Familial Evaluation in Catecholaminergic Polymorphic Ventricular Tachycardia

Disease Penetrance and Expression in Cardiac Ryanodine Receptor Mutation—Carrying Relatives

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Background—Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia syndrome associated with mutations in the cardiac ryanodine receptor gene (RYR2) in the majority of patients. Previous studies of CPVT patients mainly involved probands, so current insight into disease penetrance, expression, genotype-phenotype correlations, and arrhythmic event rates in relatives carrying a RYR2 mutation is limited.

Methods and Results—One-hundred sixteen relatives carrying a RYR2 mutation from 15 families who were identified by cascade screening of the RYR2 mutation causing CPVT in the proband were clinically characterized, including 61 relatives from 1 family. Fifty-four of 108 antiarrhythmic drug-free relatives (50%) had a CPVT phenotype at the first cardiological examination, including 27 (25%) with nonsustained ventricular tachycardia. Relatives carrying a RYR2 mutation in the C-terminal channel-forming domain showed an increased odds of nonsustained ventricular tachycardia (odds ratio, 4.1; 95% CI, 1.5–11.5; P=0.007, compared with N-terminal domain). Sinus bradycardia was observed in 19% of relatives, whereas other supraventricular dysrhythmias were present in 16%. Ninety-eight (most actively treated) relatives (84%) were followed up for a median of 4.7 years (range, 0.3–19.0 years). During follow-up, 2 asymptomatic relatives experienced exercise-induced syncope. One relative was not being treated, whereas the other was noncompliant. None of the 116 relatives died of CPVT during a 6.7-year follow-up (range, 1.4–20.9 years).

Conclusions—Relatives carrying an RYR2 mutation show a marked phenotypic diversity. The vast majority do not have signs of supraventricular disease manifestations. Mutation location may be associated with severity of the phenotype. The arrhythmic event rate during follow-up was low. (Circ Arrhythm Electrophysiol. 2012;5:748-756.)

Key Words: catecholaminergic polymorphic ventricular tachycardia • death, sudden • genetics • ion channels • tachyarrhythmias

Clinical Perspective on p 756

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia syndrome typified by physical or emotional stress–induced polymorphic ventricular tachycardia (VT) in the absence of structural heart disease and apparent 12-lead ECG abnormalities.1 CPVT is associated with mutations in the gene encoding the cardiac ryanodine receptor (RYR2) in 60% to 70% of patients.2–4 After identification of a pathogenic RYR2 mutation in a CPVT patient (the proband), relatives carrying the familial mutation can be identified after cascade screening, and preventive measures can be taken.5 Current knowledge of the clinical profile and prognosis of CPVT patients is largely based on series comprising exclusively or mainly probands. However, it is conceivable that among all CPVT patients known in clinical practice to date, most CPVT patients are relatives carrying the RYR2 mutation who were detected after cascade screening.
Two studies have previously focused on family assessment in CPVT, but they were too limited in population size to examine in much detail the phenotypic manifestations of the relatives carrying the RYR2 mutation identified in the proband. For example, associations between RYR2 mutation location and disease penetrance and expression were not studied.

In this study, we evaluated a large cohort of relatives carrying the RYR2 mutation, identified through active cascade screening, to investigate clinical and electrocardiographic variables of disease penetrance and expression, genotype-phenotype correlations, and prognosis.

Methods

Study Population

In this study, all consecutive CPVT patients carrying a RYR2 mutation (proband and relatives, including relatives of deceased probands), who presented to the departments of Cardiogenetics or Cardiology at the Academic Medical Center, Amsterdam, or the University Medical Center Groningen, Groningen (both in the Netherlands) between 1973 and January 2011, were enrolled. In 8 patients, a clinical diagnosis of exercise-induced ventricular arrhythmias or CPVT was made before 2000, when mutations in RYR2 were first discovered in patients with the CPVT phenotype. The proband was defined as the individual through whom the family was ascertained. Living probands met the diagnostic criteria of CPVT (the presence of adrenergic-mediated bidirectional or polymorphic VT in the absence of structural heart disease and apparent 12-lead ECG abnormalities, particularly prolonged heart rate–corrected QT interval [QTc]) and carried a (putative) pathogenic mutation in RYR2. Deceased probands were young victims of sudden cardiac death (SCD) with a history suggestive of CPVT (history of syncope; SCD during exercise, swimming, or emotion; and/or documented polymorphic VT), in whom familial evaluation identified a first-degree relative carrying a (putative) pathogenic RYR2 mutation. Relatives carrying a mutation were identified by cascade screening of the familial RYR2 mutation and were not known to have a clinical diagnosis of CPVT at the time of DNA testing. Almost none of the relatives carrying a mutation had been medically evaluated before the DNA test. Those with structural heart disease were not included in this study. Two families from a previously published study who were carrying a genomic deletion involving RYR2 exon-3 were not included because these families showed cardiac manifestations beyond the classic CPVT phenotype (progressive atrioventricular block, sinoatrial node dysfunction, atrial fibrillation, and atrial standstill).

