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Linking the heart and the brain: Neurodevelopmental disorders in patients with catecholaminergic polymorphic ventricular tachycardia

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BACKGROUND Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an uncommon inherited arrhythmia disorder characterized by adrenergically evoked ventricular arrhythmias. Mutations in the cardiac calcium release channel/ryanodine receptor gene (*RYR2*) are identified in the majority of patients with CPVT. RyR2 is also the major RyR isoform expressed in the brain.

OBJECTIVE The purpose of this study was to estimate the prevalence of intellectual disability (ID) and other neurodevelopmental disorders (NDDs) in RYR2-associated CPVT (CPVT1) and to study the characteristics of these patients.

METHODS We reviewed the medical records of all CPVT1 patients from 12 international centers and analyzed the characteristics of all CPVT1 patients with concomitant NDDs. We functionally characterized the mutations to assess their response to caffeine activation. We did not correct for potential confounders.

RESULTS Among 421 CPVT1 patients, we identified 34 patients with ID (8%; 95% confidence interval 6%–11%). Median age at diagnosis was 9.3 years (interquartile range 7.0–14.5). Parents for 24 of 34 patients were available for genetic testing, and 13 of 24 (54%) had a *de novo* mutation. Severity of ID ranged from mild to severe and was accompanied by other NDDs in 9 patients (26%). Functionally, the ID-associated mutations showed a markedly enhanced response of RyR2 to activation by caffeine. Seventeen patients (50%) also had supraventricular arrhythmias. During median follow-up of 8.4 years (interquartile range 1.8–12.4), 15 patients (45%) experienced an arrhythmic event despite adequate therapy.

CONCLUSION Our study indicates that ID is more prevalent among CPVT1 patients (8%) than in the general population (1%–3%). This subgroup of CPVT1 patients reveals a malignant cardiac phenotype with marked supraventricular and ventricular arrhythmias.

KEYWORDS Catecholaminergic polymorphic ventricular tachycardia; RYR2; Supraventricular arrhythmia; Ventricular arrhythmia

(Heart Rhythm 2019;16:220–228)

Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an uncommon inherited arrhythmia disorder predisposing patients to life-threatening ventricular arrhythmias (VAs), especially under circumstances of emotion- or exercise-induced stress.[1] The 12-lead resting electrocardiogram usually is normal, and patients have a structurally normal heart. Typically, patients present between the age of 7-13 years with syncope or cardiac arrest.[2]

Mutations in the ryanodine receptor 2 gene (*RYR2*), the most important gene implicated in CPVT, are identified in approximately 50%–60% of CPVT patients (CPVT1).[3] *RYR2* encodes the ryanodine receptor 2, also known as the Ca^{2+} release channel, which mediates Ca^{2+} release from the sarcoplasmic reticulum and is required for excitation–contraction coupling in the heart.[4] Mutations in *RYR2* ultimately lead to spontaneous diastolic Ca^{2+} release and, especially in the presence of catecholamines, cause delayed afterdepolarizations that can trigger VAs.[4]

Although *RYR2* is referred to as the cardiac isoform of the ryanodine receptor, it is also widely expressed in the brain and exerts a role in intracellular Ca^{2+} signaling and homeostasis in the central nervous system.[5] Studies in mice have linked mutations in *RYR2* to neuronally mediated seizures, independent of cardiac arrhythmias.[6] Mice with leaky RyR2 channels also display stress-induced cognitive dysfunction.[7] In addition, 4 case reports have reported a CPVT1 patient with either seizures or intellectual disability (ID).[8–11]

Based on these observations, we hypothesized that neurodevelopmental disorders (NDDs), particularly ID, may be more prevalent in patients with CPVT1. Our aim was to estimate the prevalence of ID/NDDs among CPVT1 patients and to study the clinical and molecular genetic characteristics of this specific patient subgroup.

Methods

Study population

For this retrospective observational cohort study, we reviewed the medical records of all consecutive patients (both probands and relatives) with *RYR2*-associated CPVT (ie, CPVT1) from the departments of cardiology, pediatric cardiology, or clinical genetics of 12 tertiary referral centers in Japan, the United States, the United Kingdom, and The Netherlands. Patient records with entries indicating a diagnosis of ID and/or other NDDs were selected for further study.

ID is characterized by significant limitations in both intellectual functioning (an intelligence quotient of approximately 70 or below) and adaptive behavior, including conceptual, social, and practical skills, with onset during the developmental period.[12] Four levels of severity can be specified: mild, moderate, severe, and profound (Supplemental Table 1). Other NDDs include communication disorders, autism spectrum disorder, attention-deficit/hyperactivity disorder, specific learning disorder, and motor disorders. We evaluated whether other causes for ID had been excluded.

Patients with an aborted cardiac arrest (ACA) were included only if the diagnosis of ID was made before this event to prevent the inclusion of patients with a NDD due to postanoxic encephalopathy. Previously performed brain computed tomography or magnetic resonance imaging scans were reassessed by an experienced neurogeneticist (GMSM) in order to exclude postanoxic encephalopathy.

The institutional review board of the participating centers approved the data collection for the study by giving a waiver for obtaining (written) informed consent or by giving formal approval for the study depending on local policies. The study complied with the principles of the Declaration of Helsinki.

