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Clinical and genetic characteristics of catecholaminergic polymorphic ventricular tachycardia combined with left ventricular non-compaction

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Original Article

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Abstract

Background: Catecholaminergic polymorphic ventricular tachycardia is an ion channelopathy, caused by mutations in genes coding for calcium-handling proteins. It can coexist with left ventricular non-compaction. We aim to investigate the clinical and genetic characteristics of this co-phenotype. *Methods:* Medical records of 24 patients diagnosed with catecholaminergic polymorphic ventricular tachycardia in two Chinese hospitals between September, 2005, and January, 2020, were retrospectively reviewed. We evaluated their clinical and genetic characteristics, including basic demographic data, electrocardiogram parameters, medications and survival during follow-up, and their gene mutations. We did structural analysis for a novel variant ryanodine receptor 2-E4005V. *Results:* The patients included 19 with catecholaminergic polymorphic ventricular tachycardia mono-phenotype and 5 catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap patients. The median age of onset symptoms was 9.0 (8.0,13.5) years. Most patients (91.7%) had cardiac symptoms, and 50% had a family history of syncope. Overlap patients had lower peak heart rate and threshold heart rate for ventricular tachycardia and ventricular premature beat during the exercise stress test ($p < 0.05$). Sudden cardiac death risk may be higher in overlap patients during follow-up. Gene sequencing revealed 1 novel ryanodine receptor 2 missense mutation E4005V and 1 mutation previously unreported in catecholaminergic polymorphic ventricular tachycardia, but no left ventricular non-compaction-causing mutations were observed. In-silico analysis showed the novel mutation E4005V broke down the interaction between two charged residues. *Conclusions:* Catecholaminergic polymorphic ventricular tachycardia overlapping with left ventricular non-compaction may lead to ventricular premature beat/ventricular tachycardia during exercise stress test at lower threshold heart rate than catecholaminergic polymorphic ventricular tachycardia alone; it may also indicate a worse prognosis and requires strict follow-up. ryanodine receptor 2 mutations disrupted interactions between residues and may interfere the function of ryanodine receptor 2.

Catecholaminergic polymorphic ventricular tachycardia, with an estimated prevalence of 1:10000,[1] is an inherited arrhythmia characterised by bidirectional or polymorphic ventricular tachycardia induced by emotional stress or exercise.[2] Mutations in genes coding for calcium-handling proteins, such as ryanodine receptor 2 or cardiac calsequestrin 2, cause untimely Ca^{2+} release in the sarcoplasmic reticulum, leading to delayed afterdepolarizations.[3] Similarly, calcium-handling protein disorders have also been linked to left ventricular non-compaction, which has been postulated to be a primary cardiomyopathy caused by failure in the compaction pathway due to arrest of myocardial morphogenesis.[4] Left ventricular non-compaction occurs when the compaction pathway is interrupted. The prevalence of left ventricular non-compaction is about 5:10000.[5]

In the past, catecholaminergic polymorphic ventricular tachycardia was thought to be structurally normal on echocardiography, but several studies have reported an overlap of catecholaminergic polymorphic ventricular tachycardia and left ventricular non-compaction. Some studies have reported that left ventricular non-compaction may worsen the clinical outcome of catecholaminergic polymorphic ventricular tachycardia and increase the risk of cardiac death.[6] Although the majority of catecholaminergic polymorphic ventricular tachycardia patients harbour ryanodine receptor 2 or cardiac calsequestrin 2 mutations, the genetic mutation in a significant amount of clinically diagnosed catecholaminergic polymorphic ventricular tachycardia patients remains elusive.[7] Previous studies have reported 2 ryanodine receptor 2 mutation patterns in cases of this co-phenotype, namely the R169Q mutation[8] and exon 3 deletion,[9,10] but most of the relevant genetics still needs to be explored.

In this study, we evaluated the clinical and genetic differences between catecholaminergic polymorphic ventricular tachycardia mono-phenotype and catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap patients to better understand and diagnose this lethal arrhythmia.

