Co-Phenotype of Left Ventricular Non-Compaction Cardiomyopathy and Atypical Catecholaminergic Polymorphic Ventricular Tachycardia in Association With R169Q, a Ryanodine Receptor Type 2 Missense Mutation

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Background: Left ventricular non-compaction (LVNC) is a cardiomyopathy characterized by prominent trabeculae and intertrabecular recesses. We present the cases of 3 girls with the same ryanodine receptor type 2 (RYR2) mutation who had phenotypes of both catecholaminergic polymorphic ventricular tachycardia (CPVT) and LVNC.

Methods and Results: Clinical characteristics and genetic background of the 3 patients were analyzed retrospectively. Age at onset was 5, 6, and 7 years, respectively. Clinical presentation included syncope during exercise in all 3 patients and cardiac arrest in 2 patients. LVNC diagnosis was confirmed on echocardiography according to previously defined criteria. Exercise stress testing provoked ventricular arrhythmia in two of the patients. Beta-blockers (n=3) and flecainide (n=2) were given, and an implantable cardioverter defibrillator was used in 1 patient. Genotyping identified the same RYR2-R169Q missense mutation and no other CPVT- or LVNC-related gene mutations. Functional analysis of the mutation using HEK293 cells with single-cell Ca2+ imaging and [3H]ryanodine binding analysis, indicated a gain of function: a reduced threshold for overload-induced Ca2+ release from the sarcoplasmic reticulum and increased fractional Ca2+ release.

Conclusions: The rare association of LVNC and CPVT phenotypes with RYR2 mutations is less likely to be coincidental. Screening for life-threatening arrhythmias using exercise or pharmacologic stress tests is recommended in LVNC patients to prevent sudden cardiac death in those with preserved LV function.

Key Words: Catecholaminergic polymorphic ventricular tachycardia; Exercise-induced syncope; Left ventricular non-compaction; Ryanodine receptor type 2; Sudden cardiac death

Left ventricular non-compaction (LVNC) is a rare cardiomyopathy characterized by prominent trabeculae and intertrabecular recesses. The determinants of the clinical course and prognosis of LVNC include left ventricular (LV) dysfunction and thromboembolism as well as ventricular arrhythmias, which occur in 17% of children with LVNC, leading to sudden cardiac death. Mutations of the genes that encode mitochondria, the cell cytoskeleton, and calcium-binding proteins were identified in 29–41% of adult patients and in 17% of pediatric patients with LVNC who underwent genetic testing. Although ryanodine receptor type 2 (RYR2) mutations are one of the typical causes of catecholaminergic polymorphic ventricular tachycardia (CPVT), RYR2 mutations have also been implicated in other fatal arrhythmias, including short-coupled torsades de pointes, and even in certain types of cardiomyopathies, such as arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D), dilated cardiomyopathy (DCM), and hypertrophic cardiomyopathy (HCM). Previous reports rarely described the CPVT-LVNC overlap syndrome associated with RYR2 mutation with either exon 3 deletion, a gain-of-function mutation, or I4855M, a loss-of-function mutation. The role of RYR2 in the pathogenesis of structural heart

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Table. Clinical Data of Three Female Patients

