tanvi Pandya, MPH; Jennifer Cunningham; Lorne J. Gula, MD, MPH; Jorge A. Wong, MD, MPH; Guy Amit, MD; Melvin M. Scheinman, MD; Andrew D. Krahn, MD; Michael J. Ackerman, MD, PhD; Silvia G. Priori, MD, PhD; Michael H. Gollob, MD; Jeff S. Healey, MD, MS; Frederic Sacher, MD; Eyal Nof, MD; Michael Glikson, MD; Arthur A. M. Wilde, MD, PhD; Hugh Watkins, MD, PhD; Henrik K. Jensen, MD, DMSc, PhD; Pieter G. Postema, MD, PhD; Bernard Belhassen, MD; S. R. Wayne Chen, PhD; Jason D. Roberts, MD, MAS

**IMPORTANCE** Sudden death and cardiac arrest frequently occur without explanation, even after a thorough clinical evaluation. Calcium release deficiency syndrome (CRDS), a life-threatening genetic arrhythmia syndrome, is undetectable with standard testing and leads to unexplained cardiac arrest.

**OBJECTIVE** To explore the cardiac repolarization response on an electrocardiogram after brief tachycardia and a pause as a clinical diagnostic test for CRDS.

**DESIGN, SETTING, AND PARTICIPANTS** An international, multicenter, case-control study including individual cases of CRDS, 3 patient control groups (individuals with suspected supraventricular tachycardia; survivors of unexplained cardiac arrest [UCA]; and individuals with genotype-positive catecholaminergic polymorphic ventricular tachycardia [CPVT]), and genetic mouse models (CRDS, wild type, and CPVT were used to define the cellular mechanism) conducted at 10 centers in 7 countries. Patient tracings were recorded between June 2005 and December 2023, and the analyses were performed from April 2023 to December 2023.

**INTERVENTION** Brief tachycardia and a subsequent pause (either spontaneous or mediated through cardiac pacing).

**MAIN OUTCOMES AND MEASURES** Change in QT interval and change in T-wave amplitude (defined as the difference between their absolute values on the postpause sinus beat and the last beat prior to tachycardia).

**RESULTS** Among 10 case patients with CRDS, 45 control patients with suspected supraventricular tachycardia, 10 control patients who experienced UCA, and 3 control patients with genotype-positive CPVT, the median change in T-wave amplitude on the postpause sinus beat (after brief ventricular tachycardia at  $\geq 150$  beats/min) was higher in patients with CRDS ( $P < .001$ ). The smallest change in T-wave amplitude was 0.250 mV for a CRDS case patient compared with the largest change in T-wave amplitude of 0.160 mV for a control patient, indicating 100% discrimination. Although the median change in QT interval was longer in CRDS cases ( $P = .002$ ), an overlap between the cases and controls was present. The genetic mouse models recapitulated the findings observed in humans and suggested the repolarization response was secondary to a pathologically large systolic release of calcium from the sarcoplasmic reticulum.

**CONCLUSIONS AND RELEVANCE** There is a unique repolarization response on an electrocardiogram after provocation with brief tachycardia and a subsequent pause in CRDS cases and mouse models, which is absent from the controls. If these findings are confirmed in larger studies, this easy to perform maneuver may serve as an effective clinical diagnostic test for CRDS and become an important part of the evaluation of cardiac arrest.

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**Author Affiliations:** Author affiliations are listed at the end of this article.

**Corresponding Authors:** S. R. Wayne Chen, PhD, 3330 Hospital Dr NW, Calgary, AB T2N 4N1, Canada (swchen@ucalgary.ca); Jason D. Roberts, MD, MAS, 237 Barton St E, Hamilton, ON L8L 2X2, Canada (jason.roberts@phri.ca).

Sudden cardiac death remains a leading cause of mortality in the world.<sup>1,2</sup> Despite contemporary investigation strategies, more than 20 000 individuals are estimated to experience an unexplained cardiac arrest each year in the US alone.<sup>3</sup> Failing to identify an underlying cause of sudden cardiac death and cardiac arrest precludes delivery of optimal care, furthers psychological distress in survivors and families, and may leave family members vulnerable to similarly catastrophic outcomes because of the possibility of a genetic contribution.<sup>4,5</sup>

Two factors contributing to unexplained cardiac arrest include undiscovered disorders and an inability to diagnose a known disease. In this context, calcium release deficiency syndrome (CRDS) is a heart condition discovered by our group in 2021 that causes sudden cardiac death.<sup>6-9</sup> Calcium release deficiency syndrome develops secondary to loss-of-function genetic variants within the *RYR2* gene, which encodes the cardiac ryanodine receptor.<sup>10</sup> The CRDS clinical phenotype is undetectable and diagnosis requires cellular-based in vitro studies after identification of a rare *RYR2* genetic variant.<sup>6</sup>

Importantly, unless previously reported, rare *RYR2* variants are most often classified as variants of unknown significance.<sup>11,12</sup> Without access to *RYR2* in vitro analyses, which are only available in research settings, clinicians cannot diagnose patients with CRDS, and cardiac events are classified as unexplained or are misdiagnosed. Equally problematic, first-degree relatives carrying *RYR2* variants remain undiagnosed, which has been documented to lead to recurrent and previously perceived unexplained familial sudden cardiac death.<sup>6-9</sup>

There is an urgent need for a simple and reliable clinical diagnostic test for CRDS. Members of our group previously reported on a family's history of catecholaminergic polymorphic ventricular tachycardia in the presence of an *RYR2*-p.M4109R variant, wherein the affected family members developed marked and transient cardiac repolarization changes (QT prolongation associated with a tall and broad T wave) after pacing-mediated tachycardia and a subsequent pause.<sup>13</sup> Catecholaminergic polymorphic ventricular tachycardia is a life-threatening arrhythmia syndrome caused by *RYR2* gain-of-function variants, and malignant arrhythmia events most often occur during intense exercise or emotion.<sup>14,15</sup>

The adrenergic-mediated ventricular arrhythmias characteristic of catecholaminergic polymorphic ventricular tachycardia can be readily reproduced on exercise stress testing (the standard clinical diagnostic test); however, the exercise stress tests conducted in this family yielded normal results.<sup>16,17</sup> Since publication of the report,<sup>13</sup> in vitro characterization of the *RYR2*-p.M4109R variant has revealed that it causes a loss of function of the protein/ion channel<sup>7</sup> and the familial clinical diagnosis has been revised to CRDS.

Driven by these observations, our group sought to determine if this provoked repolarization abnormality may be a sensitive and specific electrocardiographic signature of CRDS. We then sought to explore the underlying cellular mechanism using genetic mouse models.

