The role of stress test for predicting genetic mutations and future cardiac events in asymptomatic relatives of catecholaminergic polymorphic ventricular tachycardia probands

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Aims
Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmic disorder with a highly malignant clinical course. Exercise-stress test is the first-line approach to diagnose suspected individuals. We sought to elucidate the value of exercise-stress test for predicting mutations and future cardiac events in CPVT-family relatives.

Methods and results
The present study included 67 asymptomatic relatives (24 ± 15 years) of 17 genetically positive CPVT probands, who underwent exercise-stress test without any medication and genetic testing. Exercise-stress test, which was considered positive with the induction of ventricular tachycardia or premature ventricular contractions consisting of bigeminy or couplets, was positive in 17 relatives (25%). Genetic analysis disclosed mutations in 16 of these 17 relatives (94%) and in 16 of the 50 relatives (32%) with negative exercise-stress test; the sensitivity and specificity for a positive genotype were 50 and 97%, respectively (P < 0.001). Among 32 mutation carriers, cardiac events occurred in 7 of the 16 relatives with positive and 2 of the 16 relatives with negative exercise-stress test during the follow-up period of 9.6 ± 3.8 years, and four with positive and two with negative stress test were not on regular beta-blocker treatment at these events. In the 16 relatives with positive stress test, those on beta-blocker treatment demonstrated a trend of lower cardiac event rate (Log-rank P = 0.054).

Conclusion
In asymptomatic relatives of CPVT probands, exercise-stress test can be used as a simple diagnostic tool. Nevertheless, because of the low sensitivity for predicting mutations and future cardiac events in those with negative stress test, genetic analysis should be performed to improve patient management.

Keywords
Catecholaminergic polymorphic ventricular tachycardia • Stress test • Genetic testing • Mutation • Follow-up studies

Introduction
Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmic disorder characterized by adrenergically mediated polymorphic ventricular tachyarrhythmias without any detectable structural heart abnormalities. Since the identification of the mutations in genes encoding cardiac ryanodine type 2 receptor (RYR2) and calsequestrin 2 (CASQ2), CPVT has become recognized as a significant cause of sudden unexplained cardiac death (SCD). We have recently reported the long-term follow-up results of 101 CPVT patients, showing that CPVT is a malignant channelopathy with an estimated 8-year
cardiac event rate of 27% even in those taking beta-blockers. Our report also demonstrated that the cardiac event rates were similar between 50 probands and 51 affected family members. These results suggested that, in the family of a newly diagnosed CPVT proband, identification of the affected relatives is mandatory.

To diagnose CPVT, exercise-stress test is the first-line approach, and reproducible ventricular tachyarrhythmias in the suspected individual is considered as strong evidence of being affected with CPVT. Exercise-stress test is also used for screening of the relatives in CPVT families, but reports demonstrated that SCDs could occur in asymptomatic family members with a mutant RYR2 without any inducible arrhythmias during exercise-stress tests. So far, the sensitivity and specificity of exercise-stress tests for the identification of the CPVT causing mutation and its validity for predicting future cardiac events in the CPVT family members remain unclear. To address these issues, we retrospectively analysed the results of the initial exercise-stress tests, subsequent cascade genetic screenings, and clinical outcomes after the diagnosis in the relatives of CPVT probands with RYR2 or CASQ2 mutations. Because symptomatic relatives have a high probability of carrying a mutation, we only included asymptomatic relatives in the present analysis.