Materials and methods

Study design and participants

This is a retrospective, observational cohort study that enrolled all patients diagnosed with catecholaminergic polymorphic ventricular tachycardia at Beijing Tsinghua Changgung Hospital and Peking University People's Hospital between September, 2005, and January, 2020. Data were obtained from existing medical records and patients who failed to attend regular visits were contacted via telephone. Patients who were not genetically screened were not included in the analysis of genetic characteristics. Informed consents were obtained from all patients or their guardians if under 18 years old for clinical and genetic evaluation. The study was reviewed and approved by the Beijing Tsinghua Changgung Hospital Institution Review Board.

Definition

Catecholaminergic polymorphic ventricular tachycardia was diagnosed as previously described.[11] According to the generally accepted echocardiology criteria proposed by Oechslin,[12–14] a non-compacted/compacted myocardium ratio greater than 2:1 in adults or 1.4:1 in children at the end of systole on echocardiography was considered diagnostic of left ventricular non-compaction. We didn't use the cardiac magnetic resonance imaging criteria for the diagnosis of left ventricular non-compaction because some failed to complete cardiac magnetic resonance due to the claustrophobia or the expensive cost. Left ventricular non-compaction was diagnosed either at first visit or during regular follow-up (every 1 or 2 years), as long as the above criteria were reached. The QTc interval was calculated by heart rate-corrected QT according to the Bazett's method. The T-peak to T-end interval, an index of transmural dispersion of repolarization, was defined as the QT_{end} interval minus the QT_{peak} interval. Ventricular arrhythmias refer to ventricular premature beats, non-sustained ventricular tachycardia and bidirectional ventricular tachycardia, as a series of $\geq$ 3 successive ventricular premature beats. The VA score[15] refers to the worst VA observed: 1 point, no or isolated ventricular premature beats; 2 points, bigeminal ventricular premature beats; 3 points, couplet ventricular premature beats; 4 points, non-sustained ventricular tachycardia, bidirectional or polymorphic ventricular tachycardia. The standard Bruce protocol was used in the exercise stress test. We recorded the standard 12-lead ECGs at baseline (pre-exercise), at the end of each exercise stage, at peak exercise, and at the recovery phase for a minimum of 5 min. Physician in charge of the exercise stress test decided the endpoint based on the target heart rate or symptoms. Early exercise and peak exercise refer to the first and second half of the exercise duration; recovery refers to 5 min post-exercise.

Genetic evaluation

DNA analysis was performed by extracting genomic DNA from leukocytes. Fourteen genomic DNA samples of probands were sent for commercial genetic testing and one for laboratory-based genetic testing. Four samples of symptomatic relatives were sent to verify the positive results of the probands. In cases sent for commercial genetic testing, all exons of an extended list of disease-specific genes were entirely sequenced. The list included 86 arrhythmia or cardiomyopathy related genes, such as *ryanodine receptor 2, cardiac calsequestrin 2, CALM1, CALM2, CALM3, TRDN, TECRL, KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, KCNJ2, ANK2, ACTC1, DTNA, MYH7, TNNT2, TAZ, LDB3*, and *MIB1* (*See supplementary file*). For the case in the mono-phenotype group sent to a research lab, all the exons and flanking regions of ryanodine receptor 2 and cardiac calsequestrin 2 were tested. Ultra-rare variants, defined by a minor allele frequency $\leq$ 0.00005, were classified using the 2015 American College of Medical Genetics and Genomics guidelines[16] to allow for the contemporary assessment of prevalence and characterisation as "likely pathogenic," "likely benign," or "variant of unknown significance." In-silico analysis was conducted to explore the potential pathogenicity using 3D model of pig ryanodine receptor 2 by PyMOL software (Version 2.5.1).

Statistical analysis

All continuous variables are presented as median and interquartile range and compared using non-parametric test due to small sample size. Categorical variables are described as numbers (percentage) and compared using Fisher exact test. P-values < 0.05 were considered significant. Statistical analyses were performed using SAS Statistical Software Version 9.4 (SAS Institute, Cary, NC, USA). Survival analyses were described by Kaplan-Meier cumulative estimates and compared using log-rank test, via software Prism 8 (GraphPad, USA).

Results

Baseline demographic characteristics

A total of 24 patients diagnosed with catecholaminergic polymorphic ventricular tachycardia were enrolled. Five (20.8%) patients presented with catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap and 19 (79.2%) presented with catecholaminergic polymorphic ventricular tachycardia mono-phenotype. Among the 24 patients, 13 (54.2%) were males; 1 in the overlap group and 12 in the mono-phenotype group. The age of onset was 9.0 (8.0, 13.5) years and diagnosis was delayed by a median of 5.0 years. Onset age was similar between two groups ($p = 0.41$). The follow-up period was for a median of 7.0 (5.0, 10.0) years, and was also similar for the 2 groups ($p = 0.07$).