## Key Points

**Question** Cardiac arrest frequently occurs without explanation, even after a thorough clinical evaluation. Can a simple maneuver clinically diagnose calcium release deficiency syndrome (CRDS), a newly described cause of sudden death?

**Findings** In this international, multicenter, case-control study, a provoked measure of T-wave amplitude on an electrocardiogram ascertained cases of CRDS with high accuracy. The genetic mouse models recapitulated the human findings and suggested a pathologically large systolic calcium release from the sarcoplasmic reticulum was responsible.

**Meaning** These preliminary results suggest that the repolarization response on an electrocardiogram to brief tachycardia followed by a pause may effectively diagnose CRDS. Given the frequency of unexplained cardiac arrest, should these findings be confirmed in larger studies, this readily available maneuver may provide clinically actionable information.

## Methods

### Patient Selection

This international, multicenter case-control study included study patients from 10 centers in 7 countries. The response to brief tachycardia and a subsequent pause was evaluated in cases with CRDS and 3 control groups. The first control group included patients with suspected supraventricular tachycardia undergoing an invasive electrophysiology study. The second control group included survivors of unexplained cardiac arrest. The third control group included patients with genotype-positive catecholaminergic polymorphic ventricular tachycardia.

All CRDS cases had to possess an *RYR2* variant that had been confirmed to be a loss-of-function variant using cellular-based in vitro testing.<sup>6</sup> Patients with suspected supraventricular tachycardia were excluded if they had ventricular cardiomyopathy, known obstructive coronary artery disease (coronary stenosis >50%), ventricular preexcitation, or a diagnosis of long QT syndrome. Survivors of unexplained cardiac arrest underwent defibrillation, but their cardiac arrest remained unexplained after undergoing electrocardiographic, echocardiographic, and coronary assessments; cardiac magnetic resonance imaging; exercise stress testing; and genetic testing.<sup>18,19</sup> Genetic testing included a screening of *RYR2*. When a rare *RYR2* variant was identified, cellular-based in vitro studies were performed to confirm the variant did not have an effect on function.

Details of cellular-based in vitro studies for *RYR2* variants appear in the eMethods in Supplement 1. Patients with genotype-positive catecholaminergic polymorphic ventricular tachycardia were either heterozygous for an *RYR2* gain-of-function variant or homozygous or compound heterozygous for pathogenic *CASQ2* variants.<sup>20</sup> Individuals were excluded if they were being treated with a QT-prolonging medication (other than flecainide) at the time of their pacing-mediated or spontaneous provocation.

Table. Baseline Clinical Characteristics

| Characteristic   | Calcium release deficiency syndrome (n = 10) | Control groups                        |                                     |   |
|--|--|---------------------------------------|-------------------------------------|---|
|  |  | Supraventricular tachycardia (n = 45) | Unexplained cardiac arrest (n = 10) | Catecholaminergic polymorphic ventricular tachycardia (n = 3) |
| Age, median (IQR), y <sup>a,b</sup>                          | 16.0 (14.5-41.0)                             | 51.0 (41.5-63.0)                      | 33.0 (26.5-46.0)                    | 30.7 (1.5)  |
| Sex, No. (%)   |  |                                       |                                     |   |
| Female   | 4 (40)                                       | 17 (37.8)                             | 3 (30)                              | 2 (67)  |
| Male   | 6 (60)                                       | 28 (62.2)                             | 7 (70)                              | 1 (33)  |
| Race, No. (%) <sup>c</sup>                                   |  |                                       |                                     |   |
| Asian  | 0  | 4 (8.9)                               | 0                                   | 0   |
| Black or African American                                    | 0  | 1 (2.2)                               | 0                                   | 0   |
| White  | 10 (100)                                     | 40 (88.9)                             | 10 (100)                            | 3 (100)   |
| Body mass index, median (IQR) <sup>b,d</sup>                 | 24.6 (19.3-30.9)                             | 26.0 (22.5-29.5)                      | 25.1 (23.2-27.9)                    | 21.0 (5.8)  |
| Medical history, No. (%) <sup>e</sup>                        |  |                                       |                                     |   |
| Prior cardiac arrest   | 4 (40)                                       | 0                                     | 10 (100)                            | 3 (100)   |
| Hypertension   | 0  | 9 (20)                                | 0                                   | 0   |
| Diabetes   | 0  | 2 (4.4)                               | 0                                   | 0   |
| Medications, No. (%) <sup>a</sup>                            |  |                                       |                                     |   |
| Flecainide   | 2 (20)                                       | 0                                     | 0                                   | 1 (33.3)  |
| $\beta$ -Blockers  | 2 (20)                                       | 0                                     | 2 (20)                              | 2 (66.7)  |
| Calcium channel blockers                                     | 0  | 0                                     | 1 (10)                              | 0   |
| Electrocardiographic features, median (IQR), ms <sup>b</sup> |  |                                       |                                     |   |
| PR interval  | 151.5 (132.3-185.8)                          | 158.0 (139.0-173.5)                   | 157.5 (132.3-173.0)                 | 153.7 (24.1)  |
| QRS interval   | 90.5 (85.3-102.5)                            | 98.0 (90.0-103.0)                     | 98.5 (91.8-105.5)                   | 83.0 (9.2)  |
| QTc interval   | 427.0 (388.8-440.8)                          | 431.8 (404.2-451.2)                   | 395.5 (378.5-419)                   | 410.5 (34.7)  |

<sup>a</sup> At the time of evaluation of the provoked repolarization response.<sup>d</sup> Calculated as weight in kilograms divided by height in meters squared.<sup>b</sup> Data in last column are expressed as mean (SD).<sup>e</sup> Collected by patient report.<sup>c</sup> Participants self-identified race.

Patients with suspected supraventricular tachycardia, CRDS, unexplained cardiac arrest, or catecholaminergic polymorphic ventricular tachycardia were enrolled as part of the ongoing Evaluation of a Clinical Diagnostic Test for CRDS (DIAGNOSE CRDS) study (NCT06188689). The trial protocol was approved by the research ethics boards of Hamilton Health Sciences and select collaborating institutions. The remaining patients had either previously undergone comparable pacing maneuvers, had a spontaneous rhythm that mirrored the maneuvers, or the treating clinical team deemed the maneuvers to be part of standard care. In each instance, the study patient provided consent for their clinical data to be used for research.