**Methods**

**Study population**

The present study enrolled 67 asymptomatic relatives (24 ± 15 years, 37 males) of 17 CPVT probands who were diagnosed first on the clinical basis and later demonstrated positive results in the genetic testing [14 RYR2 and 3 CASQ2 (Table 1)]. These 17 probands and the relatives with mutation were reported in the previous studies. Asymptomatic relatives in these studies in whom exercise-stress tests were not carried out or genetic mutations were not identified in their probands were not included in the present study. Seven relatives with previous syncope and another one with a history of aborted SCD were also excluded. A twelve-lead electrocardiogram at rest, echocardiography, and exercise-stress test were performed without any medications as the initial screening in the 67 family members, and after the identification of RYR2 or CASQ2 mutation in the proband, cascade genetic screening was carried out. The relatives with the same mutation seen in their probands were defined as mutation carriers. The relatives with heterozygous R33X mutation in CASQ2 were considered as affected individuals due to the heterozygous mutation in their proband. Although cascade genetic screening in the senior relatives is essential to understand the extent of genetic transmission in CPVT families, relatives over 55 years old at the time of the diagnosis in the proband were excluded from the present analysis to reduce the chance of including age-related events unlinked to CPVT. In the present study cohort without any previous symptoms, only one gene carrier with negative exercise-stress test underwent adrenaline-stress test, which induced no ventricular arrhythmias.

**Exercise-stress test**

Exercise-stress tests were performed with a motor-driven treadmill, according to the standard Bruce protocol, or a graded continuous test to maximal effort on a cycle ergometer, starting at a workload of 25 W, which was increased by 25 W every second minute. Continuous 12-lead electrocardiogram was obtained throughout the test, and the severest arrhythmia, if induced, was recorded. The result of exercise-stress test was considered positive if bigeminal premature ventricular contractions (PVCs), PVC couplets, or ventricular tachycardia (≥ 3 of successive PVCs) was induced. Arrhythmias were further classified into four categories: unifocal, multifocal, bidirectional, and polymorphic.

<table>
<thead>
<tr>
<th>Mutations examined by cascade screening and their clinical manifestations</th>
<th>Cardiac events of the proband for the diagnosis</th>
<th>Relatives screened, n</th>
<th>Relatives with mutations, n</th>
<th>Relatives with positive stress test, n</th>
<th>Relatives with cardiac events, n</th>
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<tbody>
<tr>
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<td></td>
<td></td>
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<tr>
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<td>CASQ2</td>
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<td></td>
</tr>
<tr>
<td>E21 + 14X</td>
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<tr>
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<tr>
<td>M211 + 27X</td>
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</table>

SCD, sudden cardiac death.
Table 2: Type and morphology of arrhythmias emerged in exercise-stress test

<table>
<thead>
<tr>
<th></th>
<th>Unifocal</th>
<th>Multifocal</th>
<th>Bidirectional</th>
<th>Polymorphic</th>
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<td>2</td>
<td></td>
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</tr>
<tr>
<td>Bigeminal PVCs, n</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVC couplets, n</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Ventricular tachycardia, n</td>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

PVC, premature ventricular contraction.

Genetic analysis
Genetic testing was carried out after obtaining written informed consent from the subjects or their parents in the cases of minors. The details of the mutation screening in the probands were written in our previous report. In the 67 study subjects, a targeted mutational analysis of the exon of RYR2 or CASQ2 gene, covering the mutation identified in the proband of the family, was performed. After DNA extraction from peripheral blood leucocytes, and amplification of the region of interest by polymerase chain reaction, the screening for the familial mutation was performed using denaturing high-performance liquid chromatography (WAVE, Transgenomic Inc., Omaha, NE, USA) and direct sequencing on ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Patient follow-up
All subjects were followed every 6–12 months at the referring centres before obtaining the genetic results. Afterwards, regular follow-up was continued in the relatives with mutations, and on a volunteer basis in those without mutations. The follow-up period was 9.6 ± 3.8 years, starting at the date of exercise-stress test to that of the last visit or the patient’s death. Therapy, i.e. drugs and implantable defibrillators, depended on each individual physician. The incidence of cardiac events defined as SCD, aborted SCD, appropriate defibrillator shocks, and syncope under physical or emotional stress during the follow-up was evaluated. The clinical status of family members without mutations was obtained from the affected relatives with mutations (or their parents) who were regularly followed up at the referring centres.