Of the 24 probands and 116 relatives comprising the current study, 3 probands and 14 relatives were previously described in 2005,11 and 1 proband was included in our study of the efficacy of left cardiac sympathectomy in CPVT.6,7 and 4 probands and 13 relatives were included in our study of the efficacy of flecainide.13 All families received genetic counseling, and all investigated individuals consented to both genetic screening and cardiological evaluation.

Genetic Evaluation

Mutation analysis of RYR2 followed standard accepted protocols for genetic testing and is described elsewhere. Only individuals carrying proven pathogenic or putative pathogenic mutations in RYR2 were included. Genotype-phenotype correlation analyses were performed in single-mutation carriers using 3 prespecified mutation locators: N-terminal domain, central domain, and C-terminal channel-forming domain.

Cardiological Evaluation

Clinical evaluation included 12-lead ECG, symptom-limited exercise testing, and 24-hour Holter monitoring. Cardiac imaging was performed in all living probands and selected relatives carrying a mutation. In the remaining mutation carriers, cardiac imaging was not performed because there was no suspicion of concurrent structural heart disease. Cardiac (presumably CPVT-related) symptoms were defined as palpitations, near syncope, syncope, or aborted cardiac arrest.

Electrocardiographic Analyses

For each patient, the first available 12-lead ECG in the absence of antiarrhythmic drug therapy and all available exercise tests and Holter recordings were collected. All 12-lead ECGs were digitally measured using ImageJ 1.41o software (National Institute of Health, Bethesda, MD). Heart rate, PR interval, QRS duration, and QTc were the mean of measurements in 3 consecutive complexes with similar RR intervals in lead II or V1. Heart rate was compared with the median heart rate of established age- and sex-appropriate norms. In children aged <16 years, bradycardia was defined as heart rate below the second percentile of established age- and sex-appropriate norms. In individuals aged ≥16 years, 2 heart rate cut-offs for bradycardia were analyzed: heart rates <50 and <60 beats per minute.

The presence of a CPVT phenotype in relatives was assessed using exercise testing and 24-hour Holter monitoring. A clinical diagnosis of CPVT was defined as the presence of ≥3 ventricular premature beats per minute, bigeminal ventricular premature beats, couplets, or non-sustained VT (NSVT [≥2 consecutive ventricular premature beats]).

To exclude the interference of antiarrhythmic drug therapy on the presence of CPVT, NSVT, and supraventricular dysrhythmia phenotypes, these parameters were assessed at the first cardiological evaluation in patients who were antiarrhythmic drug free, even if this cardiological evaluation had been performed (for another reason) before genetic testing was performed. Thus, all individuals who were already receiving antiarrhythmic drugs at the first cardiological evaluation (for other reasons or because therapy had been started directly after the result of genetic testing) were excluded from the analyses of associations between clinical and genetic parameters and the presence of supraventricular and ventricular arrhythmias.

Patient Follow-Up

Patients were treated on the basis of the clinical judgment of their treating cardiologist. Clinical information about treatment, adverse events, and arrhythmic events was collected. Significant side effects of β-blocker therapy were defined as the presence of symptoms that were attributed to β-blocker use by the treating cardiologist and requiring dose decrease, discontinuation, or replacement of the β-blocker prescribed or hampered dose increase, despite insufficient suppression of ventricular arrhythmias and arrhythmic symptoms.