### Pacing Maneuvers

Among patients with suspected supraventricular tachycardia,  $\beta$ -blockers, calcium channel blockers, and antiarrhythmic drugs were withheld for 5 or greater half-lives prior to performing the maneuvers. The decision to withhold these medications in patients with CRDS, catecholaminergic polymorphic ventricular tachycardia, and unexplained cardiac arrest was at the discretion of the treating clinician and was documented. Study patients with an existing transvenous cardiac device could have the pacing maneuvers performed through their device. The pacing maneuvers in the control patients with suspected supraventricular tachycardia were performed at the time of their invasive electrophysiology study and in the ab-

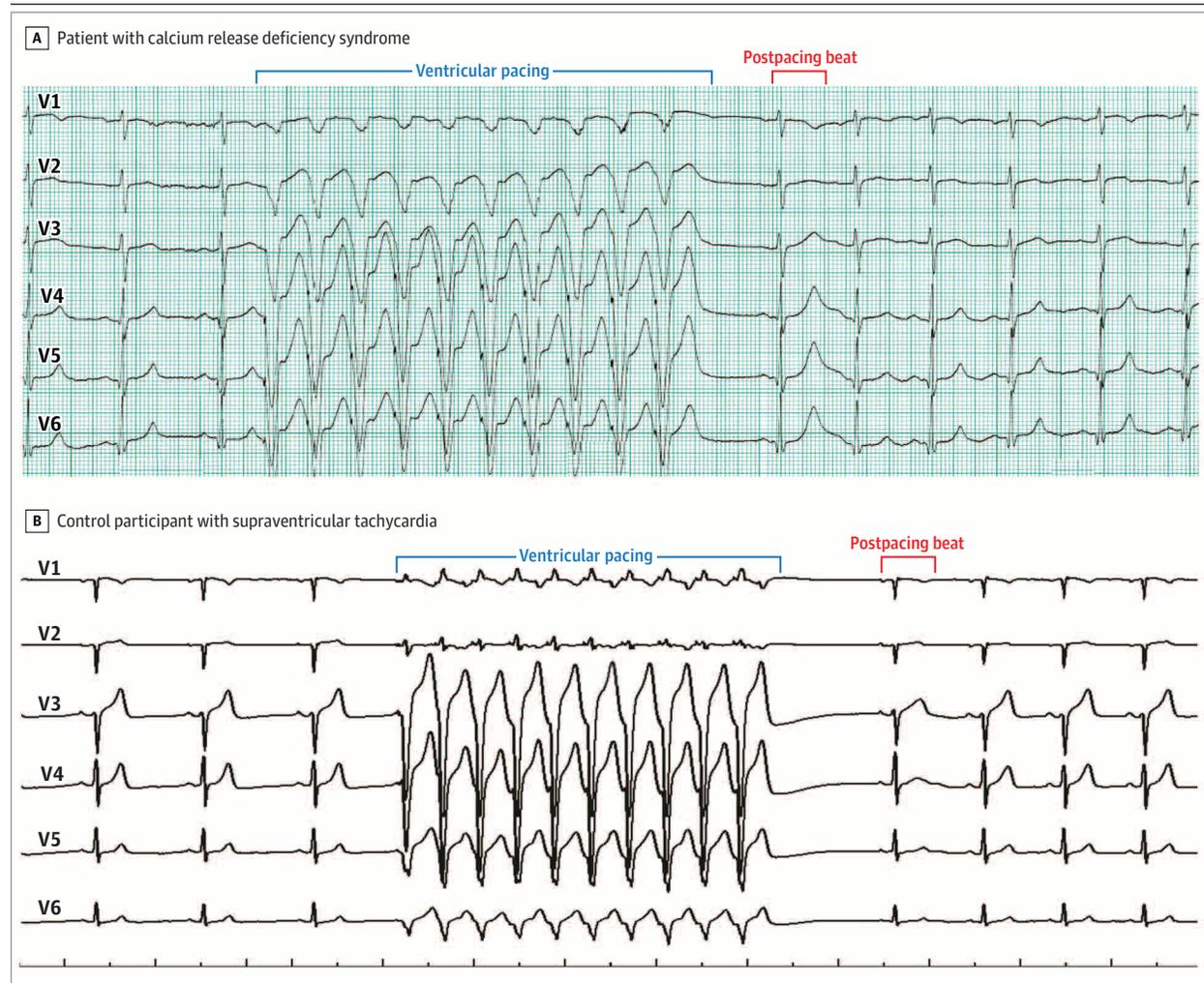
sence of isoproterenol or other  $\beta$ -agonists. Continuous 12-lead electrocardiography was required to capture (at a minimum) the last sinus beat before the pacing train, the pacing train, and 3 postpacing beats.

Among the study patients who underwent pacing maneuvers prospectively, 10 beat right ventricular and atrial pacing trains were requested to be performed at 120 beats/min and 150 beats/min. Pacing trains with atrioventricular Wenckebach, noncaptured beats, premature atrial or ventricular ectopy that interrupted the postpacing pause, or a postpacing pause of less than 700 milliseconds were excluded.

### Electrocardiographic Analyses

Electrocardiographic measurements were performed using scaled electronic calipers on the digitalized or scanned tracings by an electrophysiologist (Z.D.) blinded to case status. The QT interval was measured in leads II and V5 using the tangent method.<sup>21-25</sup> T-wave amplitude was measured as the vertical distance between the peak or nadir of the T wave and the isoelectric line (defined by the TP segment). Changes in QT interval and changes in T-wave amplitude were determined by subtracting the value of the parameter for the last beat prior to the pacing train from the value for the first postpacing beat (eFigure 1 in Supplement 1). Changes in T-wave amplitude were assessed for all 12 leads (when available) and the largest value was used for the analyses.

**Figure 1. Representative Electrocardiographic Tracings of the Cardiac Repolarization Response to Brief Tachycardia and a Pause in a Patient With Calcium Release Deficiency Syndrome and in a Control Patient With Supraventricular Tachycardia**



A. In a patient with a confirmed *RYR2* loss-of-function variant (*RYR2*-p.T4196I), a tall and broad T wave was observed in the beat immediately after ventricular pacing. B. The control participant had a structurally normal heart. Precordial

leads alone are shown for ease of illustration; however, the finding was also observed in the limb leads (12-lead electrocardiograms appear in eFigure 4 in Supplement 1).

Adjudication of a diagnosis of CRDS was performed by 2 cardiologists (C.V. and B.N.) who assessed 20 blinded tracings (10 cases of CRDS and 10 controls) to ascertain the sensitivity and specificity of the electrocardiographic findings for a CRDS diagnosis through visual review alone.