Statistical analysis
Continuous variables, expressed as the mean ± standard deviation, were compared with an unpaired t-test and categorical variables with Fisher’s exact test. The time to the first occurrence of a cardiac event was expressed with the use of Kaplan–Meier curves and with Fisher’s exact test. The time to the first occurrence of a cardiac event was compared with an unpaired t-test when appropriate. The estimated event was expressed with the use of Kaplan–Meier curves and with Fisher’s exact test. The time to the first occurrence of a cardiac event was compared with an unpaired t-test when appropriate.15

Results
Arrhythmias induced in exercise-stress test
The mean heart rate and corrected QT interval at rest were 74 ± 15 beats per min and 407 ± 22 ms, respectively, in the 67 study subjects. Echocardiography did not reveal any abnormal findings.

Exercise-stress test was performed with a cycle ergometer in 41 (61%) and a treadmill in 26 (39%) subjects. There were no symptomatic events during exercise testing. The most severe arrhythmia induced was non-sustained ventricular tachycardia, PVC couplets, and bigeminal PVCs, in 7, 7, and 3 relatives, respectively, indicating that the test was positive in 17 of the 67 family members (25%). The number of relatives with positive stress test in each mutation is shown in Table 1. The morphology of the induced arrhythmias is demonstrated in Table 2. All ventricular tachycardias showed polymorphic morphology. There were no relatives exhibiting bidirectional ventricular tachycardia, but four relatives frequently showed a bidirectional pattern of PVC couplets. Among the other 50 family members (75%), only isolated intermittent PVCs were observed in six, and no ventricular arrhythmias were induced in 44. Treadmill and cycle ergometer showed positive results in 15 and 32% of the relatives, respectively, (P = 0.16). The maximum sinus heart rate during exercise-stress test was similar between those with and without positive results (177 ± 15 vs. 176 ± 21 beats per min, P = 0.76), and the heart rate at which the PVCs emerged was 137 ± 10 beats per min in those with positive results, which was higher but not significantly different from that seen in their probands (120 ± 25 beats per min, P = 0.10). Resting heart rate tended to be lower in those with positive stress test than in those without (68 ± 17 vs. 77 ± 14 beats per min, P = 0.07).

Genetic screening
Among the 67 study subjects, cascade genetic screening, which was obtained 2.4 ± 2.0 years after the initial exercise-stress tests, disclosed 28 cases (42%) of heterozygous RYR2 mutations, four cases (6%) of heterozygous CASQ2 mutation in the family with the proband exhibiting heterozygous R33X mutation, and no patient with a homozygous CASQ2 mutation. No relatives demonstrated RYR2 mutations at the homozygous level. The relatives with RYR2 mutation were significantly younger than those with CASQ2 (R33X) mutation (17 ± 11 vs. 35 ± 14 years old, P = 0.01). Figure 1 shows the results of the genetic testing classified according to the results of exercise-stress tests, and the breakdown of induced arrhythmias in those with and without mutations is demonstrated in Table 3. Among 17 relatives with positive stress tests, all except for a 53-year-old man with induced PVC couplets, who showed no obvious heart abnormalities, demonstrated positive results in the following genetic testing. Among 50 relatives with negative stress tests, 4 with isolated intermittent PVCs and 12 with no ventricular arrhythmias exhibited positive genetic screening.
results, indicating that the mutation was more frequently identified in those with positive than in those with negative stress tests (94 vs. 32%, \( P < 0.001 \)). The sensitivity and specificity of exercise-stress test for predicting the identification of the mutation was 50 and 97%, respectively. When only ventricular tachycardia was defined as positive exercise-stress test, the mutation was identified in 100% of the relatives with positive and 42% of the relatives with negative stress test (\( P = 0.004 \)): the sensitivity and specificity of exercise-stress test for predicting the mutation in this scenario was 22 and 100%, respectively. When isolated intermittent PVCs