Cardiac symptoms were reported in 22 (91.7%) patients at presentation, with 10 (41.7%) of these patients having more than one presenting symptom. All overlap phenotype patients had cardiac symptoms, while 2 patients in the mono-phenotype group did not complain of any symptoms. The presenting symptoms included palpitations (10, 41.7%), syncope (21, 87.5%), and cardiac arrest (2, 8.3%). Triggers for symptoms were exercise, such as running (8), fighting (2), climbing stairs (4), taking physical education classes (3), or farm work (1); and emotional stress, such as anxiety (3), fright (3), or sadness (2). Among the five overlap patients, all had syncope (100%), two (40%) had palpitations, and one (20%) had cardiac arrest and received cardiopulmonary resuscitation. On the other hand, in the mono-phenotype group, 16 (84.2%) patients had syncope, eight (42.1%) had palpitations, and one (5.2%) experienced cardiac arrest and received cardiopulmonary resuscitation. Half of the 24 patients had a family

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Table 1. Clinical characteristics of patients with catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap and catecholaminergic polymorphic ventricular tachycardia mono-phenotype

	Total n = 24	CPVT-LVNC n = 5	CPVT mono-phenotype n = 19	p
Male n (%)	13 (54.2%)	1 (20.0%)	12 (63.2%)	0.14
Age at onset (years)	9.0 (8.0, 13.5)	9.0 (5.0, 12.0)	9.0 (8.0, 19.0)	0.41
Age at diagnosis (years)	14.0 (10.5, 31.0)	16.0 (12.0, 27.0)	14.0 (10.0, 35.0)	0.97
Follow-up duration (years)	7.0 (5.0, 10.0)	2.0 (2.0, 5.0)	8.5 (6.0, 10.0)	0.07
Presenting symptoms n (%)				
Palpitation	10 (41.7%)	2 (40.0%)	8 (42.1%)	1.00
Syncope	21 (87.5%)	5 (100%)	16 (84.2%)	1.00
Cardiac arrest	2 (8.3%)	1 (20.0%)	1 (5.3%)	0.38
Family history of syncope n (%)	12 (50.0%)	2 (40.0%)	10 (52.6%)	1
Resting ECG				
HR (bpm)	63 (53, 69)	59 (54, 61)	66 (52, 72)	0.25
PR duration (ms)	135.0 (123.0, 147.0)	148.0 (136.0, 156.0)	132.0 (122.0, 146.0)	0.06
QRS duration (ms)	89.0 (82.0, 96.0)	88.0 (84.0, 90.0)	90.0 (80.0, 96.0)	0.64
QTc (ms)	413.5 (405.0, 427.5)	423.0 (392.0, 426.0)	413.0 (405.0, 429.0)	0.86
Tp-ec (ms)	97.0 (82.0, 116.5)	100.0 (80.0, 124.0)	97.0 (84.0, 114.0)	0.80
LVEF%	67.0 (62.5, 70.5)	70.0 (67.0, 73.0)	66.0 (62.0, 70.0)	0.13
Gene sequencing n (%)	18 (75.0%)	4 (80.0%)	14 (73.7%)	1
Medication n (%)				
β-blockers	21 (87.5%)	4 (80.0%)	17 (89.5%)	0.38
Flecainide	1 (4.2%)	1 (20.0%)	0	0.21
CCB	5 (20.8%)	2 (40.0%)	3 (15.8%)	0.57
Surgical/device therapy n (%)				
PM	3 (12.5%)	1 (20%)	2 (10.5%)	0.52
ICD	2 (8.3%)	0	2 (10.5%)	1
LCSD	2 (8.3%)	1 (20%)	1 (5.3%)	0.38
RFCA	1 (4.2%)	0	1 (5.3%)	1
Cardiac death n (%)	4 (16.7%)	2 (40%)	2 (10.5%)	0.18

CCB = calcium channel blocker; ICD = implantable cardiac defibrillator; LCSD = left cardiac sympathetic denervation; LVEF = left ventricular ejection fraction; PM = pace maker; RFCA = radiofrequency catheter ablation.

history of syncope or sudden death, with two (40%) in the overlap phenotype group and 10 (52.6%) in the mono-phenotype group. Echocardiography of the five overlap patients specifically showed obvious hypertrabeculation in the left ventricular myocardium, and the non-compacted/compacted myocardium ratio was greater than 2.0.(Table 1; *supplementary file*).