### Murine Studies

Intracardiac pacing was performed in anesthetized mice with *RYR2*-D4646A<sup>+/-</sup> (CRDS), *RYR2*-I4855M<sup>+/-</sup> (CRDS with left ventricular noncompaction), *RYR2*-WT (wild type), and *RYR2*-R4496C<sup>+/-</sup> (catecholaminergic polymorphic ventricular tachycardia) variants (aged 8-16 weeks in both males and females) using a 1.1-F octapolar (8 electrodes) catheter (EPR-800, Millar Instruments). Details of the cardiac pacing, surface electrocardiographic tracings, and other analyses appear in the eMethods in Supplement 1.

Monophasic action potential and bipolar electrographic recordings of hearts with the *RYR2*-WT, *RYR2*-D4646A<sup>+/-</sup>, and

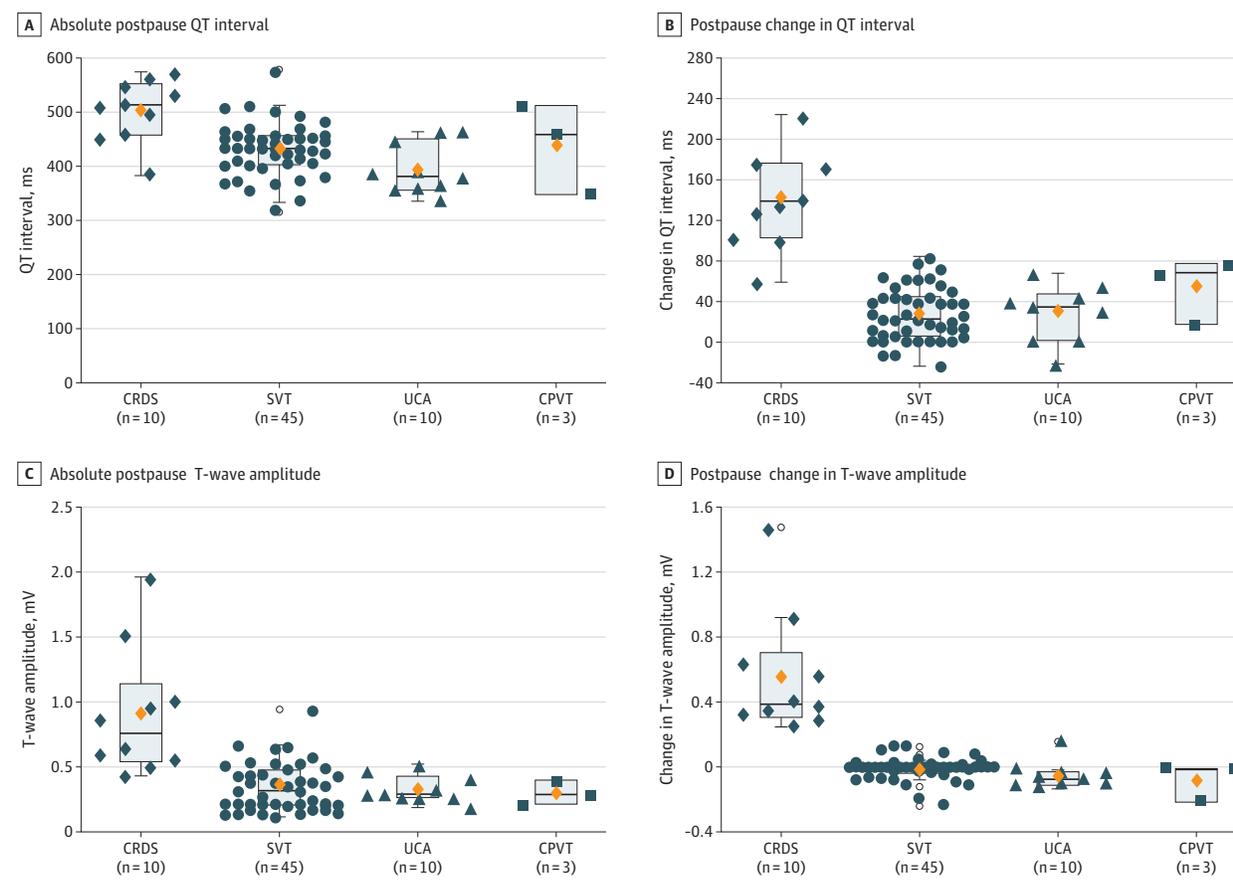
*RYR2*-R4496C<sup>+/-</sup> variants were performed between 8 and 16 weeks of age, whereas intact heart calcium imaging from these mouse models was performed at 8 to 25 weeks of age.<sup>26-30</sup> Additional details appear in the eMethods in Supplement 1.

### Statistical Analyses

Normally distributed continuous variables are presented as mean (SD). Nonnormally distributed continuous variables are presented as median (IQR). Normally distributed data were compared using the *t* test for 2 groups and 1-way analysis of variance followed by Bonferroni post hoc tests for 3 groups or more. Nonnormally distributed data were compared using the Mann-Whitney test for 2 groups and the Kruskal-Wallis test with the Dunn post hoc test for 3 groups or more. Categorical variables are presented as number (percentage) and were compared using the Fisher exact test.

The sensitivity analyses (using linear regression models) comparing human CRDS cases vs controls were performed for

Figure 2. Comparison of QT Intervals and T-Wave Amplitudes on the Sinus Beat After Brief Tachycardia and a Pause in Patients With Calcium Release Deficiency Syndrome and in Patients in 3 Control Groups



The ventricular rate was 150 beats/min or higher. The x-axis spacing represents jitter. The horizontal lines in the boxes represent the medians; the orange diamonds, the means; the upper edge of the boxes, quartile 3; the lower edge of the boxes, quartile 1; the whiskers, the upper and lower extreme values; and the open circles, the outlier values. For B, the data presented reflect the postpause change in the QT interval from the prepacing QT interval. For D, the data presented reflect the postpause change in T-wave amplitude

from the prepacing T-wave amplitude. The electrocardiographic measurements were performed using scaled electronic calipers on digitalized or scanned tracings by an electrophysiologist (Z.D.) blinded to case status. CPVT indicates catecholaminergic polymorphic ventricular tachycardia; CRDS, calcium release deficiency syndrome; SVT, supraventricular tachycardia; and UCA, unexplained cardiac arrest.

both the change in T-wave amplitude and the change in QT interval (for brief ventricular tachycardia at  $\geq 150$  beats/min) and included age, sex, and familial relatedness as covariables. Two-tailed  $P$  values  $< .05$  were considered statistically significant. The statistical analyses were performed using IBM SPSS Statistics version 26 (for Windows; IBM).