Arrhythmic events were defined as probable or proven arrhythmic syncope, aborted cardiac arrest, appropriate implantable cardioverter-defibrillator (ICD) shock, and SCD. Follow-up durations were counted from the date the clinical or genetic diagnosis of CPVT was made to the date of the last patient contact or the patient’s first arrhythmic event. Information about vital status of all relatives was obtained from the Dutch national population registry with a final check in December 2011.

Statistical Analysis

Continuous data are presented as mean±SD or median (range), and categorical variables are presented as number (percentage). The Mid-P method was used to calculate the 95% CI for proportions.

To compensate for possible correlation of characteristics between relatives within a family, generalized estimating equations with a logistic link function and an exchangeable correlation structure were applied. Associations between the following prespecified characteristics and the presence of CPVT phenotype, NSVT, and supraventricular dysrhythmias at presentation were evaluated in univariable models: sex, age at diagnosis, presence of cardiac symptoms, and mutation location. Variables with P<0.1 were included in a multivariable model to find independent predictors. Associations were expressed as odds ratios with 95% CI. Estimated (adjusted) proportions of patients with the CPVT phenotype and NSVT were calculated for both the univariable and multivariable models. The arrhythmic event rate was calculated by dividing the number of patients who had a cardiovascular event during...
the follow-up by the total number of person-years. The timing of first arrhythmic events was illustrated with a Kaplan-Meier curve. Two-sided tests with $P<0.05$ were considered statistically significant. Analyses were conducted with IBM SPSS Statistics version 19 software (IBM SPSS Inc, New York, NY).

### Results

#### Probands

We included 24 probands, of whom 6 were deceased (5 men; median age of death, 20 years [range, 6–33 years]) and 18 were alive (10 men; median age at diagnosis, 15 years [range, 2–65 years]). In 3 deceased probands, autopsy was performed, which did not show any structural abnormalities. In 2 deceased probands, postmortem material was available to identify a $RYR2$ mutation. The 18 living probands initially presented because of cardiac symptoms (n=13; combined with a family history of SCD in 4), accidentally diagnosed VT (n=3), and family screening after SCD of a relative (n=2). Thirteen probands inherited their $RYR2$ mutation, 6 probands carried a de novo mutation, 1 proband had a mother with germline mosaicism,2 and in 4 probands mutation inheritance could not be determined (Table 1).17–19

#### Relatives

A total of 127 relatives carrying a $RYR2$ mutation from 15 families were identified through cascade screening (Table 1). Of these, 11 relatives were excluded: 2 because of structural heart disease, 8 who did not undergo cardiological examination (5 asymptomatic relatives aged ≥70 years and 3 children aged ≤3 years), and 1 in whom no information about the phenotype could be retrieved. Thus, 116 relatives carrying a mutation from 15 families were included, including 61 relatives of 1 family carrying the p.R420W mutation (Table 1). Their clinical characteristics are detailed in Table 2.
Disease Penetration and Expression

At the first cardiological examination, 8 relatives were receiving β-blockers, of whom 4 had the CPVT phenotype (Figure 1A), including 1 with NSVT (Figure 1B). Fifty-four of 108 relatives not treated with antiarrhythmic drugs (50.0%) had a clinical diagnosis of CPVT (Figure 1A), including 27 (25.0%) with NSVT (Figure 1B). Among relatives with the CPVT phenotype, 30 (55.6%) were asymptomatic (Table 2). The proportion of patients with the CPVT phenotype and NSVT by age category is shown in Figure 2. The prevalence of the CPVT phenotype increased up to 20 years of age and then remained constant. Of the 54 relatives not meeting the definition of a clinical diagnosis of CPVT, 11 (20.4%) showed isolated ventricular premature beats. The presence of cardiac symptoms was significantly associated with the electrocardiographic CPVT phenotype, as well as with the presence of NSVT (Table 3).

Among the 54 relatives who were unaffected at the first clinical evaluation, 38 (70.4%) were reevaluated, usually while being treated (Figure 1A). Of these, 19 (50.0%) developed the CPVT phenotype after a median follow-up of 1.6 years (range, 0.2–19.3 years), at a median age of 24 years (range, 6–54 years). In 65 of 88 relatives (73.9%) with no NSVT at the first cardiological examination, cardiological reevaluation was performed (Figure 1B), showing NSVT in 6 (9.2%) after a median follow-up of 2.4 years (range, 0.2–7.9 years). Altogether, 73 relatives (62.9%) had a clinical diagnosis of CPVT, of whom a total of 33 (28.4%) had NSVT.