Resting ECG parameters

We compared the differences in resting ECG parameters, like heart rate, PR duration, QRS complex duration, QTc interval, and T-peak to T-end interval between the two groups. Compared to catecholaminergic polymorphic ventricular tachycardia mono-phenotype patients, catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap patients showed no significant difference in resting ECG parameters. (Table 1).

Ventricular arrhythmias in catecholaminergic polymorphic ventricular tachycardia and catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap patients

Clinical profiles and exercise stress test parameters are summarised in Table 2. One patient was unable to complete exercise stress test due to leg problem and was not included in this analysis. Two catecholaminergic polymorphic ventricular tachycardia mono-phenotype patients were not included in the analysis of verntricular tachycardia because they only had PVC during exercise stress test. Heart rate at peak exercise and also threshold for ventricular premature beat and bidirectional ventricular tachycardia/polymorphic venticular tachycardia(pVT) were lower in the catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap group than in the catecholaminergic polymorphic ventricular tachycardia mono-phenotype

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Table 2. Clinical profiles and EST parameters of patients with catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap and catecholaminergic polymorphic ventricular tachycardia mono-phenotype.

	Total n = 23	CPVT-LVNC n = 5	CPVT mono-phenotype n = 18	p
Age at EST (yrs)	13.0 (10.0, 26.0)	18.0 (12.0, 26.0)	13.0 (10.0, 21.0)	0.88
HR at baseline (bpm)	71 (65, 92)	71 (59, 79)	71.5 (65, 92)	0.43
HR at peak exercise (bpm)	166 (150, 190)	146 (139, 150)	177 (153, 193)	<0.01
Exercise duration (min)	8.8 (6.8, 10.2)	9.5 (9.5, 11.0)	8.5 (6.5, 9.4)	0.04
Maximum workload attained	8.8 (6.3, 10.2)	6.9 (5.9, 10.9)	9 (7, 10.2)	0.71
VA score				
Baseline	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	0.32
Early exercise	2.0 (1.0, 3.0)	2.0 (2.0, 4.0)	2.0 (1.0, 3.0)	0.48
Peak exercise	4.0 (4.0, 4.0)	4.0 (4.0, 4.0)	4.0 (4.0, 4.0)	0.80
Recovery	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	0.32
Threshold HR for VPB (bpm)	118 (109, 130)	106 (106, 112)	123.5 (111.5, 133.5)	0.03
Threshold HR for VT (bpm)	149 (142, 166)	126 (121, 130)	151 (148, 170)*	0.01

EST = exercise stress test; VA = ventricular arrhythmia; VPB = ventricular premature beat; VT = ventricular tachycardia.
*16 catecholaminergic polymorphic ventricular tachycardia mono-phenotype patients were included in the analysis for VT because two only had PVC.

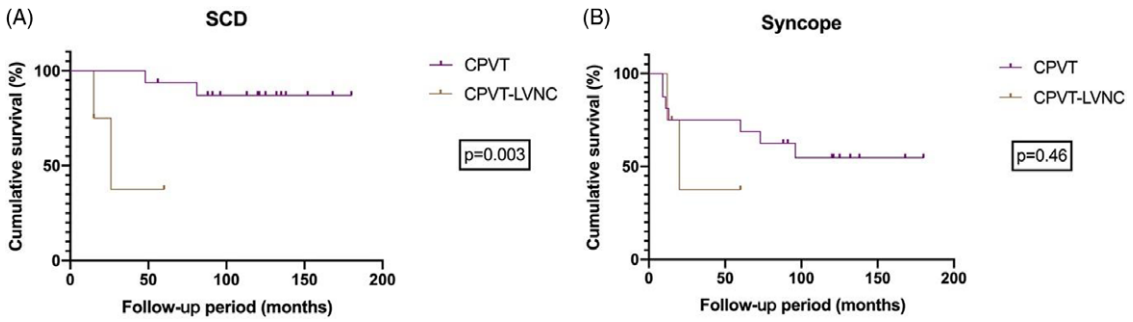

Figure 1. Kaplan-Meier analysis of cumulative sudden cardiac death-free (A) and syncope-free (B) survival in patients with catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction and catecholaminergic polymorphic ventricular tachycardia mono-phenotype. Survival risk was compared between two groups by log-rank test.