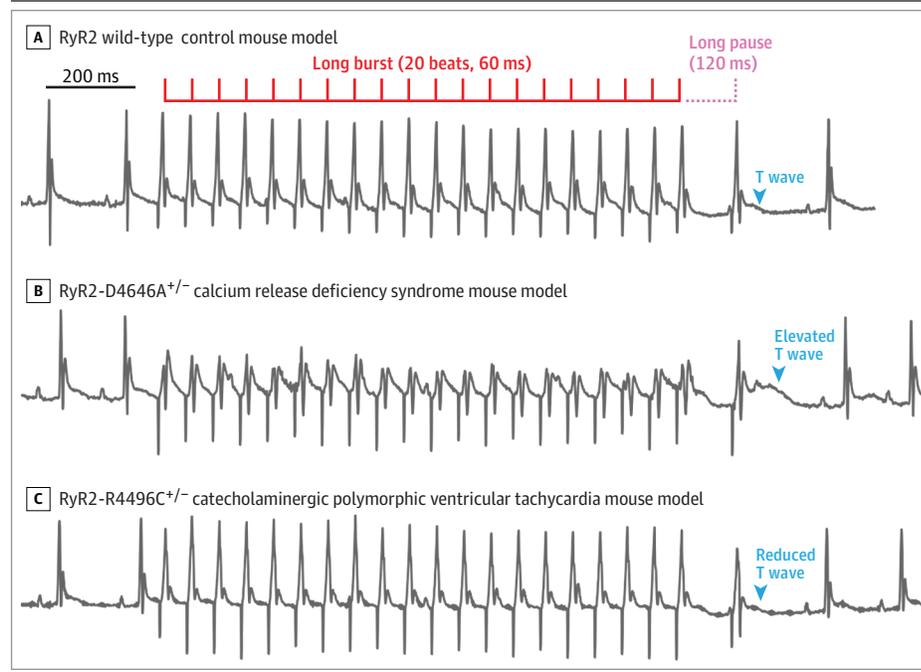
## Results

Among 10 patients with CRDS, 45 control patients with suspected supraventricular tachycardia, 10 control patients who experienced unexplained cardiac arrest, and 3 control patients with genotype-positive catecholaminergic polymorphic ventricular tachycardia, the median change in T-wave amplitude on the postpause sinus beat (after brief ventricular tachycardia at  $\geq 150$  beats/min) was higher in patients with CRDS ( $P < .001$ ). The patients had episodes of brief ventricu-

lar tachycardia followed by a pause (either through pacing or spontaneously) that permitted evaluation of a repolarization response (Table; eFigure 2 and eTable 1 in Supplement 1). The CRDS cases were derived from 6 families possessing 6 distinct *RYR2* variants previously confirmed to result in a loss of function of the protein/ion channel (eTables 1-2 in Supplement 1).<sup>6,7,9</sup>

Of the 10 patients with CRDS, 5 were asymptomatic family members, 4 had survived a cardiac arrest requiring defibrillation, and 1 had experienced unexplained syncope. Two of the 10 unexplained cardiac arrest cases carried rare *RYR2* variants (*RYR2*-p.Ala1806Thr and *RYR2*-p.Asn2386Ser) that did not have an effect on function (eFigure 3 in Supplement 1). Two catecholaminergic polymorphic ventricular tachycardia cases had *RYR2* gain-of-function variants (p.Ser2425Tyr and p.Asn628Tyr). The third case was compound heterozygous for pathogenic *CASQ2* variants (p.Tyr55Cys and p.Ser173Ile).

Figure 3. Repolarization Responses to Long-Burst, Long-Pause Electrical Stimulation in Mouse Models



The electrocardiogram traces were chosen based on the mean values of the corresponding datasets. Intracardiac pacing was performed with a catheter inserted through the internal jugular vein and advanced into the right ventricle. A long burst (20 beats at an interval of 60 ms) was used to drive more extracellular calcium into cardiomyocytes. A long pause (120 ms) was used to substantially load calcium into the sarcoplasmic reticulum. The QT interval of the signal complex after the pause was determined manually by placing cursors on the beginning of the QRS complex and on the end of the T wave.

### Repolarization Response to Brief Tachycardia and a Pause

Of the 10 cases with CRDS, 6 had previously undergone comparable pacing maneuvers, 3 prospectively underwent the ventricular pacing maneuvers, and 1 had a spontaneous episode of nonsustained ventricular tachycardia followed by a pause that enabled assessment of a repolarization response (eFigure 2 and eFigures 4-6 in Supplement 1). After ventricular burst pacing or nonsustained ventricular tachycardia at 150 beats/min or greater, the morphology of the T wave most often appeared tall and broad on the first postpacing beat (Figure 1 and eFigure 4 in Supplement 1).

The median postpause absolute QT interval in lead II was 510.5 milliseconds (IQR, 455.8-548.8 milliseconds) and the corresponding median change in QT interval was 134.5 milliseconds (IQR, 100.3-172.5 milliseconds) (eTables 3-4 in Supplement 1). The median T-wave amplitude on the first postpause beat was 0.749 mV (IQR, 0.539-1.131 mV) and the corresponding median change in T-wave amplitude was 0.388 mV (IQR, 0.311-0.700 mV) (eTables 3-4 in Supplement 1). The findings were similar from the atrial burst pacing and the ventricular burst pacing at 120 beats/min (eTables 5-7 and eFigure 5 in Supplement 1).

Examples of repolarization responses for suspected supraventricular tachycardia, unexplained cardiac arrest, and catecholaminergic polymorphic ventricular tachycardia appear in Figure 1 and eFigure 4 in Supplement 1. The summary data appear in eTables 4-7 in Supplement 1.

### Differentiating CRDS Cases From Controls

The absolute median QT interval value on the first postpause beat after brief ventricular tachycardia at 150 beats/min or greater was longer for CRDS cases relative to all 3 control groups (Figure 2A and eTable 4 in Supplement 1). Although the me-

dian change in QT interval was longer in CRDS cases ( $P = .002$ ), an overlap between the cases and controls was present. The shortest change in QT interval for a CRDS case was 58 milliseconds, and the longest change in QT interval was 82 milliseconds for a patient from 1 of the 3 control groups (Figure 2B and eTable 3 in Supplement 1).