Electrocardiographic Characteristics

In all available baseline ECGs in the absence of antiarrhythmic drugs (n=100), 3 relatives (3.0%) showed an ectopic atrial rhythm. In relatives with sinus rhythm, the average PR interval was 142±27 ms, the average QRS duration was 91±14 ms, and the average QTc was 405±24 ms. Heart rate was similar between relatives and established age- and sex-appropriate norms (mean difference of 1±16 beats per minute). To accurately determine the proportion of relatives with sinus bradycardia, only those with an available baseline ECG made in a supine position (n=75) were included, whereas relatives with resting ECGs made in a sitting or standing position before exercise testing were excluded. The proportion of relatives with sinus bradycardia was 12.0% among children (n=25) and 24.0% and 2.0% among adults (n=50), using a cut-off of 60 and 50 beats per minute, respectively. Collectively, 20.0% of relatives had sinus bradycardia using a cut-off of 60 beats per minute in adults and 5.3% using a cut-off of 50 beats per minute in adults.

At baseline, supraventricular dysrhythmias other than sinus bradycardia were present in 17 of 106 relatives in the absence of antiarrhythmic drugs (16.0% [unknown in 2]): intermittent ectopic atrial rhythm in 11, unspecified supraventricular tachycardia in 5, and sick sinus syndrome in 1. Of these, only 2 were induced by exercise testing. When considering only relatives who had Holter monitoring at baseline, 14 of 37 relatives (37.8%) had supraventricular dysrhythmias.

Genotype-Phenotype Correlations

All (putative) pathogenic RYR2 mutations are summarized in Table 1. One proband carried 3 putative pathogenic mutations (p.M2605V, p.A4510T, and c.14757-6C>T+c.14757-7T>A). Three of her relatives carried the p.M2605V and c.14757-6C>T+c.14757-7T>A mutations and were, therefore, excluded from the genotype-phenotype correlation analyses. Of the single-mutation carriers, 63 relatives from 3 families carried a mutation in the N-terminal domain (including the 61 relatives from the family carrying the p.R420W mutation), 14 from 3 families carried a mutation in the central domain, and 36 from 9 families carried a mutation in the C-terminal channel-forming domain.

Mutation location (n=75) were included, whereas relatives with resting ECGs made in a sitting or standing position before exercise testing were excluded. The proportion of relatives with sinus bradycardia was 12.0% among children (n=25) and 24.0% and 2.0% among adults (n=50), using a cut-off of 60 and 50 beats per minute, respectively. Collectively, 20.0% of relatives had sinus bradycardia using a cut-off of 60 beats per minute in adults and 5.3% using a cut-off of 50 beats per minute in adults.

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Supraventricular dysrhythmias at the first cardiological evaluation (other than sinus bradycardia) were more prevalent among central domain mutation carriers (42.9%; odds ratio, 2.5; 95% CI, 1.1–5.4; P=0.02, compared with N-terminal domain) compared with relatives carrying mutations in the N-terminal domain (11.5%) and C-terminal channel-forming domain (12.9%). No significant difference in sinus bradycardia was observed among locations of mutation.

Follow-Up of Probands and Relatives

Eighteen living probands were followed up for a median of 7.8 years (range, 0.3–37.9 years). All probands were treated with β-blockers. In addition, 8 were treated with flecainide, 2 with verapamil, 3 received an ICD, and 1 underwent left cardiac sympathetic denervation during follow-up. During follow-up, 4 probands experienced an arrhythmic event (Figure 3). One proband had several syncopal episodes associated with β-blocker noncompliance and finally died on that morning. The first rhythm recorded was ventricular fibrillation, so he was defibrillated several times. During transport to the hospital, amiodarone and epinephrine were infused in accordance with the resuscitation guidelines. When physicians at the hospital realized that it concerned a CPVT patient,
epinephrine infusion was ceased immediately, and continuous metoprolol infusion was started. However, treatment was later stopped because of severe postanoxic encephalopathy. The other arrhythmic events included aborted cardiac arrest, appropriate ICD therapy, and syncope (Table 4 and Figure 3).