group ($p < 0.05$). Exercise duration was longer in overlap group than mono-phenotype group ($p = 0.04$). There were no statistical differences in age at exercise stress test, heart rate at baseline, or maximum working load during exercise stress test between two groups. In 23 catecholaminergic polymorphic ventricular tachycardia patients, the VA scores were similar between two groups in four phases of exercise stress test.

Medication and survival

Twenty-one (87.5%) patients used β-blockers, 17 (89.5%) mono-phenotype patients took β-blockers, 1 symptomless relative reported not to be taking any medication, and another patient stopped the medication due to severe fatigue and nausea. One (20%) of the overlap phenotype patients autonomously stopped taking β-blockers. Flecainide was not widely used among the catecholaminergic polymorphic ventricular tachycardia patients, due to difficulty in accessing the drug in China. Only 1 (20%) of the overlap phenotype patients took flecainide in addition to a β-blocker because she was intolerant to the maximum dosage. Five (20.8%) patients took a calcium channel blocker, 2 (40%) in the overlap phenotype group and 3 (15.8%) in the mono-phenotype group. Pacemakers were implanted in 3 (12.5%) patients to increase their tolerance to β-blockers. Only 2 (8.3%) mono-phenotype patients received an implantable cardiac defibrillator, and 2 (8.3%) patients had undergone left cardiac sympathetic denervation. One patient received radiofrequency catheter ablation surgery due to recurrent atrial tachycardia. So far 4 of the 24 patients have died of cardiac death (16.7%), 2 in each group. Among the deceased patients, the trigger was excessive exercise, like skiing, stairs-climbing, and fighting (Table 1; *supplementary file*). Survival analysis was conducted in 20 probands instead of all patients, as we considered probands should have worse survival than family members. It showed catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap patients had higher risk of sudden cardiac death (heart rate 179, $p = 0.003$) despite medication, while syncope risk was comparable between the two groups ($p = 0.46$) (Fig. 1).

Genetic characteristics

Genetic tests were administered to 18 of the 24 patients. Nine different ryanodine receptor 2 missense mutations were detected in 13 (72.2%) patients (Table 3). Of the nine ryanodine

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Table 3. Ryanodine receptor 2 variants in patients with catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction and catecholaminergic polymorphic ventricular tachycardia mono-phenotype

Variant	Amino acid change	Phenotype	dbSNP ID (NCBI)	ACMG
c.1298T > C	L433P	CPVT	rs121918602	LP
c.5278C > T	R1760W#	CPVT-LVNC	rs776550479	VUS
c.6949G > A	A2317T	CPVT	rs1553263743	VUS
c.11836G > A	G3946S	CPVT	rs794728777	LP
c.12014A > T	E4005V*	CPVT-LVNC	–	VUS
c.12272C > T	A4091V	CPVT	rs794728783	LP
c.13786C > T	P4596S	CPVT	–	VUS
c.14311G > A	V4771I	CPVT	rs794728804	LP
c.14461G > A	V4821I	CPVT	–	VUS

ACMG = the American College of Medical Genetics and Genomics; LP = likely pathogenic; VUS = variant of uncertain significance.
*Novel.
#First reported in catecholaminergic polymorphic ventricular tachycardia.

receptor 2 missense mutations, c.12014A > T (p.E4005V) is the best of our knowledge, reported here for the first time. While c.5278C > T (p.R1760W)[17] was previously reported in long QT syndrome patients but not catecholaminergic polymorphic ventricular tachycardia patients. The remaining seven mutations: c.1298T > C (p.L433P) [17–19], c.6949G > A (p.A2317T),[20] c.11836G > A(G3946S),[21,22] c.12272C > T (p.A4091V),[17,19] c.13786C > T(p.P4596S) [23,24] c.14311G > A(V4771I),[19,22] and c.14461G > A(V4821I)[21] were previously reported in catecholaminergic polymorphic ventricular tachycardia patients in other studies. No patients carried mutations of the genes pathognomonic of left ventricular non-compaction, like *DTNA, MYH7, TNNT2*, and *TAZ*. The structural features of these ryanodine receptor 2 mutations are shown in Figure 2.