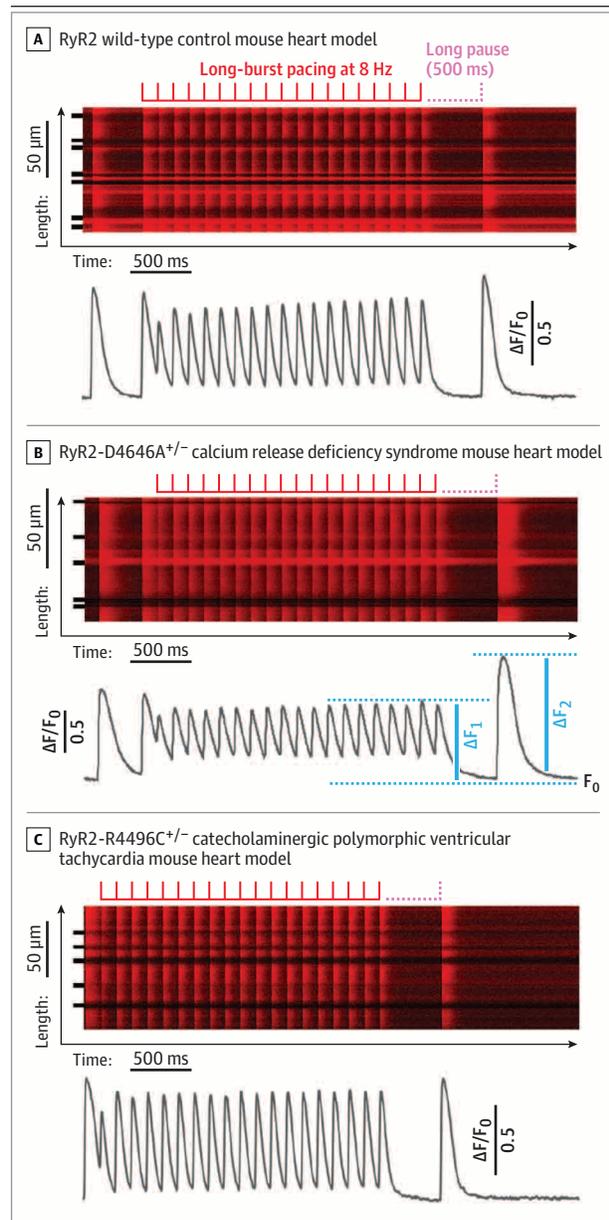
Improved discrimination appeared present for T-wave amplitude when assessed qualitatively. The median T-wave amplitude was statistically greater in CRDS cases relative to all 3 control groups ( $P < .001$ ; Figure 2C and eTable 4 in Supplement 1); however, there was overlap between the individual values from the CRDS cases and controls. No overlap was observed for the individual change in T-wave amplitude values for CRDS cases relative to controls (Figure 2D). The smallest change in T-wave amplitude was 0.250 mV for a CRDS case compared with the largest change in T-wave amplitude of 0.160 mV for a control patient, indicating 100% discrimination (Figure 2D and eTable 3 in Supplement 1). The sensitivity analyses used linear regression models and adjusted for age, sex, and familial relatedness, revealing consistent findings for the comparisons of CRDS cases relative to controls in mean change in QT interval and change in T-wave amplitude (eTables 8-9 in Supplement 1).

Blinded visual adjudication of tracings for the 10 CRDS cases and 10 controls by 2 cardiologists (without performing detailed measurements) revealed a combined sensitivity of 90% and a specificity of 95% for correctly ascertaining a CRDS diagnosis.

### Repolarization Responses to Brief Tachycardia in Genetic Mouse Models

Similar studies were performed in genetic mouse models known to recapitulate the human cardiac phenotypes (eFigure 7 in

**Figure 4. Effect of Long-Burst, Long-Pause Electrical Stimulation on Calcium Transient Amplitude in Mouse Models**



The images were chosen based on the mean values of the corresponding datasets. The hearts were intact. The scale bar (50 μm) depicts the length of the confocal scanning line used for calcium imaging of the heart. The short black bands indicate cell boundaries. The red color indicates the fluorescence of the calcium indicator (Rhod2-AM), whose intensity reflects the level of calcium. F<sub>0</sub> indicates baseline fluorescence intensity just before the first postpause stimulation; F<sub>1</sub>, peak fluorescence intensity during 8-Hz stimulation; and F<sub>2</sub>, peak fluorescence intensity of the first postpause stimulation. ΔF indicates a given fluorescence intensity minus the baseline fluorescence (F - F<sub>0</sub>); ΔF<sub>1</sub>, F<sub>1</sub> minus F<sub>0</sub>; and ΔF<sub>2</sub>, F<sub>2</sub> minus F<sub>0</sub>. A, For the RyR2 wild-type control mouse heart model, the median ΔF<sub>1</sub>/F<sub>0</sub> = 0.85 (IQR, 0.74-0.86) and the mean ΔF<sub>2</sub>/F<sub>0</sub> = 1.07 (SD, 0.20). B, For the RyR2-D4646A<sup>+/−</sup> calcium release deficiency syndrome mouse heart model, the mean ΔF<sub>1</sub>/F<sub>0</sub> = 0.88 (SD, 0.04) and the mean ΔF<sub>2</sub>/F<sub>0</sub> = 1.48 (SD, 0.14). C, For the RyR2-R4496C<sup>+/−</sup> catecholaminergic polymorphic ventricular tachycardia mouse heart model, the mean ΔF<sub>1</sub>/F<sub>0</sub> = 0.75 (SD, 0.09) and the mean ΔF<sub>2</sub>/F<sub>0</sub> = 0.85 (SD, 0.12).

Supplement 1).<sup>6,31,32</sup> Consistent with the findings in humans, pacing-mediated tachycardia and a subsequent pause provoked significantly increased mean absolute QT intervals and mean changes in QT intervals in the CRDS RYR2-D4646A<sup>+/−</sup> mouse model relative to the wild-type mouse model (Figure 3A and Figure 3B; eTable 10 in Supplement 1).

Similar findings were also observed in the mouse model expressing the human RYR2-I4855M<sup>+/−</sup> loss-of-function variant, which recapitulates the overlapping phenotype for CRDS and left ventricular noncompaction (eFigure 8 in Supplement 1).<sup>31</sup> In contrast, significantly reduced mean absolute QT intervals and changes in QT intervals were observed in the RYR2-R4496C<sup>+/−</sup> catecholaminergic polymorphic ventricular tachycardia mouse model compared with the wild-type mouse model (Figure 3A and Figure 3C; eTable 10 in Supplement 1). Increasing the duration of the burst and the pause were both found to increase the absolute QT interval and the change in QT interval on the first postpacing beat in the RYR2-D4646A<sup>+/−</sup> CRDS mouse model (eFigures 9-11 in Supplement 1).