Ninety-eight relatives carrying a RYR2 mutation (84.4%) were followed up for a median of 4.7 years (range, 0.3–19.0 years); 7 (6.0%) were discharged from follow-up because of the absence of significant ventricular arrhythmias, and 11 (9.5%) were lost to follow-up. Of the 98 relatives, 80 (81.6%) received β-blocker therapy (mostly metoprolol, atenolol, and bisoprolol), which caused significant side effects in 23 (28.8%). Fifteen relatives (15.3%) were treated with flecainide (mostly added to β-blocker), 4 (4.1%) received an ICD, and 1 (1.0%) underwent left cardiac sympathetic denervation. During follow-up, 2 asymptomatic relatives experienced syncope (Table 4 and Figure 3). One untreated relative collapsed while working in the garden. Both the first cardiological evaluation at the age of 19 years and the second evaluation 6 months before the event had been unremarkable. One relative with previous evidence of the CPVT phenotype experienced syncope while swimming after β-blocker noncompliance.

Collectively, the arrhythmic event rate was 21.7 per 1000 person-years among probands and 4.4 per 1000 person-years among relatives with RYR2 mutations.

Among the 7 probands and relatives with a transvenous ICD, adverse events occurred in 3 patients: 2 patients required lead reposition because of dislocation, including 1 patient in whom the previous lead had been replaced after lead fracture, and 1 patient received inappropriate shocks.
In the present study, the characteristics of 116 RYR2 mutation carriers from 15 families, who were identified through active cascade screening, are reported, representing the largest series in the literature so far. Our study provided several important observations: first, we found a marked diversity in disease phenotype. At the first cardiological evaluation, 50% of mutation carriers showed signs of disease, including 25% with NSVT. Second, our data indicate that the vast majority of mutation carriers do not have signs of supraventricular disease manifestations, including sinus bradycardia. Third, we observed a significant association between mutation location and disease severity, with relatives carrying mutations in the C-terminal channel-forming domain showing a more severe phenotype. Finally, the arrhythmic event rate in relatives during follow-up was low, particularly in comparison with probands.

**Phenotypic Characteristics**

On the basis of cardiological examination at presentation, half of relatives carrying mutations had evidence of the CPVT phenotype. This increased to 63% when including all cardiological examinations performed during follow-up (while being treated), which is still somewhat lower than previous studies, in which 65% to 79% of RYR2 mutation carriers had a clinical diagnosis of CPVT. This discrepancy might be explained by the low number of families and different RYR2 mutations included in all studies. Mutation-specific effects may be present and affect the overall disease penetrance. For example, our study included 61 relatives of the same family carrying the p.R420W mutation, which shows incomplete penetrance and a relatively mild disease expression. Similar to the other channelopathies, future studies will shed light on possible modifier genes and other factors that may influence disease penetrance in CPVT but have not been identified at present.

**Figure 2.** Relatives with the catecholaminergic polymorphic ventricular tachycardia (CPVT) phenotype and nonsustained ventricular tachycardia (NSVT) at the first cardiological examination by age category. Values are proportion and 95% CI.

**Discussion**

In the present study, the characteristics of 116 RYR2 mutation carriers from 15 families, who were identified through active cascade screening, are reported, representing the largest series in the literature so far. Our study provided several important observations: first, we found a marked diversity in disease phenotype. At the first cardiological evaluation, 50% of mutation carriers showed signs of disease, including 25% with NSVT. Second, our data indicate that the vast majority of mutation carriers do not have signs of supraventricular disease manifestations, including sinus bradycardia. Third, we observed a significant association between mutation location and disease severity, with relatives carrying mutations in the C-terminal channel-forming domain showing a more severe phenotype. Finally, the arrhythmic event rate in relatives during follow-up was low, particularly in comparison with probands.