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Characteristics of the asymptomatic relatives with and without mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With mutations (( n = 32 ))</td>
</tr>
<tr>
<td>Male gender, ( n ) (%)</td>
<td>18 (56)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>19 ± 12</td>
</tr>
<tr>
<td>HR at rest</td>
<td></td>
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<tr>
<td>All relatives (per min)</td>
<td>69 ± 17</td>
</tr>
<tr>
<td>Age 0–10 years old (per min)</td>
<td>80 ± 10</td>
</tr>
<tr>
<td>Age 11–20 years old (per min)</td>
<td>62 ± 13</td>
</tr>
<tr>
<td>Age ≥ 21 years old (per min)</td>
<td>65 ± 17</td>
</tr>
<tr>
<td>Severest arrhythmia in stress test</td>
<td></td>
</tr>
<tr>
<td>Ventricular tachycardia, ( n ) (%)</td>
<td>7 (22)</td>
</tr>
<tr>
<td>PVC couplets, ( n ) (%)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Bigeminal PVCs, ( n ) (%)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Isolated intermittent PVCs, ( n ) (%)</td>
<td>4 (13)</td>
</tr>
<tr>
<td>None, ( n ) (%)</td>
<td>12 (38)</td>
</tr>
<tr>
<td>Maximum sinus HR in stress test (per min)</td>
<td>174 ± 17</td>
</tr>
</tbody>
</table>

HR, heart rate; PVC, premature ventricular contraction.
were defined as positive stress test, the mutation was identified in 87% of those with positive and 27% of those with negative stress test, respectively ($P < 0.001$): the sensitivity and specificity of exercise-stress test for predicting the mutation in this scenario was 63 and 91%, respectively.

Table 3 shows the characteristics of the subjects with and without mutations. Compared with the relatives without mutations, those with mutations had a lower heart rate at rest and more frequently demonstrated ventricular tachycardia and PVC couplets as the severest arrhythmia during exercise-stress test. When the relatives were divided into three groups according to their age (0–10, 11–20, and ≥ 21 years old), resting heart rate tended to be lower in those with mutations than in those without in any of these subgroups (Table 3).

### Cardiac events during the follow-up

During a follow-up of $9.6 \pm 3.8$ years, 14 relatives were lost to follow-up: 6 of 17 relatives (35%) with positive and 8 of 50 relatives (16%) with negative stress test ($P = 0.16$). The length of the follow-up period did not differ between those with positive and those with negative stress test ($10.1 \pm 3.4$ vs. $9.4 \pm 3.9$ years, $P = 0.57$).

Cardiac events developed in nine relatives with a positive genotype: eight experienced syncope and another one suffered from SCD. Among the eight subjects with syncope, one subsequently experienced an aborted SCD (Figure 1). The number of relatives with cardiac events in each mutation is shown in Table 1. All cardiac events emerged in those with RYR2 mutation. The estimated 4- and 8-year cardiac event rates in 32 mutation carriers were 13 and 34%, respectively (Supplementary material online, Figure S1). None of the relatives without mutation experienced cardiac events during the follow-up period. Figure 2 compares the cardiac event rates between the mutation carriers with positive and negative results in the initial exercise-stress test. Cardiac events developed in 7 of 16 mutation carriers (44%) with positive stress test and in 2 of 16 mutation carriers (13%) with negative stress test. The estimated 4- and 8-year cardiac event rates in 16 subjects with positive exercise results were 13 and 47%, respectively, and were 13 and 13%, respectively in 16 subjects with negative stress test ($P = 0.14$). The severest induced arrhythmia in seven relatives with positive exercise-stress test who later experienced cardiac events were ventricular tachycardia, PVC couplets, and bigeminal PVCs in 2, 3, and 2, respectively. Ventricular arrhythmias were not induced at all in the other two subjects who subsequently suffered from syncope during the follow-up period without beta-blockers: a girl, who was 5 years old at the time of the stress test, experienced syncope during exercise in school at 9 years of age, and a female, who was 26 years old at the time of the stress test, had two syncopal episodes during emotional stress at 29 years of age. The relatives with cardiac events were significantly younger at the time of the stress test than those without cardiac events ($12 \pm 6$ vs. $22 \pm 13$ years old, $P = 0.03$).