Cardiologic characteristics at baseline and during follow-up

Clinical data were obtained by reviewing existing patient records for clinical history, treatment, arrhythmic events, and current vital status. Arrhythmic events were defined as probable or proven arrhythmic syncope, ACA, appropriate implantable cardioverter–defibrillator shock, and sudden cardiac death. The endpoint of follow-up was defined as the date of last contact or the date of death.

Genetic testing

All patients received genetic counseling and consented to genetic testing. The mutation nomenclature recommendations from the Human Genome Variation Society were followed.[13] A detailed description of the mutational analysis can be found in the Supplemental Material.

Construction of *Ryr2* missense mutations

Missense mutations in the mouse *Ryr2* cDNA were generated by the overlap extension method using polymerase chain reaction (Supplemental Material).[14,15]

Caffeine-induced Ca^{2+} release in HEK293 cells

The free cytosolic Ca^{2+} concentration in transfected HEK293 cells was measured using the fluorescence Ca^{2+} indicator dye Fluo-3 (Molecular Probes, Eugene, Oregon).[16] Details of the experiments can be found in the Supplemental Material.

Statistical analysis

Statistical analyses were performed using IBM SPSS statistics version 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp). Continuous data are given as median (interquartile range [IQR]) or mean ± standard error of mean (SEM) where appropriate, and categorical variables as given as number (percentage; 95% confidence interval [CI]). To test for differences between groups, we used the Student *t* test (2-tailed). $P < .05$ was considered significant.

Results

Study population

Among 421 patients with CPVT1, we identified 34 patients (8%; 95% CI 6%–11%) with concomitant ID with or without other NDDs (Table 1 and Supplemental Table 2). Median age at diagnosis was 9.4 years (IQR 7.0–15.5). Thirty-one patients (91%) had a history of cardiac symptoms before the diagnosis of CPVT, including 20 patients (59%) with syncopal episodes and 5 patients (15%) with ACA. Three patients were identified through cascade screening within their family. In 1 patient (no. 10), CPVT1 was diagnosed postmortem by confirming the presence of the familial *RYR2* mutation.

Table 1 Characteristics of CPVT patients with concomitant ID (n = 34)

Male	17 (50)
Age at diagnosis (y)	9.4 (7.0–15.5)
History of cardiac symptoms	31 (91)
Syncope	20 (59)
Aborted cardiac arrest	5 (15)
Arrhythmia at Holter or exercise testing	
Supraventricular	17 (50)
Ventricular	33 (97)
Therapy	
β-Blocker	32 (97)
Flecainide	19 (58)
ICD	14 (42)
Left cardiac sympathetic denervation	11 (33)
Follow-up (y)	8.4 (1.8–12.0)
Arrhythmic event during follow-up	15 (45)
Syncope	3 (9)
Appropriate ICD shock	4 (12)
Aborted cardiac arrest	5 (15)
Sudden cardiac death	3 (9)

Values are given as n (%) or median interquartile range.

CPVT = catecholaminergic polymorphic ventricular tachycardia; ICD = implantable cardioverter–defibrillator; ID = intellectual disability.

ID and other NDDs

The severity of ID ranged from mild to severe but was mainly in the mild range (Supplemental Table 2). ID was accompanied by other NDDs in 9 patients (26%), including 4 patients with autism spectrum disorder, 3 with attention-deficit/hyperactivity disorder, and 2 with both disorders (Supplemental Table 2).

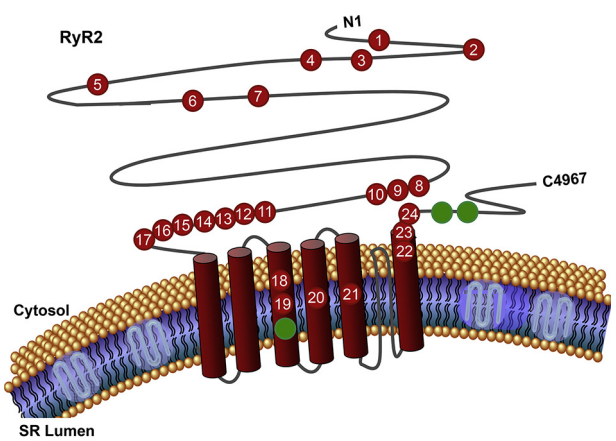

Figure 1 Location of *RYR2* mutations. *Red dots* indicate the distinct mutations found in this cohort. *Green dots* indicate the mutations that have previously been linked to intellectual disability and epilepsy.[10,11,13] SR = sarcoplasmic reticulum.