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**CPVT has been associated with sinus bradycardia** and supraventricular rhythm and conduction disturbances, but the exact prevalence among patients with RYR2 mutation–associated CPVT is unknown. In the present study, the proportion of relatives with sinus bradycardia was 20% using a lenient cut-off and 5% using a strict cut-off. In addition, 16% of all relatives and more than a third of the relatives who had

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**Table 3. Predictors of the CPVT Phenotype and Nonsustained Ventricular Tachycardia at the First Cardiological Evaluation**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>CPVT Phenotype</th>
<th>Nonsustained Ventricular Tachycardia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariable</td>
<td>Multivariable</td>
</tr>
<tr>
<td></td>
<td>Estimated Proportion OR (95% CI)</td>
<td>Estimated Proportion OR (95% CI)</td>
</tr>
<tr>
<td>Sex*</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>41.8% Reference</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>53.6% 1.6 (0.8–3.2)</td>
<td>28.8% 1.7 (0.7–4.0)</td>
</tr>
<tr>
<td>Age at diagnosis, y (per decade)*</td>
<td>1.3 (1.0–1.8) 0.06</td>
<td>1.3 (0.9–1.7) 0.1</td>
</tr>
<tr>
<td>15</td>
<td>39.4% 42.0%</td>
<td>23.5%</td>
</tr>
<tr>
<td>45</td>
<td>60.1% 59.1%</td>
<td>26.3%</td>
</tr>
<tr>
<td>Cardiac symptoms*</td>
<td>0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>No</td>
<td>40.4% Reference</td>
<td>41.4% Reference</td>
</tr>
<tr>
<td>Yes</td>
<td>67.9% 3.1 (1.6–6.0)</td>
<td>64.2% 2.5 (1.1–5.6)</td>
</tr>
<tr>
<td>RYR2 mutation location</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>N-terminal domain (n=59)</td>
<td>47.4% Reference</td>
<td></td>
</tr>
<tr>
<td>Central domain (n=12)</td>
<td>41.7% 0.8 (0.4–1.5) 0.5</td>
<td>16.7% 1.0 (0.4–2.7) 1.0</td>
</tr>
<tr>
<td>C-terminal channel-forming domain (n=34)</td>
<td>61.8% 1.8 (0.8–4.1) 0.2</td>
<td>44.1% 3.9 (1.4–10.5) 0.008</td>
</tr>
</tbody>
</table>

CPVT indicates catecholaminergic polymorphic ventricular tachycardia; OR, odds ratio; RYR2, cardiac ryanodine receptor gene.

*n=108
Holter monitoring at baseline showed other supraventricular dysrhythmias. Thus, it is conceivable that supraventricular disease manifestations are underdiagnosed in CPVT patients who do not undergo Holter monitoring, although supraventricular dysrhythmias may also occur in healthy individuals. Intermittent ectopic atrial rhythms, which were presumably initiated because of sinus node dysfunction, were most prevalent. Conversely, Sy et al. 20 observed supraventricular tachyarrhythmias only among 26% of 27 patients, but their patient population consisted of probands and relatives carrying a RYR2 mutation and patients negative for a mutation. In a previous article from our group, sinus bradycardia was more pronounced in probands compared with relatives. 11 Taken together, this suggests that only a minority of relatives carrying a mutation show a marked diversity of supraventricular disease manifestations and that this might be different in probands.

### Genotype-Phenotype Correlations

Current knowledge of genotype-phenotype correlations consists of differences observed between carriers of RYR2 and cardiac calsequestrin gene mutations and patients with nongenotyped CPVT. Although the number of homozygous and compound heterozygous patients carrying a cardiac calsequestrin gene mutation with CPVT is low, there are clear observations that their phenotype tends to be more malignant than RYR2 mutation–related CPVT. 23,24 Compared with patients with nongenotyped CPVT, Priori et al. 18 showed a male predominance and younger age at first syncope among patients with CPVT related to a RYR2 mutation.

In this study, we observed an increased prevalence of NSVT among relatives carrying RYR2 mutations in the C-terminal channel-forming domain compared with those carrying mutations in the N-terminal and central domains. Notably, no significant differences were observed in disease penetrance. Our data suggest that mutations in all domains cause a RYR2 gain of function but that the degree of the RYR2 defect and the subsequent ventricular arrhythmia burden may depend on mutation location. This is consistent with a study by Tester et al. 25 who functionally characterized a central domain mutation (p.R2267H) and a C-terminal channel-forming domain mutation (p.S4565R), which were identified in 2 cases of sudden infant death syndrome. Their data suggested that the p.S4565R mutation produces chronically leaky RYR2 channels, which is enhanced during increased sympathetic activity. In contrast, the p.R2267H mutation only showed abnormal functionality during stress. Indeed, recent in vitro experiments suggest that RYR2 mutation location may direct the mode of calcium release dysfunction and accompanying channel instability and that mutations in the C-terminal channel-forming domain may lead to more postactivation channel instability. 26 Larger multicenter studies are needed to validate this association between mutation location and disease severity. In particular, analyzing whether mutation location could play a role