Three of the four (75%) catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap patients who were administered the genetic test carried ryanodine receptor 2 mutations; the newly reported mutation E4005V was detected in a male patient and his symptomatic mother. The site of this mutation appears to be close to the cytosolic calcium sensor region.[25] Another girl with the overlap phenotype harboured the R1760W mutation, which was previously reported in long QT syndrome patients.[17] The SNP of cardiac calsequestrin 2 (p.T66A) was detected in the fourth overlap phenotype girl.

In the mono-phenotype group, 14 patients were subjected to genetic testing. Ten (71.4%) patients had ryanodine receptor 2 mutations, and one harboured the cardiac calsequestrin 2-T66A SNP. The ryanodine receptor 2 mutation site A2317T was detected in a 21-year-old female patient and was located at the site of FKBP12.6 binding. The L433P mutation, located in the ryanodine receptor 2 N-terminal domain, was found in a family, where it was also carried by a sister, a brother, and the sister's son. The G3946S mutation was detected in a mother and her son, both having experienced exercise-induced syncope. The P4596S, V4821I, A4091V, and V4771I mutations are located near the transmembrane regions, while V4821I is close to the pore area.[25]

The novel mutation E4005V resulted in a glutamic acid changed to valine at the 4005th position of the ryanodine receptor 2 protein. We performed in-silico analysis and modelling based on the pig 3D ryanodine receptor 2 structure,[26] and found the missense mutation broke down the interaction between charged residues (Fig. 3). In closed state of ryanodine receptor 2, the negative charged glutamic acid (E4005) was strongly banded to positively charged lysine located in close subunit (K173), and thus may stabilise the closed state of pore area and strengthen the 3D structure of ryanodine receptor 2. While the strong interaction might be disrupted by E4005V mutation because the valine was not charged. This interaction was not seen in ryanodine receptor 2 open state, which might indicate its importance in stabilisation of closed ryanodine receptor 2.

Discussion

Our study presents the clinical and genetic characteristics of 24 Chinese catecholaminergic polymorphic ventricular tachycardia patients. To the best of our knowledge, this is the first study to evaluate the differences between patients with catecholaminergic polymorphic ventricular tachycardia mono-phenotype and catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap. We evaluated the clinical information and expanded the genetic spectrum of catecholaminergic polymorphic ventricular tachycardia, in particular catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction patients, and explored the unique characteristics of this rare syndrome.

Although catecholaminergic polymorphic ventricular tachycardia has been traditionally defined as structurally normal, some case reports have already disclosed clinical cases of overlap between catecholaminergic polymorphic ventricular tachycardia and left ventricular non-compaction. As early as in 2013, Szentpali et al.[9] reported the first case of morphologically typical left ventricular non-compaction and bidirectional ventricular tachycardia during exercise stress test with a deletion of exon 3 in ryanodine receptor 2 (c.169-198_273 + 823del). Later, two more case reports also indicated that the deletion of exon 3 in ryanodine receptor 2 caused catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap syndrome.[6,10] Besides large deletions in exon 3, Roston et al.[27] and Nozaki et al.[8] described missense mutations (ryanodine receptor 2-I4855M, ryanodine receptor 2-R169Q) in catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction patients. Our study expanded the genetic spectrum by identifying one more missense mutations ryanodine receptor 2-E4005V in this overlap syndrome. Ryanodine receptor 2-E4005V is specifically a novel mutation reported for the first time. Our study found that ryanodine receptor 2-E4005V broke down the interaction between charged residues in closed state, which indicated the variant might be pathogenetic. While ryanodine receptor 2-R1760W was reported for the first time in catecholaminergic polymorphic ventricular tachycardia.