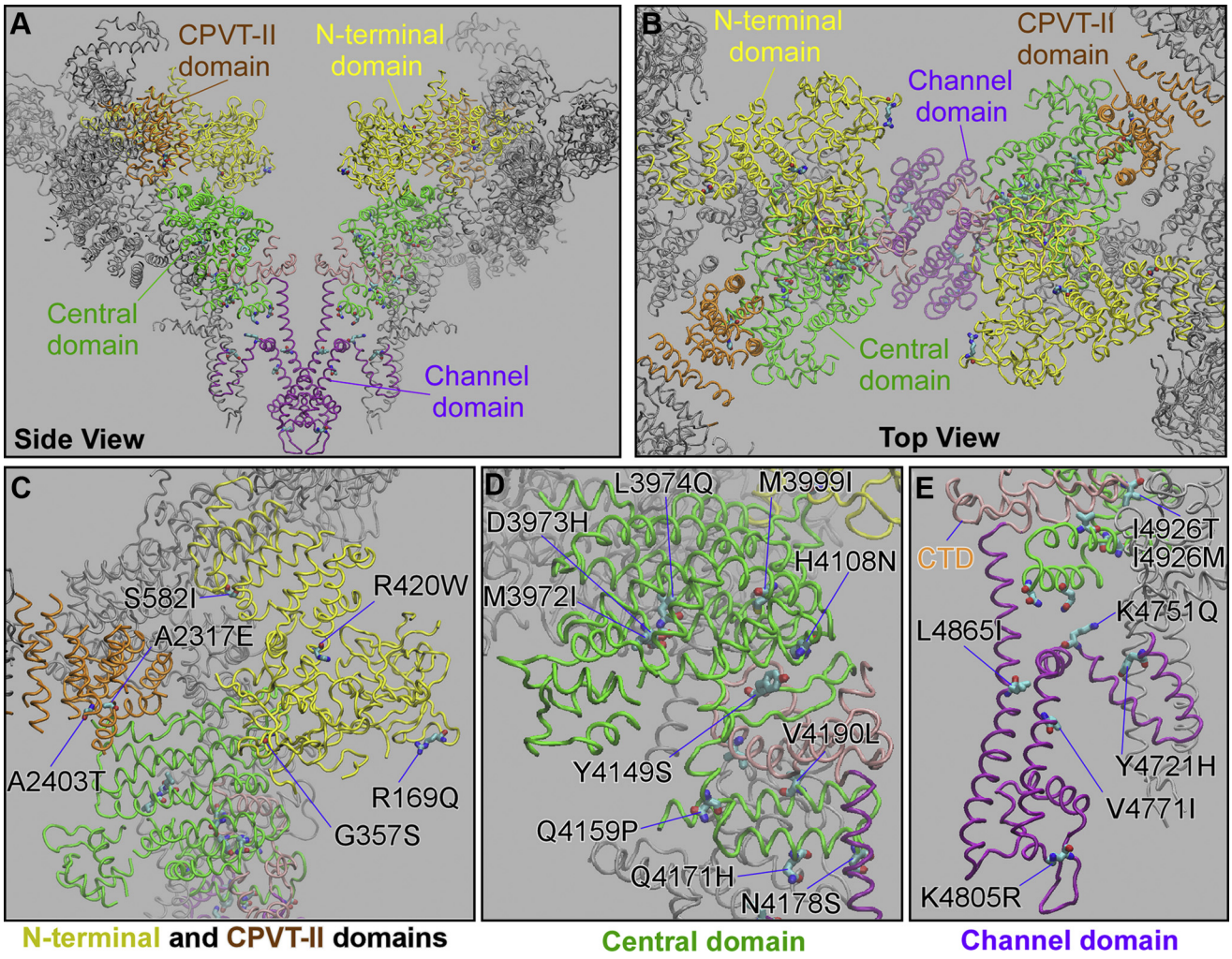

Figure 2 Location of *RYR2* mutations associated with intellectual disability in the 3-dimensional structure of RyR2 protein. Four major regions that contain *RYR2* mutations are highlighted by different colors: hotspot-I (N-terminal domain; *yellow*), hotspot-II (*orange*), hotspot-III (central domain; *green*), and hotspot-IV (channel domain; *purple*). The side **(A)** and top **(B)** views of 2 RyR2 monomers are shown.[34] *RYR2* mutations associated with neurodevelopmental disorders located in the N-terminal and CPVT-II domains **(C)**, the central domain **(D)**, and the channel domain **(E)** are depicted. Note that most of these mutations are located in the central domain, which is known to be critical for cytosolic Ca^{2+} activation of RyR2.[33,35] CPVT = catecholaminergic polymorphic ventricular tachycardia.

Brain imaging studies were available in 10 patients (29%). In general, no gross structural abnormalities or signs of post-anoxic encephalopathy were observed before the onset of cardiac arrhythmias. Minor abnormalities were identified in 5 patients (Supplemental Material and Supplemental Figure 1).

Genetics

The 34 *RYR2* mutations identified in these patients included 23 missense mutations and 1 splice site mutation and clustered to the known hotspot regions in *RYR2* (Figure 1 and Supplemental Table 3).[3] In a 3-dimensional structure of RyR2, 10 mutations (43%) clustered in the central domain (Figure 2).

Parents of 24 patients were available for genetic testing; in 13 patients (54%) a *de novo* mutation was identified. In 4 families, the *RYR2* mutation cosegregated with the ID (Figure 3). In 2 families (Figures 3B and 3D), the *RYR2* mutation was inherited from a parent in whom mosaicism for the mutation was detected.[3]

Sensitivity analysis

To correct for a potential familial enrichment driving the association, we calculated the prevalence of ID excluding the 11 familial cases. This yielded 23 patients with ID among 410 screened patients and an ID prevalence of 6% (95% CI 4%–8%). In 3 patients (no. 17, 31, and 33), an additional genetic substrate that may have contributed to the neurologic phenotype was identified. Excluding these 3 patients and the 11 familial cases yielded an ID prevalence of 5% (95% CI 3%–7%).

