This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.

2. Original statistical analysis plan, final statistical analysis plan, summary of changes.

# A Prospective Randomized Crossover trial of Oral Flecainide for Catecholaminergic Polymorphic Ventricular Tachycardia

**Table of Contents:**

## 1.0 Background

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a genetic arrhythmia syndrome characterized by frequent ventricular ectopy and polymorphic, classically bidirectional ventricular tachycardia with physical or emotional stress, which also carries a risk of ventricular fibrillation and sudden death, despite no structural heart abnormality.(Liu et al.) The disease is caused by mutations in the cardiac ryanodine receptor gene (RYR2) or cardiac calsequestrin gene (CASQ2).(Priori et al.;Postma et al.) Exercise can elicit ectopy or VT in the majority of CPVT patients. Treatment consists of beta-blockers and/or calcium channel blockers, but up to 30% of patients require implantable cardioverter-defibrillators (ICDs) due to recurrent symptoms on medical therapy(Priori et al.).

## 2.0 Rationale and Specific Aims

Additional forms of therapy for CPVT are needed, as current medications are not completely effective in all patients. By studying mouse models of CPVT (CASQ null mice), we have observed beneficial effects of flecainide, a class IC sodium channel blocker. In a retrospective clinical study in patients with CPVT we have also shown improvement of ventricular ectopy on exercise tests when flecainide is added to standard therapy.

The Specific Aims of this protocol are:

1: to test the hypothesis that the addition of oral flecainide to standard therapy will reduce cardiac events, defined as either VT treated by ICD or cardiac death, compared to placebo plus standard therapy, in patients with CPVT.

2. to test the hypothesis that ventricular ectopy and/or VT on treadmill exercise test in patients with CPVT on standard therapy is reduced by flecainide, but not by placebo.

3. to test the hypothesis that reduction in ventricular ectopy on exercise test is associated with reduction in cardiac events.

This will be a single-blind (blinded subjects) randomized cross-over study, in which each patient will receive treatment A (flecainide or placebo) for 18 months and, after a 1 week wash-out, treatment B (placebo or flecainide). The event rate and time to event will be assessed during each treatment period. Any events that occur during treatment A will result in early crossover to treatment B after 1 week of wash-out. Any events during treatment B will result in the end of the study for that subject.

## 3.0 Animal Studies and Previous Human Studies

Since the causal association between stress and arrhythmic symptoms of CPVT was recognized, beta--blockers have been the mainstay of therapy. Moreover, since polymorphic VT is reproducibly induced with exercise in the majority of patients with CPVT, it is common practice to use repeated exercise testing to evaluate the efficacy of β-blocker therapy. While beta-blockers are very effective for preventing exercise-induced sustained polymorphic VT, the majority of patients with CPVT continue to have different degrees of ventricular ectopy during exercise despite maximally tolerated dosages. In addition, studies report high mortality rates and a high incidence of recurrent polymorphic VT despite beta-blocker therapy.

The addition of calcium channel blockers to beta-blockers was reported in 6 children with CPVT, with an improvement in exercise-induced ectopy in all, and a marked clinical improvement in 1 (reduction in ICD shocks).(Rosso et al.) Left cardiac sympathetic denervation has also been reported as an effective alternative in 5 patients when symptoms persist despite the maximum tolerable dose of β-blocker. (Wilde et al., Collura et al.)

Oral flecainide has also been reported to reduce ventricular tachycardia in another genetic arrhythmia syndrome, Andersen-Tawil syndrome (ATS). (Bokenkamp et al.) Two siblings with ATS who failed therapy with beta-blockers and calcium channel blockers had marked improvement with oral flecainide. ATS shares some features with CPVT, in that the characteristic bidirectional ventricular tachycardia (only seen in CPVT, ATS, and digoxin toxicity) is frequently observed, again with minimal symptoms, yet patients are at risk for ventricular fibrillation.