Action potential duration is an important determinant of QT interval.<sup>33</sup> To gain insight into the underlying mechanism of the observed QT prolongation, we performed monophasic action potential and bipolar electrographic recordings ex vivo in intact Langendorff-perfused hearts. Pacing-mediated tachycardia and a subsequent pause markedly prolonged the monophasic action potential duration and the corresponding QT interval on the first postpacing beat in the CRDS RYR2-D4646A<sup>+/−</sup> variant hearts compared with the wild-type hearts (eFigure 12A, 12B, 12F, and 12G in Supplement 1). In contrast, this provocation significantly reduced the monophasic action potential duration and QT interval on the first postpacing beat in the catecholaminergic polymorphic ventricular tachycardia RYR2-R4496C<sup>+/−</sup> variant hearts (eFigure 12A, 12C, 12F, and 12G in Supplement 1).

The amplitude of calcium transients can influence the action potential duration via the sodium-calcium exchanger current.<sup>34</sup> Pacing-mediated tachycardia and a subsequent pause markedly increased the peak of calcium transients on the first postpause beat in the CRDS RYR2-D4646A<sup>+/−</sup> variant hearts compared with the wild-type hearts (Figure 4A and Figure 4B; eTable 11 in Supplement 1). In contrast, this provocation significantly reduced the amplitude of calcium transients on the first postpacing beat in the catecholaminergic polymorphic ventricular tachycardia RYR2-R4496C<sup>+/−</sup> variant hearts (Figure 4A and Figure 4C; eTable 11 in Supplement 1).

## Discussion

Driven by an urgent need and prior report of a family whose diagnosis has been revised to CRDS,<sup>7,13</sup> we evaluated the repolarization response to brief tachycardia and a subsequent pause as a clinical diagnostic test for CRDS. Among the CRDS cases and the mouse models with or without structural heart disease, marked increases in QT interval and T-wave amplitude were consistently observed on the first postpause beat, whereas this finding was absent from the controls. Electrophysiology studies in isolated mouse hearts suggested this was

secondary to a large calcium transient. Beyond providing important pathophysiological insight, this unique electrocardiographic signature appears promising as a sensitive, specific, and simple method for clinically diagnosing CRDS. If these findings are confirmed in larger studies, we envision these simple pacing maneuvers becoming standard in the diagnostic workup of unexplained cardiac arrest.<sup>4,18,19</sup>

Cardiac arrest remains common and is invariably devastating, particularly in young and previously healthy individuals. Despite the critical importance of identifying an underlying cause of cardiac arrest to enable optimal care of survivors and their family members, failure to do so remains common. In this context, establishing a clinical diagnostic test for CRDS has been considered crucial since its discovery in 2021.<sup>6</sup> Initial reports of CRDS were particularly alarming given that patients had often undergone clinical evaluations by expert cardiology teams for syncope and had been reassured that their clinical test results were normal, only to then experience a cardiac arrest or sudden cardiac death.<sup>6,8,9</sup> These devastating outcomes, which may have been prevented through insertion of an implantable cardioverter-defibrillator in symptomatic patients, were often further exacerbated by recurrent tragedies among family members.<sup>6,8,9</sup>

The requirement for cellular-based in vitro functional analysis to confirm a CRDS diagnosis has been extremely problematic given this type of analysis has only been available in research settings and those data are not accessible to most clinicians. A maneuver involving ventricular pacing that uses a 3-component stimulation sequence (termed *long-burst, long-pause, and short-coupled ventricular extra stimulus*) showed initial promise; however, this maneuver has been found to have limited sensitivity and carries the undesirable need to induce ventricular fibrillation for a positive test.<sup>6,9</sup> The findings in this report will hopefully serve as an important step toward empowering clinicians with the ability to diagnose CRDS and help avert future catastrophic outcomes.

The median absolute QT intervals and T-wave amplitudes after brief ventricular tachycardia and a pause among the human CRDS cases were significantly greater relative to the 3 control groups; however, overlap was present (Figure 2A and Figure 2C). Recognizing the existence of a broad range of normal variation in QT intervals and T-wave amplitudes, we elected to adjust for this variation through calculation of change in QT interval and change in T-wave amplitude.

Comparison of change in QT interval revealed improved discrimination between CRDS cases and controls; however, overlap was still present (Figure 2B). Remarkably, no overlap was observed for change in T-wave amplitude (Figure 2D). Beyond being readily available and easily performed, the clinical feasibility of this provocation maneuver was further emphasized by 2 cardiologists ascertaining a CRDS diagnosis with

a sensitivity of 90% and a specificity of 95% through blinded visual review of 20 tracings.

Findings in the CRDS mouse models mirrored those observed in humans, reinforcing the likelihood that these initial observations are reflective of a bona fide phenomenon. The mouse models additionally enabled evaluation of the underlying cellular mechanism and suggested that an increased calcium transient on the first postpause beat was responsible for the observed action potential duration prolongation (Figure 4). Although additional work will be necessary to fully delineate the molecular details, we hypothesize that the sarcoplasmic reticulum becomes progressively overloaded with calcium during the brief tachycardia and subsequent pause.

The resultant large calcium gradient between the sarcoplasmic reticulum and the cytosol results in a pathologically large efflux of calcium on the next beat despite the reduced activity intrinsic to these variant channels. The large release of calcium from the sarcoplasmic reticulum into the cytosol and the associated activation of the electrogenic sodium-calcium exchanger at the cell membrane are anticipated to prolong phase 2 of the action potential duration and manifest as QT interval prolongation on the surface electrocardiogram.<sup>34-38</sup>

### Limitations

This study has limitations. First, although compelling, and the mouse models provide insight into the underlying cellular mechanism, larger-scale human studies are necessary before definitively concluding this provocation maneuver as a CRDS clinical diagnostic test. The current study involves a modest number of patients, and hence it is uncertain if the full range of change in QT interval and change in T-wave amplitude values are captured. Second, the electrocardiographic tracings used for these analyses were collected both prospectively and retrospectively, creating the potential for bias.

The full results of the ongoing DIAGNOSE CRDS (NCT06188689) study should provide clarity; however, the study will take multiple years to complete. In this context, and given the urgent clinical need for a CRDS diagnostic test, a preliminary report communicating these findings was considered important.

### Conclusions

There is a unique repolarization response on an electrocardiogram after provocation with brief tachycardia and a subsequent pause in CRDS cases and mouse models, which is absent from the controls. If these findings are confirmed in larger studies, this easy to perform maneuver may serve as an effective clinical diagnostic test for CRDS and become an important part of the evaluation of cardiac arrest.