The mechanism by which ryanodine receptor 2 mutations cause catecholaminergic polymorphic ventricular tachycardia has been studied for years. Mutations in ryanodine receptor 2 result in inappropriate calcium leakage from the sarcoplasmic reticulum, associated with impairment of the intracellular Ca^{2+} release mechanism might induce arrhythmias, and imbalances in intracellular Ca^{2+} homeostasis could trigger cellular apoptosis or necrosis.[8,18] Ryanodine receptor 2 NH2-terminal mutations are related to arrhythmogenic right ventricular cardiomyopathy/dysplasia and dilated cardiomyopathy, in which Ca^{2+} dynamics

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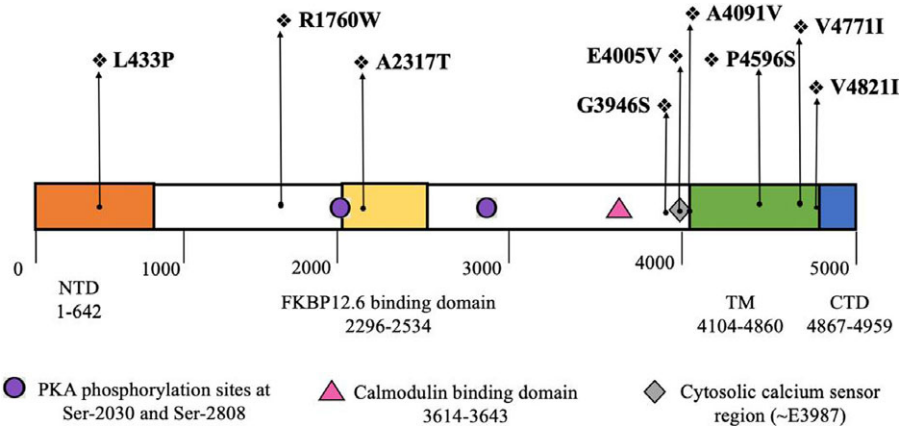

Figure 2. Ryanodine receptor 2 mutation sites in patients with catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction and catecholaminergic polymorphic ventricular tachycardia mono-phenotype. Orange box: N-terminal region; yellow box: FKBP12.6 binding domain; green box: transmembrane region; blue box: C-terminal region. (Refer to http://triad.fsm.it/cardmoc/).

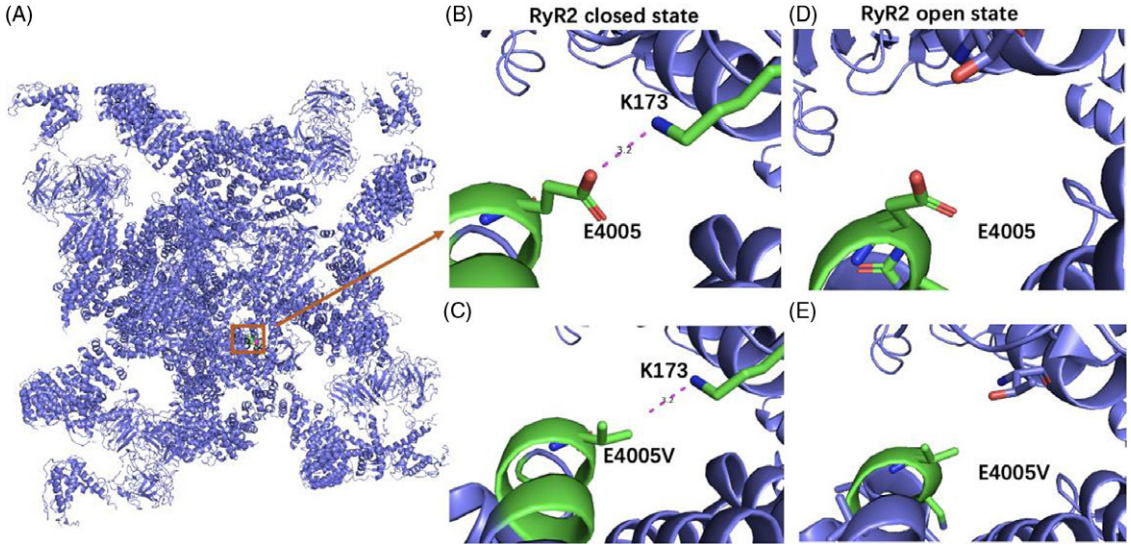

Figure 3. Ryanodine receptor 2-E4005V mutation disrupted the banding of charged residues. ***a***: 3D structure of the pig ryanodine receptor 2 by pyMol (version 2.5.1) and PDB file (PDB code: 5goa for ryanodine receptor 2 in open state; 5go9 for ryanodine receptor 2 in closed state). ***b***: E4005 and K173 were strongly banded in the closed state of ryanodine receptor 2. ***c***: the banding was disrupted when glutamic acid was mutated to valine (E4005V) in the closed state of ryanodine receptor 2. ***d***: E4005 was not banded to any amino acid in the open state of ryanodine receptor 2. E: E4005V in the open state of ryanodine receptor 2.