Functional impact of *RYR2* mutations

To gain insights into the functional impact of the *RYR2* mutations associated with ID/NDDs, we generated 16 of the 24 *RYR2* mutations identified in our cohort using site-directed mutagenesis and assessed their responses to caffeine activation. Figure 4 shows intracellular Ca^{2+} elevations in HEK293 cells expressing the murine Ryr2 wild-type (WT)

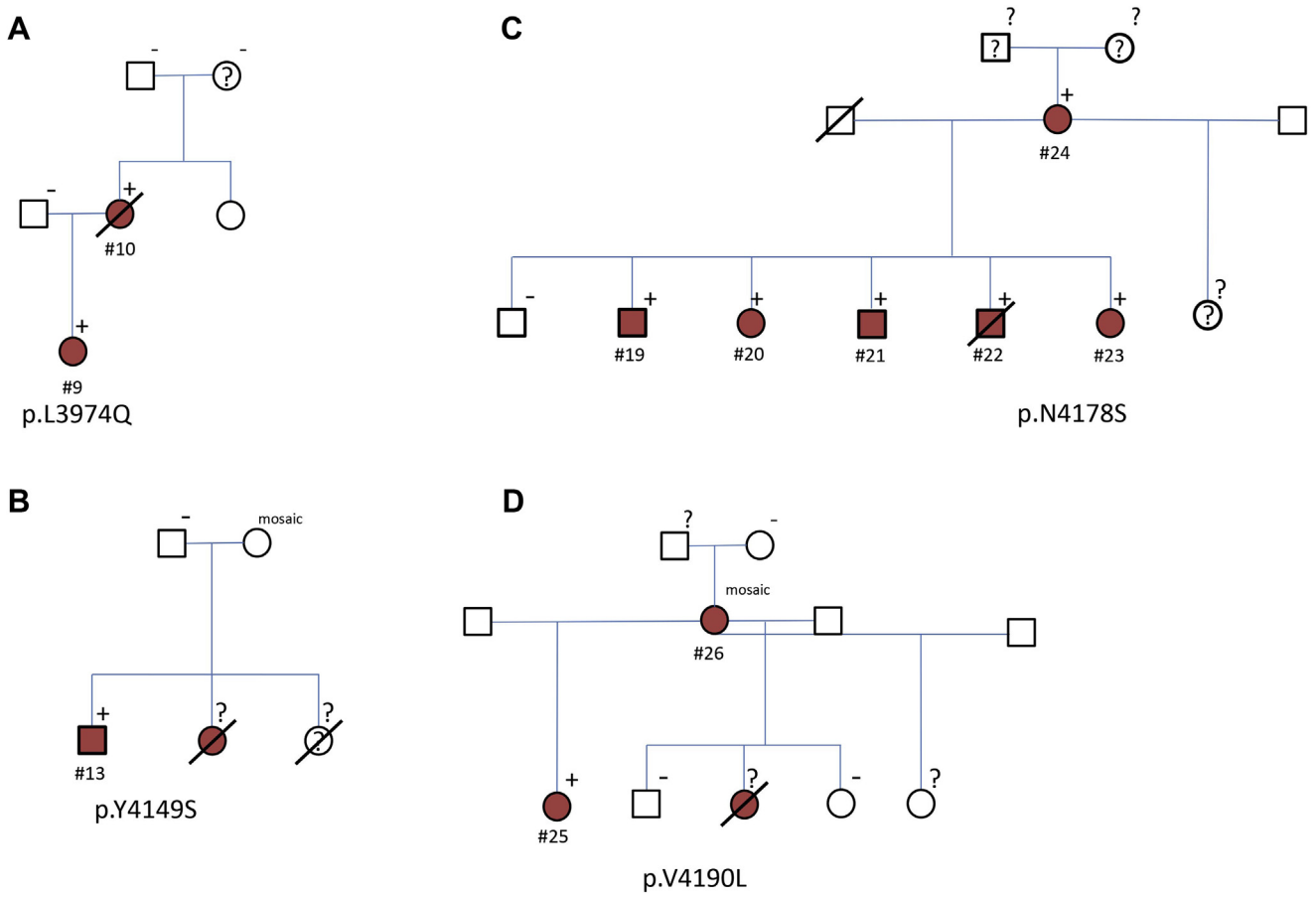

Figure 3 Pedigrees of family members with catecholaminergic polymorphic ventricular tachycardia and ID. The causal *RYR2* mutation is shown for each family. *Squares* and *circles* indicate males and females, respectively. *Diagonal lines* indicate deceased individuals. *Open symbols* indicate persons without ID. *Solid filled symbols* indicate patients with ID. *Plus* and *minus signs* indicate presence or absence of an *RYR2* mutation, respectively. *Question mark* indicates that no details were available. ID = intellectual disability.

or mutants in response to repetitive additions of increasing concentrations of caffeine (from 0.025 to 5 mM). The amplitude of each caffeine-induced Ca^{2+} release in mutant-expressing HEK293 cells was determined and normalized to the maximum release amplitude, and compared with those of WT-expressing HEK293 cells. As shown in Figure 5, all ID-associated *RYR2* mutations tested, including p.S582I, p.M3972I, p.D3973H, p.M3972I/D3973H, p.L3974Q, p.H4108N, p.Y4149S, p.Q4159P, p.Q4171H, p.N4178S, p.K4751Q, p.V4771I, p.K4805R, p.L4865I, and p.I4926T, except for p.R420W and p.A2403T, markedly enhanced the response of RyR2 to activation by caffeine, especially at low concentrations of caffeine. In contrast, the *RYR2* mutations p.M3978I, p.E4076K, and p.N4178Y that cause CPVT without ID in 3 large independent CPVT families (unpublished data) exerted little or no impact on the caffeine response of RyR2 (Figures 5 and 6).