#### ARTICLE INFORMATION

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**Author Affiliations:** Libin Cardiovascular Institute, Department of Physiology and Pharmacology, University of Calgary, Calgary, Alberta, Canada (Ni, Tian, Sun, Estillore, Wang, Chen); Section of Cardiac Electrophysiology, Division of Cardiology, Department of Medicine, Hamilton Health Sciences

and McMaster University, Hamilton, Ontario, Canada (Dadon, Alak, Acosta, Divakara Menon, Tan, Vivekanantham, Pandya, Wong, Amit, Healey, Roberts); Jesselson Integrated Heart Center, Eisenberg R&D Authority, Shaare Zedek Medical Center, and Hebrew University Faculty of Medicine,

Jerusalem, Israel (Dadon, Glikson); Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, England (Ormerod, Watkins); Oxford Heart Centre, John Radcliffe Hospital, Oxford, England (Ormerod); Department of Cardiology, Faculty of Medicine and Health Sciences, Antwerp University Hospital, Antwerp, Belgium (Saenen); Cardiovascular Research, Departments of Genetics, Pharmacology and Physiopathology of Heart, Blood Vessels and Skeleton, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium (Saenen); Member of the European Reference Network for Rare, Low Prevalence, and Complex Diseases of the Heart (ERN GUARD-Heart) (Saenen, Mazzanti, van der Werf, Wilde, Jensen, Postema); Department of Clinical Cardiology, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands (Hoeksema, van der Werf, Wilde, Postema); Heart Failure and Arrhythmias, Amsterdam Cardiovascular Sciences, Amsterdam, the Netherlands (Hoeksema, van der Werf, Wilde, Postema); Section of Cardiac Electrophysiology, Division of Cardiology, Department of Medicine, Western University, London, Ontario, Canada (Antiperovitch, Khan, Gula); Montreal Heart Institute and Université de Montréal, Montreal, Quebec, Canada (Tadros); Department of Cardiology, Aarhus University Hospital, Aarhus N, Denmark (Christiansen, Jensen); Institut Universitaire de Cardiologie et de Pneumologie de Québec, Laval University, Québec City, Québec, Canada (Steinberg); Department of Cardiac Pacing and Electrophysiology, Hôpital Cardiologique du Haut-Leveque, Centre Hospitalier Universitaire de Bordeaux, Pessac, France (Arnaud, Sacher); Division of Cardiology and Centre for Cardiovascular Innovation, University of British Columbia, Vancouver, Canada (Roston, Krahn); Department of Molecular Cardiology, IRCCS Istituto Clinico Scientifici Maugeri, Pavia, Italy (Mazzanti, Priori); Department of Molecular Medicine, University of Pavia, Pavia, Italy (Mazzanti, Priori); Windland Smith Rice Genetic Heart Rhythm Clinic, Division of Heart Rhythm Services, Department of Cardiovascular Medicine, Mayo Clinic, Rochester, Minnesota (Giudicessi, Ackerman); Department of Cardiovascular Medicine, Mayo Clinic, Rochester, Minnesota (Siontis); Section of Cardiac Electrophysiology, Division of Cardiology, University of Washington Medical Center, Seattle (Nazer); Population Health Research Institute, Hamilton Health Sciences, Hamilton, Ontario, Canada (Cunningham, Healey, Roberts); Section of Cardiac Electrophysiology, Division of Cardiology, Department of Medicine, University of California, San Francisco (Scheinman); Windland Smith Rice Sudden Death Genomics Laboratory, Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, Minnesota (Ackerman); Division of Pediatric Cardiology, Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, Minnesota (Ackerman); Inherited Arrhythmia and Cardiomyopathy Program, Arrhythmia Service, Division of Cardiology, Toronto General Hospital and the University of Toronto, Toronto, Ontario, Canada (Gollub); Leviev Heart Institute, Chaim Sheba Medical Center, Ramat Gan, Israel (Nof); Tel Aviv University, Tel Aviv, Israel (Nof, Belhassen); Oxford Biomedical Research Centre and Wellcome Centre for Human Genetics, University of Oxford, Oxford, England (Watkins); Department of Clinical

Medicine, Aarhus University, Aarhus C, Denmark (Jensen); Heart Institute, Hadassah University Hospital, Jerusalem, Israel (Belhassen).

**Author Contributions:** Drs Chen and Roberts had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Ni and Dadon contributed equally and are co-first authors.

Drs Chen and Roberts contributed equally and are co-corresponding authors.

**Concept and design:** Ni, Dadon, Antiperovitch, Tian, Sun, Roston, Mazzanti, Ackerman, Glikson, Watkins, Belhassen, Chen, Roberts.

**Acquisition, analysis, or interpretation of data:** Ni, Dadon, Ormerod, Saenen, Hoeksema, Antiperovitch, Tadros, Christiansen, Steinberg, Arnaud, Tian, Sun, Estillore, Wang, Khan, Giudicessi, Siontis, Alak, Acosta, Divakara Menon, Tan, van der Werf, Nazer, Vivekanantham, Pandya, Cunningham, Gula, Wong, Amit, Scheinman, Krahn, Priori, Gollub, Healey, Sacher, Nof, Wilde, Watkins, Jensen, Postema, Belhassen, Chen, Roberts.

**Drafting of the manuscript:** Ni, Dadon, Ormerod, Antiperovitch, Arnaud, Tian, Sun, Nazer, Scheinman, Belhassen, Chen, Roberts.

**Critical review of the manuscript for important intellectual content:** Ni, Dadon, Ormerod, Saenen, Hoeksema, Antiperovitch, Tadros, Christiansen, Steinberg, Tian, Sun, Estillore, Wang, Khan, Roston, Mazzanti, Giudicessi, Siontis, Alak, Acosta, Divakara Menon, Tan, van der Werf, Nazer, Vivekanantham, Pandya, Cunningham, Gula, Wong, Amit, Scheinman, Krahn, Ackerman, Priori, Gollub, Healey, Sacher, Nof, Glikson, Wilde, Watkins, Jensen, Postema, Belhassen, Chen, Roberts.

**Statistical analysis:** Ni, Dadon, Tian, Sun, Ackerman, Roberts.

**Obtained funding:** Ni, Dadon, Wilde, Chen, Roberts.

**Administrative, technical, or material support:** Ni, Dadon, Antiperovitch, Tadros, Arnaud, Estillore, Wang, Khan, Roston, Alak, Divakara Menon, Nazer, Vivekanantham, Cunningham, Wong, Amit, Krahn, Glikson, Jensen, Chen.

**Supervision:** Khan, Acosta, Tan, Ackerman, Nof, Watkins, Belhassen, Chen, Roberts.

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