### Table 4. Arrhythmic Events During Follow-Up in Probands and Relatives

<table>
<thead>
<tr>
<th>Proband/Relative</th>
<th>Age at Diagnosis, y</th>
<th>Gender</th>
<th>First Arrhythmic Event During Follow-Up</th>
<th>Age at the Event, y</th>
<th>Therapy at the Event*</th>
<th>Therapy Compliance at the Event</th>
<th>Therapy After the Event</th>
<th>Recurrent Arrhythmic Event During Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband</td>
<td>8</td>
<td>Male</td>
<td>Syncope</td>
<td>13</td>
<td>Atenolol (0.4)</td>
<td>No</td>
<td>Atenolol was continued, after next syncope changed to metoprolol</td>
<td>Synopces and SCD, all associated with noncompliance</td>
</tr>
<tr>
<td>Proband</td>
<td>8</td>
<td>Male</td>
<td>Aborted cardiac arrest</td>
<td>12</td>
<td>Atenolol (1.6)</td>
<td>Yes</td>
<td>Bisoprolol was added, thereafter verapamil was added, finally verapamil was replaced by flecainide</td>
<td>No</td>
</tr>
<tr>
<td>Proband</td>
<td>26</td>
<td>Female</td>
<td>Syncope</td>
<td>34</td>
<td>None (bisoprolol [0.04] during exercise only)</td>
<td>NA</td>
<td>None (refused to take β-blocker)</td>
<td>No</td>
</tr>
<tr>
<td>Proband</td>
<td>16</td>
<td>Male</td>
<td>Appropriate ICD shock</td>
<td>16</td>
<td>None (refused to take β-blocker)</td>
<td>NA</td>
<td>Metoprolol and flecainide were started</td>
<td>No</td>
</tr>
<tr>
<td>Relative</td>
<td>20</td>
<td>Female</td>
<td>Syncope</td>
<td>23</td>
<td>None</td>
<td>NA</td>
<td>Bisoprolol was dose increased</td>
<td>No</td>
</tr>
<tr>
<td>Relative</td>
<td>7</td>
<td>Female</td>
<td>Syncope</td>
<td>13</td>
<td>Bisoprolol (0.05)</td>
<td>No</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

SCD indicates sudden cardiac death; NA, not applicable; ICD, implantable cardioverter-defibrillator.

*Doses are daily dose in mg/kg body weight.

![Figure 3](http://circap.ahajournals.org/)

**Figure 3.** Kaplan-Meier curve of the timing of first arrhythmic events in probands and relatives.
in risk stratification among RYR2 mutation carriers could be of great importance because current risk stratification in CPVT is poorly defined.

Finally, mutations in the central domain were significantly associated with supraventricular disease manifestations, although caution is warranted with this observation because of the low number of relatives carrying mutations in this domain. To our knowledge, no studies describing a possible mechanism that could explain this association are available.

Follow-Up

Overall, the incidence of arrhythmic events among relatives was low: 2 events occurred in 2 relatives who were untreated because they were not being treated or were noncompliant. The cause of death of one 85-year-old man could not be retrieved, but CPVT-related SCD at such an old age is unlikely. Other studies including relatives carrying a RYR2 mutation also reported low event rates,6,7,11 except for the study by Hayashi et al.1 In this study, arrhythmic events occurred in 12 of 51 relatives (23.5%) during a follow-up of nearly 8 years. However, only 3 events were (near-) fatal, and in all of these cases the victim was not being treated with a β-blocker at an optimal dose. Thus, optimally treated relatives carrying a mutation presumably have a low risk of potentially fatal events. Yet fatal events have been observed in relatives without a clinical diagnosis of CPVT at Cardiological evaluation,6,7 underscoring the importance of treating all mutation carriers until risk stratification tools become available.