Cardiac phenotype

Thirty-three patients (97%) showed VAs during exercise testing, Holter monitoring, or a provocative drug challenge. One patient (no. 10) was diagnosed with CPVT1 by postmortem genetic testing. An exercise test before the fatal event showed VAs and atrial flutter. Unfortunately, CPVT was not recognized and treated, and the patient died suddenly 4 months later while walking.

Seventeen patients (50%) had supraventricular tachyarrhythmias, including 11 (32%) with paroxysmal or persistent atrial fibrillation. Supraventricular arrhythmias were the first presenting cardiac symptom in 4 patients (12%).

Patient follow-up

Thirty-three patients (excluding no. 10) were followed for a median of 8.4 years (IQR 1.8–12.0). All but 1 patient were treated with β-blockers (Table 1). β-Blocker therapy was combined with flecainide in 19 patients (58%). Fourteen patients (42%) received an implantable cardioverter–defibrillator. Eleven patients (33%) underwent left cardiac sympathetic denervation because of persistent symptoms and/or persistent VAs. Of these patients, 9 (82%) have been asymptomatic ever since, 1 (no. 32) received additional renal denervation due to persistent VAs, and 1 (no. 33) died in-hospital from therapy-refractory VAs.

During follow-up, 15 patients (45%) experienced an arrhythmic event, including 5 (15%) with ACA and 3 (9%) fatalities. Four patients (12%) suffered from multiple arrhythmic events.