|                     | Case 1 | Case 2 | Case 3 |
|---------------------|--------|--------|--------|
| **Age (years)**     | 5      | 6      | 7      |
| **Exercise-related syncope** | (+)    | (+)    | (+)    |
| **Cardiac arrest**  | (+)    | (−)    | (+)    |
| **Family history of sudden death** | (−)    | (+)    | (−)    |
| **Resting HR (beats/min)** | 50     | 53     | 69     |
| **QTc (s)**         | 0.461  | 0.400  | 0.400  |
| **bVT**             | (+)    | (−)    | (+)    |
| **pVT**             | (−)    | (−)    | (+)    |
| **VF**              | (+)    | (−)    | (+)    |
| **Sinus bradycardia** |        |        |        |
| **Sinus bradycardia** |        |        |        |
| **Epi/Ventricular** |        |        |        |
| **bVT**             | (+)    | (−)    | (+)    |
| **pVT**             | (−)    | (−)    | (+)    |
| **VF**              | (+)    | (−)    | (+)    |
| **Other arrhythmias** |        |        |        |
| **Initial diagnosis** |        |        |        |
| **Exercise stress test** | pVPCs, bVT | pVT | ND† |
| **Pharmacologic stress test** | ND | AVNRT by ISP | ND |
| **Echocardiogram**  |        |        |        |
| **LVEF**            | 76%    | 51%    | 67%    |
| **NC/C of Jenni’s criterion** | 2.6  | 2.1   | 2.3    |
| **X/Y of Chin’s criterion** | 0.28 | 0.44  | 0.32   |
| **Gene analysis**   |        |        |        |
| **Nucleotide change** | c.506 g>a | c.506 g>a | c.506 g>a |
| **Protein change**  | R169Q  | R169Q  | R169Q  |
| **Genotyped family members** | Trio | Only proband | Trio |
| **Inheritance**     | De novo | NA  | De novo |
| **Time from onset to diagnosis of CPVT** | 3 months | 2 weeks | 1 year |
| **Treatment**       |        |        |        |
| **Pharmacologic treatment** | Carvedilol, flecainide, enalapril | Propranolol | Carvedilol, flecainide |
| **Device treatment** | ICD    | (−)   | (−)† |
| **Outcome**         | Alive  | Alive  | Alive  |

*Resting heart rate was less than the second-degree percentile of established age- and sex-appropriate norms.23 †Already bedridden when adrenergic VT was suspected. ‡Maximum ratio of noncompacted to compacted myocardium at end-systole in the parasternal short-axis view. †X is the distance from the epicardial surface to the trough of the trabecular recess, and Y is the distance from the epicardial surface to the peak of trabeculation at end-diastole. 17 An implantable cardioverter defibrillator was not applied, but an automated external defibrillator was available at home. Age, age at onset of CPVT; AVNRT, atrioventricular nodal reentrant tachycardia; bVT, bidirectional VT; CPVT, catecholaminergic polymorphic ventricular tachycardia; ICD, implantable cardioverter-defibrillator; ISP, isoproterenol; LVEF, left ventricular ejection, fraction; NA, not applicable; ND, not done; pVPC, polymorphic VPC; pVT, polymorphic VT; VF, ventricular fibrillation; VPC, ventricular premature contraction; VT, ventricular tachycardia.

disease (SHD), however, remains unclear. In this report, we present the clinical features of 3 unrelated individuals with comorbid LVNC and atypical CPVT and carrying the same RYR2 mutation, R169Q (“atypical” here means positive for SHD, as opposed to typical CPVT, which occurs in a structurally normal heart). This mutation was previously reported as pathogenic in CPVT patients without SHD on the basis only of family history analysis.15 Although the mutation is located in a region that has been implicated in CPVT,16 its function has not been investigated. In this study, we performed functional analysis of the mutation using HEK293 cells and demonstrated that the activities of both spontaneous Ca2+ release via RYR2 and Ca2+-induced Ca2+ release (CICR) were increased.

**Methods**

**Clinical Assessment**

This report presents the cases of 3 patients with the phenotypes of both atypical CPVT and LVNC with an RYR2 mutation, R169Q. The retrospective review includes the clinical presentation at diagnosis, clinical signs and symp-
Genomic Analysis

Genomic DNA was isolated from peripheral white blood cells in each patient. Testing of 71 genes related to inherited arrhythmia and/or cardiomyopathy, including CPVT-associated genes (RYR2, CASQ2, CALM1, and TRDN) and LVNC-associated genes (LDB3, ACTC1, MYH7, TNNT2, TPM1, MYBPC3, and TAZ) was undertaken (Supplementary Table). All novel putative pathogenic variants were confirmed to be absent in the Genome Aggregation Database (http://gnomad.broadinstitute.org/about) and in the Human Genetic Variation Database, which includes more than 1,000 Japanese individuals.18