Beta-blockers were prescribed on a regular basis in 11 of 16 (69%) affected relatives with positive exercise results and in 6 of 16 (38%) affected relatives with negative exercise results. Among the 16 relatives demonstrating positive exercise results, cardiac events developed in three patients with beta-blocker treatment and in four without (Figure 1). The cardiac event rate in those with and without beta-blocker treatment was 9 and 20% at 4 years, and 31 and 80% at 8 years, respectively (Figure 3A), demonstrating a tendency for lower cardiac event rate in those with regular beta-blocker treatment compared with those without ($P = 0.054$). Among three relatives who experienced cardiac events under beta-blockers, two with syncope took nadolol at doses of 1.8 and 1.4 mg/kg, respectively, and the other one suffering from SCD took nadolol at a dose of only 0.6 mg/kg. Among the 16 relatives demonstrating negative exercise results, cardiac events developed in two without beta-blocker treatment (Figure 1). The cardiac event rate in this subset was 20% at 4 and 8 years ($P = 0.26$) (Figure 3B). None of our subjects were prescribed flecainide nor verapamil, nor underwent any surgery for left cardiac sympathetic denervation.

A defibrillator was implanted in two patients after syncopal episodes while taking nadolol, but no appropriate shocks were delivered during the following period of 8.1 and 6.2 years, respectively.

### Exercise-stress test during the follow-up

Among the 32 mutation carriers, exercise-stress tests were repeated in 14. In four of these relatives, the repeated stress tests were performed after the first cardiac event during the follow-up period. Eleven relatives with initially positive stress test underwent the second test on beta-blockers, which still demonstrated positive results in eight (73%). Among these 11 relatives, the cardiac events after the second stress test were seen in two: one with positive result who intentionally stopped beta-blockers, and another with negative result who continued beta-blockers. The repeated stress tests were performed in three relatives with initially negative results, which turned into positive in all subjects. One of them who was not taking beta-blockers experienced syncope during the follow-up.

![Figure 2](https://academic.oup.com/europace/article-abstract/14/9/1344/510619/fig2)

**Figure 2** Kaplan–Meier survival curves of the cardiac events in the 32 genetically positive relatives demonstrating positive (solid line) and negative results (broken line) in exercise-stress test. Cardiac events developed in seven relatives with positive stress test and were also seen in two relatives with negative stress test.
Discussion

Main findings

Among the 67 asymptomatic relatives of the CPVT probands with a RYR2 or CASQ2 mutation, the initial exercise-stress tests showed positive results in 17 (25%), and genetic testing was positive for the family mutations in 32 (48%). Although the specificity of the stress test for predicting a mutation was high (97%), the sensitivity was low (50%). Among the 32 gene carriers, cardiac events developed not only in 7 of 16 relatives with positive stress test, but also in 2 of 16 relatives with negative stress test, during the 9.6 ± 3.8 years of follow-up, and all relatives with negative stress test and four relatives with positive stress test did not take beta-blockers at the time of the cardiac events. In the gene carriers with positive stress test, the beta-blocker treatment was associated with a trend of lower cardiac event rates.

Diagnostic validity of exercise-stress testing in catecholaminergic polymorphic ventricular tachycardia relatives

To diagnose patients with symptoms suspected of CPVT, exercise-stress test is frequently performed and considered to be mandatory. With regard to the diagnosis of asymptomatic relatives, a recent survey demonstrated that 82% of large European centres performed exercise-stress tests in this category of individuals. The sensitivity and specificity of exercise-stress test; however, in asymptomatic CPVT relatives remained to be elucidated. Triggering of ventricular tachyarrhythmias during exercise-stress test has been considered as the hallmark of ‘affected’ CPVT relatives, irrespective of the presence of previous symptoms. The present study bolsters this theory with a 97% specificity of exercise-stress test for predicting RYR2 or CASQ2 mutations in asymptomatic CPVT relatives. On the other hand, our data demonstrate that the sensitivity of exercise-stress test for predicting mutations is low (50%). These results are consistent with those presented by Bauce et al. Although they also included symptomatic subjects, the sensitivity and specificity of exercise-stress test for predicting the mutations were 60 and 100%, respectively, in subjects belonging to the families with pathogenic RYR2 mutations. Another study reported a higher sensitivity (76%) of exercise-stress test for a positive genotype in the relatives with the CPVT causing mutations, which was presumably due to the inclusion of many symptomatic relatives (more than one-third of the study subjects).