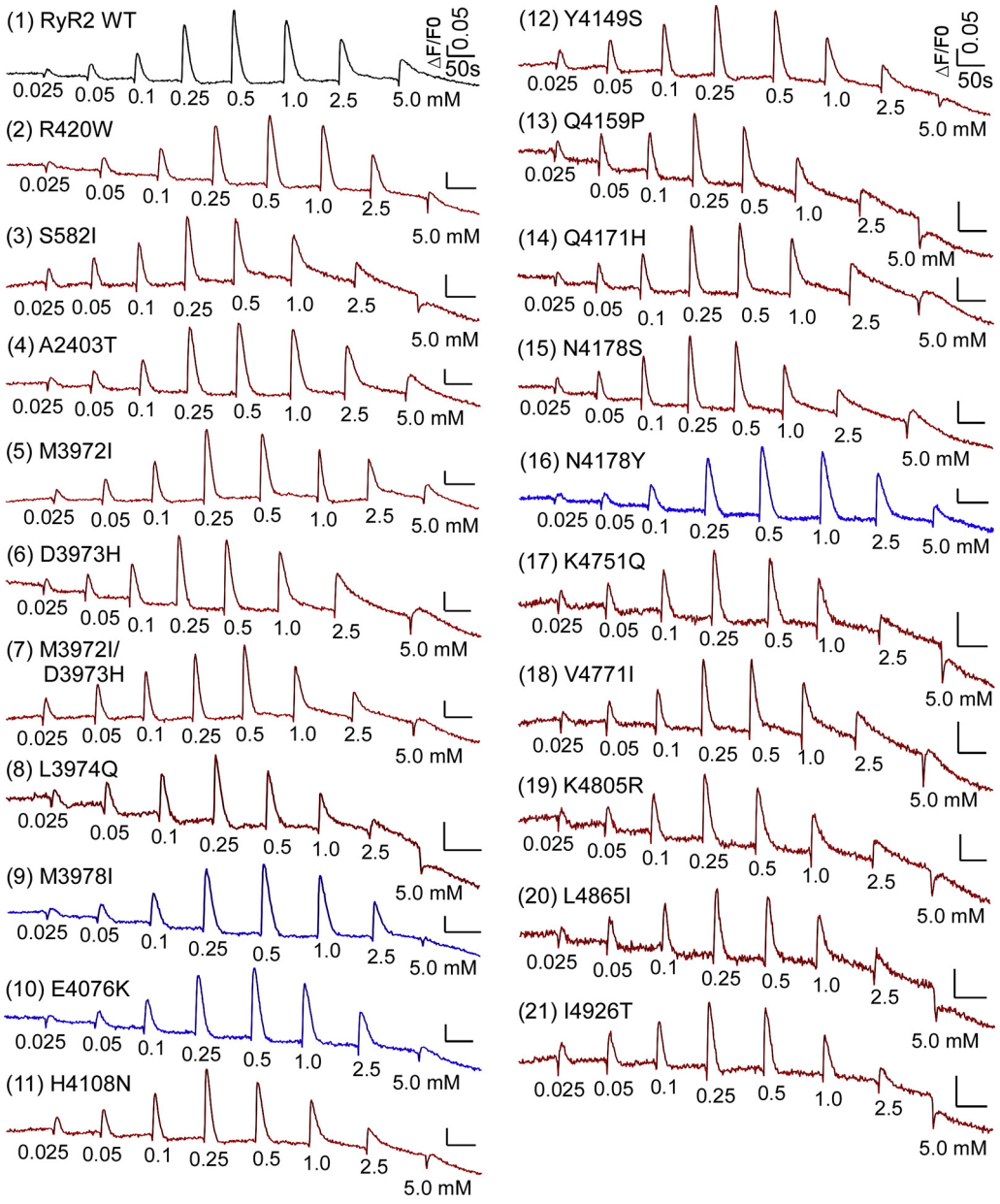

Figure 4 Caffeine-induced Ca^{2+} release in HEK293 cells expressing RyR2 WT and mutants. HEK293 cells were transfected with RyR2 WT (1) or RyR2 mutants: p.R420W (2), p.S582I (3), p.A2403T (4), p.M3972I (5), p.D3973H (6), p.M3972I/D3973H (7), p.L3974Q (8), p.M3978I (9), p.E4076K (10), p.H4108N (11), p.Y4149S (12), p.Q4159P (13), p.Q4171H (14), p.N4178S (15), p.N4178Y (16), p.K4751Q (17), p.V4771I (18), p.K4805R (19), p.L4865I (20), and p.I4926T (21). Fluorescence intensity of the Fluo-3–loaded transfected cells before and after repeated additions of increasing concentrations of caffeine (0.025–5 mM) was monitored continuously. Mutations that are associated with CPVT with ID are labeled in *red,* mutations associated with CPVT without ID in *blue,* and WT in *black.* CPVT = catecholaminergic polymorphic ventricular tachycardia; ID = intellectual disability; WT = wild type.

Discussion

This is the first study to evaluate the prevalence of ID and other NDDs in a large cohort of patients with CPVT1. Eight percent of 421 CPVT1 patients had evidence of concomitant ID compared with an estimated 1%–3% prevalence of ID in the general population.[17,18] Therefore, ID seems to be 2–8 times more prevalent among patients with CPVT1. This subgroup of patients with CPVT1 and ID showed a severe cardiologic phenotype with marked supraventricular arrhythmias and a high arrhythmic event rate despite receiving guideline-recommended therapies.

NDDs in CPVT1

Previous anecdotal reports have suggested a potential association between CPVT1 and NDDs. LaPage et al[8] described a 15-year-old girl referred for seizurelike episodes that occurred since the age of 11 years that were unresponsive to multiple antiepileptic medications. The patient had cognitive delay, short stature, and a low body weight. During a typical seizurelike episode, bidirectional ventricular tachycardia was documented, and the patient was diagnosed with CPVT. Genetic testing revealed a *de novo* mutation in *RYR2* (p.L4188P).