Figure 1. (A, B) Electrocardiogram (ECG), (C, D) echocardiogram and (E, F) cardiac magnetic resonance imaging (MRI) in case 1. (A) Waveforms of bidirectional ventricular tachycardia followed by ventricular fibrillation were recorded with an automated external defibrillator. (B) Standard ECG at rest shows isolated sinus bradycardia with a heart rate disproportionate to the patient’s age. (C, D) On short-axis view on the echocardiogram, the thickness ratio of the non-compacted layer to the compacted layer met the Jenni et al criteria1 at (C) end-systole, and the proportion of the distances from the epicardial surface to the trough of the trabecular recess and to the peak of trabeculation met the Chin et al criteria17 at (D) end-diastole. (E, F) The 2-chamber and 4-chamber views on cardiac MRI showed typical findings of left ventricular non-compaction with a ratio of non-compacted to compacted myocardium of 5.2, which met the Petersen et al criteria.24
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Functional Analysis of RYR2-R169Q
Using the Flp-In T-REx system (Invitrogen), we generated stable HEK293 cells that can be induced to express mouse RyR2-wild type (WT) and R169Q.\textsuperscript{19,20} Cytoplasmic Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{cyto}) and endoplasmic reticulum (ER) luminal Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{ER}) signals were monitored with G-GECO1.\textsuperscript{21} and R-CEPIA1er,\textsuperscript{22} respectively.\textsuperscript{6,20} The [\textsuperscript{3}H]ryanodine binding assay was carried out with microsomes isolated from the HEK293 cells.\textsuperscript{6,20} Details of these methods are described in Supplemental Material.

Ethics
The study protocol was approved by the University of Tsukuba Hospital ethics committee, and informed consent was obtained from each patient (or from the parents if the patient was aged <15 years or incapable of communication) by a coordinator in charge at each institution before registration of the patient data.

Results
Clinical Features and Genetic Testing
Case 1 A 5-year-old girl fainted during exercise, but physical examination and ECG on arrival at hospital were normal. Two months after the episode, she had another syncopal episode. Clinical examination was again negative and epilepsy-related syncope was suspected, although electroencephalogram was normal (Table). One month later,
she had a cardiac arrest during exercise and received bystander cardiopulmonary resuscitation (CPR), followed by automated external defibrillation (AED). The AED device recorded typical bidirectional ventricular tachycardia (VT) and ventricular fibrillation with successful defibrillation (Figure 1). The patient recovered completely from the arrhythmic storm without any neurologic sequelae. ECG recorded at rest showed regular sinus bradycardia with a heart rate (HR) of 50 beats/min in the awake state and as low as 30 beats/min during sleep (Figure 1B). Exercise stress testing induced ventricular bigeminy as well as polymorphic ventricular premature contractions (VPC) and bidirectional VT. Subsequent echocardiography showed LVNC with a preserved LV ejection fraction (LVEF) of 76% (Figure 1C,D). Cardiac magnetic resonance imaging (MRI) showed a thick non-compacted and a thin compacted myocardium layer with a ratio of the former to the latter of 5.2, which met the Petersen et al criteria (Figure 1E,F). LVEF was preserved (66%), and late gadolinium enhancement was not evident. The patient was treated with flecainide and carvedilol, and an implanted cardioverter defibrillator (ICD) with atrial pacing at 80 beats/min was subsequently implanted. Under this therapeutic regimen, exercise stress testing did not induce VT, and the ICD did not trigger appropriate or inappropriate shock. Gene testing identified the de novo RYR2-R169Q mutation. The parents of the patient underwent neither echocardiography nor exercise stress testing because they had had no symptoms from childhood and their ECG findings were normal. On genetic testing the parents were negative for the RYR2 mutation.