Recently, we discovered that the class 1C antiarrhythmic agent flecainide directly blocked *RyR2* channels, prevented *RyR2*-mediated premature $Ca^{2+}$ release, and suppressed triggered beats in myocytes isolated from mouse hearts lacking

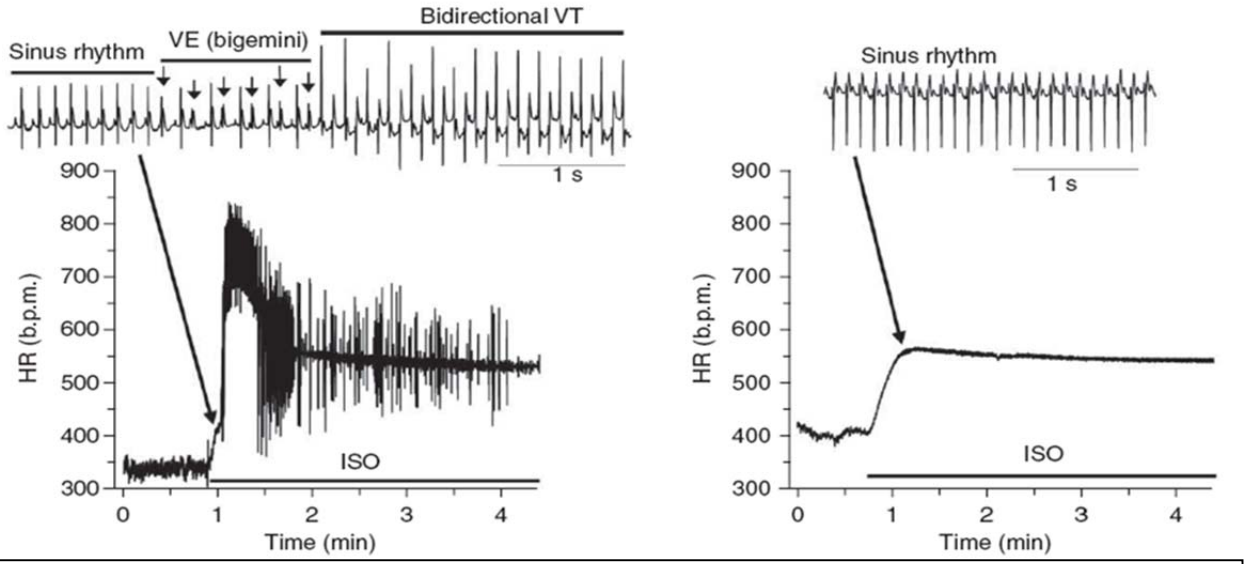


Figure 1. Ventricular arrhythmias during exercise in CASQ null mice (left) are completely abolished with flecainide (right)

calsequestrin, an animal model of CPVT.(Watanabe, et al.) Flecainide treatment completely suppressed ventricular arrhythmias during exercise in this mouse model (Figure1). Flecainide's mechanism of action can be attributed to an open state block of *RyR2* channels, thereby directly targeting the molecular defect responsible for the arrhythmogenic $Ca^{2+}$ waves that trigger CPVT *in vivo*. (Hilliard et al.) Flecainide also appeared to be effective during short-term treatment in two highly symptomatic CPVT patients.

Based on these animal studies, we have collaborated with international centers to perform am open-label, nonrandomized therapeutic trial of oral flecainide for patients with CPVT and persistent ventricular ectopy on exercise testing on standard therapy. In this trial, 86% of patients had improvement of ventricular ectopy on exercise, and there was no worsening of ectopy with flecainide added to standard therapy.

## 4.0 Inclusion/Exclusion Criteria

Inclusion criteria:

- Clinical diagnosis of CPVT, based on:
  - reproducible polymorphic or bidirectional ventricular tachycardia with exercise

  OR
  - Ventricular ectopy on exercise test with *RYR2* or *CASQ2* mutation
- Functioning ICD in place
- On stable dose of standard therapy defined as the maximal tolerated dose of beta-blocker and may include a calcium channel blocker
  - Patients on flecainide are also eligible for enrollment after a 1 week "washout" period during which flecainide is discontinued, and standard therapy alone is used.