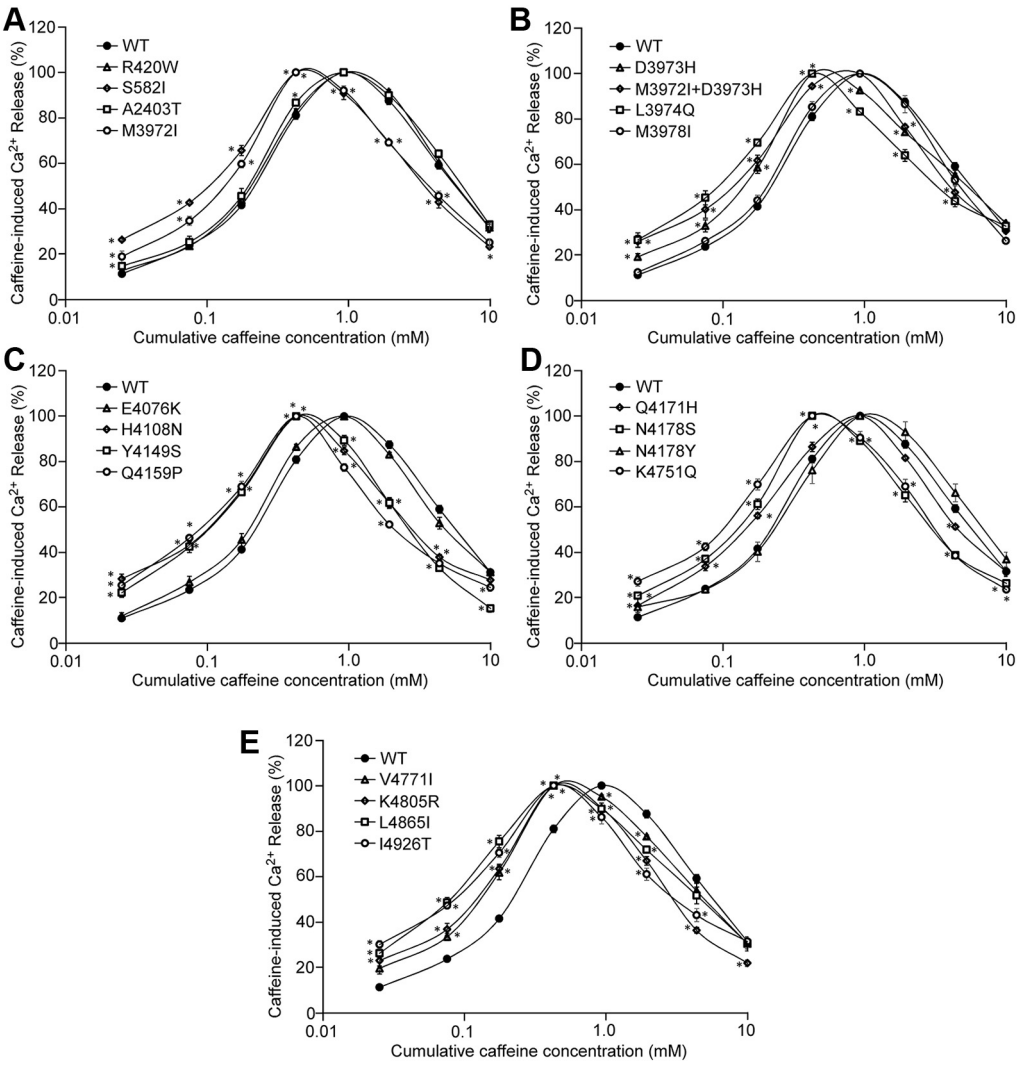

Figure 5 Effect of *RYR2* mutations on activation of RyR2 by caffeine. The relationship between caffeine-induced Ca^{2+} release and the cumulative caffeine concentration in HEK293 cells transfected with RyR2 WT and mutants: **A:** p.R420W, p.S582I, p.A2403T, and p.M3972I; **B:** p.D3973H, p.M3972I/D3973H, p.L3974Q, and p.M3978I; **C:** p.E4076K, p.H4108N, p.Y4149S, and p.Q4159P; **D:** p.Q4171H, p.N4178S, p.N4178Y, and p.K4751Q; and **E**: p.V4771I, p.K4805R, p.L4865I, and p.I4926T. The amplitude of each caffeine peak was normalized to that of the maximum peak for each experiment. Data shown as mean ± SEM (n = 3–8). *P <.05 vs WT. WT = wild type.

Using whole exome sequencing in 41 patients with moderate to severe ID, Hamdan et al[9] identified a *de novo RYR2* mutation (p.G4955E) in a 9-year-old boy with global developmental delay and attention-deficit/hyperactivity disorder. At the age of 22 months, the boy was admitted to the hospital for generalized tonic–clonic seizures. Atrial tachycardia was identified and treated with β-blockers. At the age of 4 years, he was hospitalized for tachycardia and a secondary dilated cardiomyopathy. Even though it is unknown whether this patient has CPVT, this case pointed toward a possible link between the cardiac and the neuropsychiatric phenotype.

Nagrani et al[10] reported an 18-year-old man with a history of mild developmental delay and CPVT (exact *RYR2* mutation not reported). He was treated with mexiletine and nadolol. Routine electroencephalography revealed epileptic brain activity in the absence of cardiac arrhythmias. At the age of 18 years, he presented after an episode of unresponsiveness, which was attributed to a complex partial seizure and was treated with antiepileptic medication.

Johnson et al[11] reported a girl with exercise-induced electroencephalographic-confirmed epileptic seizures since the age of 4 years that responded well to antiepileptic medication. She had normal developmental milestones and a normal brain magnetic resonance imaging scan. Seizure activity returned, and cardiologic workup showed no complex arrhythmias on Holter or exercise testing. At the age of 8 years, the patient died during 1 of her seizures. At molecular autopsy, a *de novo RYR2* mutation (p.G4935R) was identified.

Reassessment of brain imaging in our patients revealed no identifiable cause for their ID. However, we observed subtle abnormalities in areas with relatively high *RYR2* expression, including the hippocampus and cerebellum.[19]

To study the link between CPVT and seizures in mice, Lehnart et al[6] generated a knock-in mouse model of a *RYR2*

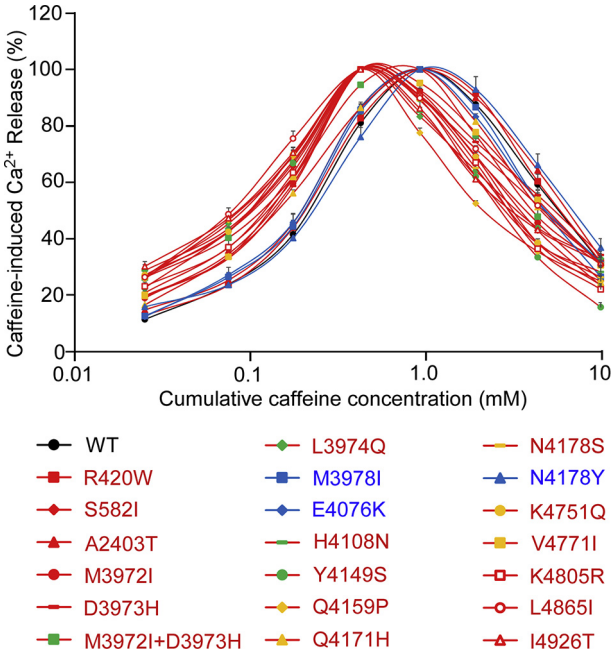

Figure 6 Responses of RyR2 WT and mutants to caffeine activation. The relationship between caffeine-induced Ca^{2+} release and the cumulative caffeine concentration in HEK293 cells transfected with RyR2 WT and mutants. The amplitude of each caffeine peak was normalized to that of the maximum peak for each experiment. Data shown as mean ± SEM (n = 3–8). Mutants that are associated with ID were labeled in *red,* mutants associated with CPVT without ID in *blue,* and WT in *black.* ID = intellectual disability; WT = wild type.

missense mutation (p.R2474S). The mutant mice exhibited both exercise-induced cardiac arrhythmias and spontaneous generalized tonic–clonic seizures in the absence of cardiac arrhythmias. Histologic examination of the mutant mice brains did not show any abnormalities.