Genetic testing to diagnose catecholaminergic polymorphic ventricular tachycardia relatives

Identifying the mutations in individuals suspected of CPVT sometimes takes time because the gene encoding RYR2 is one of the largest and most complex in the human genome. The comprehensive examination of the RYR2 gene, however, is not required for cascade screening. Compared with genetic testing, exercise-stress test is more rapid in confirming the results and less expensive, but it also had 6% false-positive and 32% false-negative rates in the present study. This result suggests that, to avoid unnecessary medical treatment and strict restriction of strenuous physical activity in non-mutation carriers and to effectively prevent serious arrhythmic consequences in affected but silent mutation carriers, cascade genetic screening should be performed in the undiagnosed relatives regardless of the previous symptoms if a causative mutation is identified in the proband.

On the other hand, exercise-stress test is still valuable in the present era of molecular biology because genetic testing does not always identify a mutation in CPVT patients. All subjects included in the present study were relatives of genetically-positive

Figure 3  Kaplan–Meier survival curves of cardiac events in the genetically positive relatives demonstrating positive (A) and negative results (B) in exercise-stress test. The cardiac event rates were compared between the subjects with (solid line) and without (broken line) regular beta-blocker (BBL) treatment. (A) In those with positive stress test, beta-blocker treatment tended to be associated with lower cardiac event rates. (B) Even in those with negative stress test, two subjects who did not take beta-blockers experienced cardiac events.
CPVT probands, which represents ~70% of the clinically diagnosed CPVT probands.5,19 In asymptomatic relatives of the CPVT probands without identification of any mutations, risk stratification has to be performed by means of exercise-stress (or occasionally catecholamine-stress) test. Although risk stratification in these relatives has not been fully elucidated and the sensitivity of the stress test for predicting the mutation in the present study subjects was low, the positive stress test results would be helpful to us in deciding which relatives to treat. In those with negative stress test, we consider that the repeated stress test might be useful, as, in the present study, the repeated test results turned into positive in three gene carriers with initially negative results. We believe that, at present, exercise-stress test and genetic testing should both be performed to improve CPVT diagnosis. Beta-blockers should be given to these relatives with positive stress test. It remains unclear as to whether beta-blockers should also be given to these relatives with negative stress test. In our institute, beta-blockers are often prescribed empirically in case of several SCD victims in the family or the relatives favour treatment.

**Therapeutic strategy in asymptomatic catecholaminergic polymorphic ventricular tachycardia relatives**

In the present study, beta-blocker treatment was associated with a trend towards a lower cardiac event rate in genetically affected asymptomatic relatives with positive stress test, indicating that beta-blocking treatment should be started in such relatives. Additional therapy exhibiting encouraging results16–18 would possibly further decrease cardiac events in this subset of affected relatives. Further, among all mutation carriers, cardiac events developed in 18% of the relatives with beta-blockers and 40% of those without (Figure 1). Although it did not reach statistical difference in the present study including small number of the CPVT relatives, we speculate that, with larger population, cardiac event rates would be lower in mutation carriers who are on beta-blockers (and possibly drugs such as flecainide16), irrespective of the results of the stress test. The dose of beta-blockers is also important, because one gene carrier with beta-blockers who suffered from SCD took only 0.6 mg/kg of nadolol, which was not sufficient to prevent arrhythmias in CPVT.5

The negative stress test in the mutation carriers should not simply be considered as incomplete penetrance. Considering the discrepancy of the event rates between mutation carriers with and without positive stress test shown in Figure 2, the difference could be statistically significant if larger number of the relatives were included in the analysis. Nonetheless, what, we consider, is important is that 2 of 16 mutation carriers with negative stress test, who were not treated with beta-blockers, experienced cardiac events during the follow-up period. Bauce et al.3 reported a CPVT relative with a negative result at the initial exercise-stress test, who later died suddenly without beta-blocker treatment. Haugaa et al.14 also demonstrated an SCD in a patient with a RYR2 mutation in whom the initial stress test was negative. Considering these results and the fact that repeated exercise-stress tests would disclose ventricular arrhythmias in those with negative results in the first test, as was also shown in the present study and others,3,20 we suggest the therapeutic strategy in asymptomatic CPVT relatives with pathogenic mutations as follows:

1. **Mutation carriers should receive beta-blockers** (or maybe another promising treatment16 in the future) irrespective of the results of the stress test.