**Case 2** A 6-year-old girl with a family history of sudden death (2 siblings of the proband and 2 cousins of the proband’s mother; Figure 2A) fainted during exercise. Electroencephalogram and brain MRI were normal. ECG at rest showed sinus bradycardia, monomorphic VPC, and normal corrected QT interval (QTc; 0.40 s). On the first cardiac event, LV function was normal on echocardiography and no cardiomyopathy was identified. Exercise stress test provoked polymorphic non-sustained VT, and a pharmacologic stress test using i.v. isoproterenol provoked atrioventricular nodal re-entrant tachycardia. Following treatment with oral propranolol, the patient did not have syncopal attacks and exercise stress testing did not induce VT. The generalized low-voltage T waves on ECG progressed gradually, and a non-compacted structure in the mid-to-apical portion of the LV was found on repeat echocardiography conducted at age 21. LVEF was slightly reduced, to 51% (Figure 2B–E). The previous echocardiograms did not focus on the LV apical portion, and whether LVNC was present could not be determined. Cardiac MRI at age 23 showed a thick non-compacted and a thin compacted myocardium layer (Figure 2F), with a ratio of the former to the latter of 3.1. LVEF was preserved (60%), but late gadolinium enhancement was observed in the mid-myocardial layer of the mid-to-apical anteroseptal segment (Figure 2G,H). Genetic testing identified the RYR2-R169Q missense mutation. The parents of the patient did not
undergo echocardiography or exercise stress testing because they had been asymptomatic from childhood and ECG was normal. Although genetic analysis of the family members was not performed, 2 cousins of the proband underwent exercise stress tests, which were negative for ventricular arrhythmia (Figure 2A, IV-5, IV-6).

Case 3 A 7-year-old girl was diagnosed with epilepsy after 2 episodes of fainting, and valproic acid was started at a neighboring clinic. Diagnosis of attention-deficit hyperactivity disorder was also made later. At the age of 8 years, she had a cardiac arrest during exercise and received bystander CPR followed by defibrillation with an AED, which detected ventricular fibrillation (VF) and provided defibrillation shock. The family history was negative for life-threatening arrhythmia and sudden death. ECG at rest showed a regular sinus rhythm (HR, 69 beats/min) with a QTc of 0.40 s (Figure 3A). Typical bidirectional VT (Figure 3B) and VF (Figure 3C) were noted repeatedly after arrival at hospital. Echocardiography showed LVNC with preserved LVEF (67%; Figure 3D,E). Despite intensive care, the patient developed severe neurologic disabilities and became bedridden. ICD was not applied, but an AED was set up at home. After commencement of treatment with carvedilol and flecainide, 24-h Holter ECG monitoring showed no ventricular arrhythmias. Genetic testing identified a de novo RYR2-R169Q mutation. The parents of the patient had no symptoms. Both ECG and echocardiography were normal, although they did not undergo exercise stress testing. On genetic testing the parents were negative for the RYR2 mutation.

Functional Analysis
Single cell Ca²⁺ imaging in HEK cells expressing WT or R169Q was carried out 24–28 h after induction with doxycycline.
cycloheximide. Figure 4A shows representative [Ca\textsuperscript{2+}]_{cyto} and [Ca\textsuperscript{2+}]_{ER} measurements using G-GECO1.1 and R-CEPIA1er, respectively. WT cells showed spontaneous Ca\textsuperscript{2+} oscillations with corresponding periodic decrease in [Ca\textsuperscript{2+}]_{ER} in normal Krebs solution (Figure 4A Left), as reported previously.\[9\text{,}19\text{,}20\] Application of 10 mmol/L caffeine induced transient Ca\textsuperscript{2+} oscillations with amplitude similar to spontaneous oscillations (Figure 4A). The R169Q cells showed very small and more frequent Ca\textsuperscript{2+} oscillations in normal Krebs solution (Figure 4A Right). Both the threshold and nadir [Ca\textsuperscript{2+}]_{ER} in the R169Q cells were markedly reduced compared with the WT cells (Figure 4A Right C).

The Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR) activity was assessed on Ca\textsuperscript{2+}-dependent [H\textsubscript{3}]ryanodine binding.\[8\text{,}14\] WT RYR2 exhibited biphasic Ca\textsuperscript{2+}-dependent [H\textsubscript{3}]ryanodine binding and R169Q showed greater activity than WT at all Ca\textsuperscript{2+} concentrations tested (Figure 4D). On the basis of our calculation (Supplementary Figure), the estimated CICR activity of R169Q at resting Ca\textsuperscript{2+} (100 nmol/L) was 13-fold greater than that of WT at resting (100 nmol/L) Ca\textsuperscript{2+} (Figure 4E). This indicates that the R169Q mutation greatly enhanced CICR activity to facilitate spontaneous Ca\textsuperscript{2+} oscillation and to reduce [Ca\textsuperscript{2+}]_{ER} in HEK cells.