Exclusion criteria:

- Pregnant females
- Children < 5 years of age
- Patients unable to perform treadmill exercise
- Patients with significant structural heart disease
- Patients with features consistent with Andersen-Tawil syndrome
  - Periodic paralysis or unexplained weakness
  - Dysmorphic facies
  - Known *KCNJ2* mutation
- Known hypersensitivity to flecainide
- Inability to comply with follow-up

## 5.0 Enrollment/Randomization

*Patient Enrollment:* The treating physicians at each center will identify potential subjects and present a brief overview of the study; if the subject (or his/her parent/legal guardian for patients < 18 years) is interested, an investigator will be contacted and will

approach them. Informed consent will be obtained by the investigator after discussing the study in detail, including the voluntary nature of participation and notification the subject can withdraw at any time. Ample time for questions and answers will be allowed. The investigator will give the subject and his/her legal guardian the opportunity to take the consent home to think about it more, with the option to call or meet with the investigator to ask additional questions. If the subject and his/her parent/legal guardian agree to participate, the investigator will ask them to sign a written, informed consent and assent. A copy of the assent and consent will be given to the subject and his/her parent/legal guardian. Subjects who are < 18 years of age at the time of enrollment who turn 18 years of age during the study period will be re-consented after their 18$^{th}$ birthday. Subjects who wish to participate in the clinical trial without providing a DNA sample will be allowed to do so.

Patients already on flecainide will have a serum level drawn. If the level is > 0.5 mcg/ml, his/her previous dose will be the final study dose (no up titration). If the level is < 0.5, the previous dose will be the starting dose and up titration will occur as below. All subjects on flecainide will discontinue it for 1 week prior to the baseline assessment, continuing on standard therapy alone.

*Randomization Procedure:* This will be a single-blind placebo-controlled randomized crossover study with 2 treatments: oral flecainide or oral placebo. Each enrolled patient will receive both treatments for 18 months, with a 1 week washout period between. The order of treatments will be randomized 1:1 across all centers.

## 6.0 Study Procedures

All patients enrolled in the study will undergo the following baseline assessment and data collection:

- Demographics (age, gender, race)
- Review of data confirming clinical diagnosis of CPVT (recordings of polymorphic VT, exercise tests, genetic tests). Required components include:
    - Normal imaging study (echocardiogram or cardiac MRI)
    - No evidence of coronary ischemia on ECG or exercise testing, unless subsequent stress imaging study was not suggestive of ischemia
    - For subjects with no family history of CPVT nor a putative pathogenic *RYR2* or *CASQ2* mutation who were > 35 years of age *at the time of initial presentation* with polymorphic VT, a coronary angiogram demonstrating no coronary artery disease must be documented.
- Previous anti-arrhythmic treatments
- Prior ECG and echocardiogram reports

Randomization

There will be a 50:50 randomization across all centers, with half the subjects randomized to flecainide then placebo, and half randomized to placebo then flecainide.

Patients previously on flecainide with a serum level > 0.5 mcg/ml will receive a study dose equal to their previous dose. Patients previously on flecainide with a serum level < 0.5 mcg/ml will receive a starting dose equal to their previous dose, with uptitration as below. For all subjects not previously on flecainide, the starting dose will be based on age and weight. Children < 12 years of age will be dosed 3 times per day (every 8 hours) and patients > 12 years of age will be dosed 2 times per day (every 12 hours), based on plasma elimination half-life (Perry et al.) For children < 12 years of age an oral suspension will be used, the starting dose will be 2 mg/kg/day and the maximum dose will be 8 mg/kg/day. For patients > 12 years of age the starting dose will be 100 mg per day (50 mg every 12 hours) and the maximum dose will be 300 mg/day. Study drugs will be prepared and distributed by a central investigational pharmacy; flecainide and placebo will be similar in appearance.