2. In mutation carriers who are opposed to taking medication because of the negative result of the initial stress test, the stress test should be carried out periodically, although it remains unclear as to what the ideal frequency of testing should be, and if it should be carried out indefinitely.

**Criterion for the positive results in exercise-stress test**

The present study defined positive result of exercise-stress test as bigeminal PVCs, PVC couplets, or ventricular tachycardia. This criterion was the same as that used in the report by Swan et al.,20 in which subjects in two distinct families with six SCD victims underwent exercise-stress tests and bigeminal PVCs were induced in 14 with the genetic abnormality. At present, the positive criterion of exercise-stress test in diagnosing CPVT remains to be established, and might be different in each individual according to their symptom or situation, e.g. proband or relatives. In reports2,8,21 including many symptomatic individuals (>80% of all study subjects), triggering of ventricular tachycardia during exercise-stress test was high, ranging from 80 to 100%, but a study19 which enrolled 30 genetically positive CPVT relatives with 19 asymptomatic subjects reported its emergence much lower (13%). In the present study including fully asymptomatic relatives, ventricular tachycardia was induced in only 22% of the mutation carriers, indicating very low sensitivity for predicting the mutation. Further, among nine relatives experiencing cardiac events during the follow-up, only two showed ventricular tachycardia in exercise-stress test. On the other hand, considering isolated intermittent PVCs as a positive criterion for the stress test decreased the specificity from 97 to 91%, which would have resulted in an overdiagnosis with unnecessary therapy and lifestyle modifications. The present study also demonstrated that two subjects with negative stress tests and following cardiac events had no ventricular arrhythmias during the initial exercise-stress test, indicating that these events could not be prevented by including isolated intermittent PVCs into positive criterion.

**Limitations**

This study has several limitations. First, as exercise-stress tests were repeated in a limited number of the patients, we cannot confirm the usefulness of the repeated stress tests categorically. Further study is needed to elucidate its value especially for predicting the mutations or future cardiac events in those with negative results in the initial exercise-stress test. Secondly, 10 of the 32 mutation carriers have the same mutation (P4902T). Including such a large family might influence the results of the study, but the sensitivity and specificity of the stress test for a positive genotype without including this family were 45 and 97%, respectively, which were almost similar with those of all 67 relatives. Thirdly, we demonstrated no significant difference in the cardiac event...
rates between mutation carriers with and without positive results of the exercise-stress test, but this might be due to a lack of statistical power because of smaller sample sizes. Similarly, other parameters, such as the rates of positive stress test between the relatives underwent treadmill and those underwent cycle ergometer, and the heart rate at which the PVCs emerged between the probands and the relatives with positive stress test, might differ significantly with larger patient population. Fourthly, the result of exercise-stress test or the mutation screening was not completely reflected to the treatment. This is mostly attributable to the retrospective fashion of the present analysis, in which the treatment depended on each individual physician. Fifthly, only one patient in the present study subjects underwent adrenaline-stress testing. Performing adrenaline infusion testing in the relatives with negative exercise-stress test might have increased the sensitivity of the stress test, as was shown in the recent report by Sy et al.22

Conclusion

In asymptomatic relatives of CPVT probands with RYR2 or CASQ2 mutations, positive exercise-stress test was significantly associated with the identification of the mutation with a 97% specificity. The sensitivity, however, was only 50%, and in two of nine mutation carriers experiencing cardiac events during the follow-up period, the stress test induced no arrhythmia. These results indicate that exercise-stress test can be used as a simple risk-stratifying tool in the relatives of CPVT families, but genetic testing and following cascade screening should be performed to improve CPVT diagnosis and better prevent future cardiac events.

Supplementary material

Supplementary material is available at Europace online.

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