similar to that occurring in catecholaminergic polymorphic ventricular tachycardia is speculated to take place.[28] Left ventricular non-compaction is known to be caused by mutations in sarcomeric genes and also other genes linked to cardiomyopathies. A large deletion of exon 3 in ryanodine receptor 2 appears to increase fractional sarcoplasmic reticulum Ca^{2+} release and reduces the threshold for Ca^{2+} release termination, allowing excess of Ca^{2+} into the cytoplasm, which may induce left ventricular non-compaction.[10,28] Missense mutations of ryanodine receptor 2 also change the Ca^{2+} dynamics, and thus may induce left ventricular non-compaction.[8,27] Further studies are needed to explore whether Ca^{2+} processing abnormalities can cause left ventricular non-compaction in vivo.

In our study, responses to exercise stress test differed between the two groups. Patients with overlap phenotype showed lower threshold heart rate for ventricular tachycardia and ventricular premature beat than those with mono-phenotype ($p<0.05$). Survival analysis indicated overlap patients had higher risk of sudden cardiac death. Thus, it appears that left ventricular non-compaction may worsen the prognosis of catecholaminergic polymorphic ventricular tachycardia. Matthew et al.[6] also described a case in which a girl with catecholaminergic polymorphic ventricular tachycardia-left ventricular non-compaction overlap experienced a series of sudden cardiac arrests despite adequate medication and implantable cardiac defibrillator therapy. Some studies have proposed that left ventricular non-compaction reflects early myocardial morphogenesis abnormality. Ventricular arrhythmias are estimated to occur in about 20–30% of isolated left ventricular non-compaction cases, and its origination from an arrhythmogenic substrate secondary to endomyocardial fibrosis not limited to only the trabeculated tissue has been considered.[29] Literature has been reviewed to explain the lower threshold for ventricular arrhythmias in catecholaminergic polymorphic ventricular tachycardia-left ventricular non-

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compaction overlap patients, but there are limited studies. We hypothesise that it may be related to cardiac vagal activity, which could shorten the ventricular effective refractory period and lower the ventricular tachycardia threshold if acutely inhibited.[30] As vagal neurones are located in the epicardium of the atria and the ventricular septum,[30] which might be damaged by ventricular non-compaction and thus affect exercise stress test performance. The deeper relationship should be explored via basic experiment to better understand and explain clinical manifestations.

Limitations

Our study included clinically diagnosed catecholaminergic polymorphic ventricular tachycardia patients in two Chinese hospitals, but the diagnosis could not be confirmed in all cases. Medication, including the types of β-blockers, was different in the 24 patients when exercise stress test was conducted. Genetic tests were not done for all patients and those for whom they were done, the tests were not performed in the same lab, although the standards for genetic screening were comparable. We conducted in-silico structural analysis to predict function but not cytological experiment, which may lack accuracy. The interpretation of our findings might be limited by the sample size, and our results need to be confirmed on a large-scale multi-centre research.

Conclusions

Cases of catecholaminergic polymorphic ventricular tachycardia overlapping with left ventricular non-compaction were found, and had lower threshold heart rate for ventricular tachycardia and ventricular premature beat during exercise stress test than monophenotype cases and may have higher sudden cardiac death risk during follow-up in this small-scale study, which needs further verification. We found one novel ryanodine receptor 2 missense mutation E4005V, which may be associated with the overlap and broken down charged residues in 3D structure of ryanodine receptor 2 closed state. Functional analysis was needed to dig into the novel variant. The appearance of left ventricular non-compaction in catecholaminergic polymorphic ventricular tachycardia patients may prompt a worse prognosis and requires strict clinical follow-up. Further large-scale investigations are needed to confirm these findings.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1047951123003086.

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Competing interests. None.

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