Intracellular Ca^{2+} homeostasis is essential to neuron survival and function. This is supported by the large number of Ca^{2+} handling genes previously implicated in NDDs.[20] Interestingly, the *CACNA1C* gene, which encodes the L-type voltage-gated Ca^{2+} channel alpha 1c subunit, has also been associated with cardiac arrhythmias. Gain-of-function mutations in *CACNA1C* cause Timothy syndrome type 1, which is characterized by pronounced QT-interval prolongation and signs of autism spectrum disorder, ID, and/or seizures.[21]

Further support for the role of the RyR2 channel in NDDs comes from studies on the *CLIC2* gene. This gene encodes a chloride intracellular channel protein that modulates the activity of RyR2.[22] Functionally, this *CLIC2* mutation leads to excessive release of Ca^{2+} from the sarcoplasmic reticulum by keeping the RyR2 channel in an open state, which is comparable to the effect of *RYR2* mutations. A mutation in *CLIC2* has been linked to X-linked ID and seizures, in addition to cardiac abnormalities including cardiomegaly, congestive heart failure, and atrial fibrillation.[23]

Taken together, mutations in *RYR2* could confer susceptibility to ID and NDDs by decreased neuronal survival or neuronal function through altered Ca^{2+} handling.

Genetics and functional studies

We found a high number of *de novo RYR2* mutations in this cohort (54%). Previous studies have reported *de novo* mutation rates of 20%–65% in CPVT.[3,24] *De novo* mutations are often more deleterious than inherited mutations because they have not been subjected to evolutionary selection.[25] The mutations clustered in the known hotspot regions of the *RYR2*-encoded Ca^{2+} release channel.[3] In the 3-dimensional structure of RyR2, most of the mutations were located in the central domain, which is known to be critical for cytosolic Ca^{2+} activation of RyR2.[26–28] Some of the mutations found in this cohort have been described previously in patients without documented ID (eg, p.R420W, p.G357S),[29,30] and some of the mutations (p.H4108N and p.Q4159P) recently have been characterized functionally.[28]

All of the ID-associated *RYR2* mutations tested, except for p.R420W and p.A2403T, markedly enhanced the response of RyR2 to activation by caffeine, especially at low concentrations of caffeine. Consistent with these observations, one of the ID-associated *RYR2* mutations identified in our cohort (p.K4751Q) has also been shown to be hypersensitive to stimuli.[31] Importantly, the *RYR2* mutations p.M3978I, p.E4076K, and p.N4178Y, which cause CPVT1 in 3 large independent families but are not associated with ID, show caffeine responses similar to that of the WT. Thus, these data suggest that *RYR2* mutations associated with ID may cause more severe functional impact on RyR2 function compared with those associated with only a cardiac phenotype.

Cardiac phenotype

We found a large number of symptomatic patients with a relatively high recurrent arrhythmic event rate (45%) on conventional therapy during a median follow-up period of 8.4 years. Previous studies, which included a large number of symptomatic patients, have described arrhythmic event rates of 25% and 37% during a follow-up period of 3.5 and 8 years, respectively.[2,32] Additionally, a large subset of our patients (33%) underwent left cardiac sympathetic denervation compared to other cohorts,[2] indicating a subset of patients who either remained symptomatic or have persistent severe VAs despite medical therapy.

Furthermore, our cohort showed a high rate of supraventricular arrhythmias (50%). The link between CPVT and supraventricular arrhythmias has been recognized previously in cohort studies,[1,33] which have reported a prevalence of approximately 16%.[29] It has been hypothesized that microfibrosis in the sinus node may result in altered electrical impulse generation and propagation and leads to sinus node dysfunction and supraventricular tachyarrhythmias.[34] A second hypothesis is that the supraventricular arrhythmias occur due to dysfunctional Ca^{2+} handling in atrial cardiomyocytes (comparable to the ventricular phenotype).[35]

Study limitations

Patients from the participating centers were not systematically assessed for NDDs. However, that only patients who

had obvious signs of NDDs based on chart review were included in our study, this would give an underestimation of the true prevalence of NDDs in CPVT patients rather than an overestimation. In some patients, the existence of a syndromic genetic disease in addition to CPVT contributing to the neurologic phenotype cannot be fully excluded.

We did not have access to an age- and gender-matched control group that would enable valid comparison of the prevalence of ID and NDDs. However, the prevalence of ID has been evaluated thoroughly in the general population; therefore, we compared the prevalence of ID in CPVT to the prevalence of ID in the general population.

Finally, because of the retrospective nature of this study, not all information was available for assessment, making it impossible to correct for possible confounders for the reported association between CPVT and ID.

Conclusion

Our study indicates that ID is more prevalent in patients with CPVT1 than in the general population. This suggests that CPVT and ID may share common underlying pathophysiological mechanisms involving caffeine-hyperresponsive, *RYR2*-mediated intracellular Ca^{2+} handling in the heart and the brain.

Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.hrthm.2018.08.025.

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