**Discussion**

We have here presented 3 unrelated cases of phenotypes of both atypical CPVT and LVNC involving the same gene mutation, RYR2-R169Q. All 3 patients had clinical features of CPVT, including exercise/emotion-related syncopal attacks and bidirectional or polymorphic VT on exercise stress testing or Holter ECG monitoring. Also, in all 3 cases, the echocardiography findings met the criteria for LVNC proposed by Jenni et al and Chin et al.\[1\text{,}7\]

The association between RYR2 mutations and LVNC has also been described in previous reports. Kanemoto et al reported an infant with LVNC and extracardiac manifestations, including facial dysmorphism and psychomotor delay.\[23\] On chromosome analysis the infant had a karyotype of 46,XX.del (1)(q43q43), with the deleted region containing RYR2, although WT was not described in that patient.\[28\] Furthermore, Szentpali et al described a case of morphologically typical LVNC and bidirectional VT during exercise stress testing involving the RYR2 mutation (c.169-198_c.273+823 del) in exon 3.\[10\] Our group also reported 2 probands with CPVT in unrelated families, with LVNC features and deletion in exon 3 of RYR2.\[11\] Genetic analysis showed the same deletion in 8 of the 12 members of the families. LVNC was also diagnosed in 7 of the 8 gene mutation carriers. Furthermore, 4 of those 7 patients showed a CPVT phenotype.\[11\] The morbidity and mortality of CPVT-LVNC overlap syndrome are uncertain because this syndrome has been only rarely reported in the literature.\[10\text{–}12\text{,}14\] In one of these cases, a family history of sudden unexplained death was reported,\[14\] as in the present case 2, although the probands were reported to be alive and being treated with medication and ICD.\[10\text{–}12\text{,}14\]

LVNC has been postulated to be a primary cardiomyopathy caused by arrest of myocardial morphogenesis between 32 and 70 days of fetal life, resulting in persistent immature myocardium, even after birth.\[1\] During the fetal period, septation of the atria and ventricles progresses, and the ventricular myocardium evolves from the trabeculated morphology into a compact structure. Typically reported gene mutations observed in LVNC include LDB3, ACTC1, MYH7, TNNT2, TPM1, MYBPC3, and TAZ, but all such mutations were not found in any of the present 3 patients. As for RYR2, in 1 animal study the expression of RYR2 mRNA in the rat myocardium was significantly lower in trabeculations than in the compact layers in the early stage of heart development,\[26\] suggesting that RYR2 may be involved in myocardial maturation.

The role of the RYR2 protein in intracellular Ca\textsuperscript{2+} dynamics and the mechanisms by which the mutated proteins induce certain types of arrhythmias or cardiomyopathies have been elucidated to some extent. Functional analysis of the RYR2-R169Q mutation in this study indicated reduced thresholds for overload-induced Ca\textsuperscript{2+} release from the sarcoplasmic reticulum and increased fractional Ca\textsuperscript{2+} release. This suggests that the mutation is associated with apparent gain of function of the channel, including delayed after-depolarization, which provokes tachyarrhythmias in CPVT.\[27\text{,}28\] With regard to RYR2-related cardiomyopathies, RYR2 NH2-terminal mutations are associated with ARVC/D, in which calcium dynamics similar to CPVT are speculated.\[7\] In that study, it was stated that impairment of the intracellular Ca\textsuperscript{2+} release mechanism associated with RYR2 mutations might induce arrhythmias and that any imbalance of intracellular calcium homeostasis could trigger apoptosis and/or cellular necrosis.\[2\] Conversely, the RYR2-A1107M mutation associated with HCM shows the opposite action (i.e., an increased threshold for Ca\textsuperscript{2+} release termination and reduced fractional release).\[9\] The precise mechanisms underlying the association of Ca\textsuperscript{2+} dynamics with cardiac morphology, however, remain unclear.