In the present study, only 1 compliant proband and none of the compliant relatives receiving β-blocker therapy experienced an arrhythmic event. These data underscore the efficacy of β-blockers as first-line therapy for CPVT. However, more than a quarter of actively treated relatives receiving β-blocker therapy suffered from significant side effects. Side effects may hamper individual titration to an optimal dose or may reduce drug adherence, which can be fatal in CPVT, as shown in the only incident SCD case described in this study. We recently described a promising effect of flecainide in addition to maximal or suboptimal β-blocker doses in reducing ventricular arrhythmias and preventing arrhythmic events during a median follow-up of 20 months.13 Hence, it seems appropriate to recommend maintaining β-blockers as first-choice therapy but to promptly add flecainide when β-blocker doses cannot be optimally titrated because of side-effects or when the uptitrated β-blocker dose is not sufficiently effective.

Furthermore, 50% of relatives carrying a RYR2 mutation with no CPVT phenotype at the initial Cardiological evaluation developed the phenotype during follow-up, in all but 1 of the cases in the presence of β-blocker therapy. This underscores the importance of regular reevaluation of RYR2 mutation carriers even in the absence of the clinical phenotype.

Limitations

First, of the 63 relatives carrying a RYR2 mutation in the N-terminal domain, 61 relatives belonged to a single family carrying the p.R420W mutation. We used generalized estimating equations to compensate for possible correlation of characteristics between relatives within a family. However, this does not exclude the possibility that the genotype-phenotype correlations that were observed in this study are a mutation-specific rather than a gene domain–specific effect. In addition, we cannot rule out the possibility of survival bias in our analysis showing a significant association between mutation location and the presence of NSVT because the multiple SCD cases from this large family carrying the p.R420W mutation that occurred before the proband presented to our center could not be included.

Second, assessment of clinical manifestations of CPVT is largely based on 1 exercise test and Holter recording performed after genetic testing because treatment is usually initiated after 1 baseline Cardiological examination. Although we previously showed that exercise testing results are reproducible in patients with CPVT treated with β-blockers only or in addition to flecainide,13 its reproducibility is unknown in untreated individuals.

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Disclosures

Dr Wilde is consultant for Transgenomic that has released the FAMILION genetic test for cardiac ion channel abnormalities. The other authors have no conflicts to report.

References

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is associated with mutations in the gene encoding the cardiac ryanodine receptor (RYR2) in 60% to 70% of patients. After identification of a pathogenic RYR2 mutation in a patient with CPVT (the proband), relatives carrying the familial mutation can be identified after cascade screening. However, current knowledge of the clinical characteristics and prognosis of relatives carrying a RYR2 mutation is limited. In this study, we evaluated a large cohort of 116 relatives carrying a RYR2 mutation from 15 families. Half of the relatives had a CPVT phenotype at the first cardiological examination, including a quarter with nonsustained ventricular tachycardia. We found a possible association between mutation location and phenotype severity. The vast majority of relatives did not have supraventricular disease manifestations. The arrhythmic event rate during follow-up was low. We conclude that relatives carrying a RYR2 mutation show a marked phenotypic diversity and that the CPVT phenotype seems to be less severe in relatives compared with probands. However, because risk stratification tools in CPVT are unavailable at present, we still recommend to actively treat all relatives carrying a RYR2 mutation.

CLINICAL PERSPECTIVE

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is associated with mutations in the gene encoding the cardiac ryanodine receptor (RYR2) in 60% to 70% of patients. After identification of a pathogenic RYR2 mutation in a patient with CPVT (the proband), relatives carrying the familial mutation can be identified after cascade screening. However, current knowledge of the clinical characteristics and prognosis of relatives carrying a RYR2 mutation is limited. In this study, we evaluated a large cohort of 116 relatives carrying a RYR2 mutation from 15 families. Half of the relatives had a CPVT phenotype at the first cardiological examination, including a quarter with nonsustained ventricular tachycardia. We found a possible association between mutation location and phenotype severity. The vast majority of relatives did not have supraventricular disease manifestations. The arrhythmic event rate during follow-up was low. We conclude that relatives carrying a RYR2 mutation show a marked phenotypic diversity and that the CPVT phenotype seems to be less severe in relatives compared with probands. However, because risk stratification tools in CPVT are unavailable at present, we still recommend to actively treat all relatives carrying a RYR2 mutation.
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