On the basis of structural analysis, R169Q is considered to diminish the size of the side chains and reduces the positive charge and stacking interaction.\[29\text{,}30\] Thus, the mutation could affect allosteric regulation without induction of a conformational change or structural instability of RYR2. It is worth noting that the same mechanism, impaired allosteric regulation, has been proposed in patients with exon 3 deletion of RYR2,\[29\] in which possible association of LVNC with CPVT has been postulated.\[10\text{–}12\] Further studies are needed to elucidate how such mutations contribute to the combined phenotypes of CPVT and LVNC.

Sinus bradycardia, atrial fibrillation, and tachycardia–bradycardia syndrome are uncommon in children with LVNC,\[2\text{,}16\text{,}31\text{,}33\] whereas resting ECG in childhood CPVT tends to show sinus bradycardia,\[33\] and in 12% of children with the RYR2 mutation, the resting HR was often lower than the second percentile of age- and sex-adjusted normal values.\[33\] In the above-cited publications, patients with LVNC combined with RYR2 exon 3 deletion developed various types of arrhythmias, including atrial arrhythmias, sinoatrial dysfunction, and VT.\[10\text{–}11\text{,}13\text{,}34\] It is noteworthy that 2 of the present 3 patients had bradycardia disproportionate to age\[23\] and VT.

The diagnosis of CPVT remains challenging.\[6\] Syncpe triggered by exertion or emotion is a characteristic symptom, but the symptoms of CPVT may occur during daily activity, while sitting quietly, or even during sleep.\[33\] A family history of syncpe or sudden cardiac death can provide a clue to the diagnosis in some cases. De novo mutations, however, were identified in 60–70% of genetically confirmed CPVT patients.\[5\text{,}8\] In the present study, only 1 patient had an apparent family history of CPVT. This highlights the need for thorough investigation owing
to the difficulty in the diagnosis of CPVT in early childhood. It is also possible that routine echocardiography might overlook the apical site of the LV, the most common site of non-compaction.\textsuperscript{39} In case 2 of the present study, the diagnosis of LVNC was established 16 years after the diagnosis of CPVT, which was in turn based on the syncopal episodes and on a positive family history. To clarify the association between CPVT and LVNC, follow-up echocardiography is needed, with special reference to the apical lesion of LV in CPVT populations.

**Study Limitations**

Although several cases of LVNC–CPVT overlap syndrome caused by either gain-of-function mutation or loss-of-function mutation of \textit{RYR2} have been reported,\textsuperscript{10–14} how \textit{RYR2} is associated with SHD or how Ca\textsuperscript{2+} handling in myocardial cells affects the cardiac structure remains to be investigated. Given that the present sample size was very small, it is also essential to study a large number of patients to elucidate whether development of LVNC occurs only in the case of some specific \textit{RYR2} mutations. Furthermore, we had limited clinical data for the deceased relatives of the probands.

**Conclusions**

In 3 unrelated patients with coexistent phenotypes of atypical CPVT and LVNC, the same \textit{RYR2}-R169Q mutation was identified. Functional analysis demonstrated a prominent gain-of-function property of the channel, suggesting that this mutation could be the cause of the malignant tachyarrhythmia observed in the present 3 cases. Given that the coexistence of atypical CPVT and LVNC may not be coincidental, patients with LVNC, especially those with arrhythmia-related symptoms, should undergo screening for adrenergic ventricular arrhythmias, including CPVT, and other arrhythmias using exercise or pharmacologic stress testing to prevent sudden cardiac death, even when the standard ECG is completely normal. Conversely, echocardiography screening for cardiomyopathy is mandatory for patients with CPVT.

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**Disclosures**

This study was conducted in the capacity of our normal salaried positions as employees of each institution. Patient costs were covered by the Japanese National Health Insurance scheme. The authors declare no conflict of interest.

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Supplementary Files
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