After enrollment (and after a 1 week washout for subjects previously on flecainide), patients will undergo a baseline exercise test on standard therapy (Exercise test 0). Subjects with baseline ECG or exercise test evidence of ischemia must be excluded, unless stress imaging studies are performed and are not suggestive of ischemia. Patients will then initiate treatment A (flecainide or placebo) in a blinded fashion. After 1 week, blood samples for DNA isolation and storage, and determination of a flecainide level will be drawn. Each time a serum sample is obtained for a flecainide level, and ECG will be obtained. For patients on flecainide, the target serum level will be 0.5-0.8 mcg/ml. Patients with levels < 0.35 mcg/ml will have the dose doubled, and those with levels between 0.35 and 0.5 will increase the dose by 50%, unless the maximum dose has been achieved. Patients requiring a dose adjustment (and an equal number of randomly chosen subjects on placebo) will have a second serum sample drawn at 1 month. Any further dose adjustments and serum samples will be made prior to the 3 month visit. Serum levels will be obtained locally and dose adjustments will be done by the central pharmacy, after confirmation with the treating physician that there are no side effects or significant QRS widening.

The dose escalation will continue until either:

1. The trough flecainide level is > 0.5 mcg/ml
2. The QRS width is > 120 ms or prolonged by > 50% of the baseline QRS
3. The maximum dose is achieved.

At 3 months, all subjects undergo a repeat exercise test (exercise test A) and a serum sample drawn for a flecainide level. After 18 months of treatment A, subjects will discontinue the study drug, and after 1 week of standard therapy alone, start treatment B. Determination of flecainide levels will be done as above. After 3 months of treatment B, another exercise test (exercise test B) will be performed. After 18 months of treatment B, the subject will be removed from the study.

Patients will be followed every 6 months or as clinically indicated for ICD interrogation. Patients who receive therapy (shock or anti-tachycardia pacing) from the ICD will be

carefully assessed and the ICD data downloaded. Therapies will be categorized as "appropriate" if delivered for ventricular tachyarrhythmias, or "inappropriate" if delivered for other reasons. In the event of an appropriate ICD therapy, a serum sample will be drawn for a flecainide level. Subjects that have events during treatment A will discontinue treatment A, start the 1 week washout period, and crossover to treatment B. Subjects with events during treatment B will be removed from the study and unblinded. Further treatment will be determined by the treating physician.

The primary endpoint will be cardiac event defined as appropriate ICD therapy (shock or anti-tachycardia pacing for VT) or death.

Secondary endpoints include reduction in ventricular ectopy at exercise test compared to baseline during treatment with flecainide but not placebo.

Exercise tests will be scored using the following scale:

Exercise test scoring system:
0= no ventricular ectopy
1=PVC's, < 1 in 2 beats, and < 10/min
2= PVC's in bigeminal pattern or > 10/minute
3= ventricular couplets
4= nonsustained VT (3 or more consecutive beats)

## 7.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Any adverse events (AEs) will be recorded on the adverse event form (see attached) and sent to the Data Coordinating Center within 72 hours of the event. AEs will be reported to the IRB according to the IRB policies and procedures. The data coordinating center will notify the DSMB of any major adverse events. Any unanticipated problems involving risk to the participants or others will be discussed with the PI and DSMB.

Administration of flecainide is associated with the potential for serious side effects. In our preliminary studies, 8% of CPVT patients were unable to take flecainide due to bradycardia or fatigue and dizziness. Patients who discontinue the study drug (flecainide or placebo) due to side-effects will continue to be followed, with an intent-to-treat analysis. Adverse events related to the administration of flecainide will be reported. All unanticipated problems/events such as breach of confidentiality will be reported.

**Serious Adverse Events (SAEs) will have to be reported according to the following special procedure:**

The occurrence of serious adverse events will be reported to the Investigator by telephone or fax; they must be reported to him/her within 24 hours after becoming aware of their occurrence. The Investigator will report SAEs to the Vanderbilt Institutional Review Board per policy.

## 8.0 Study Withdrawal/Discontinuation

Subjects may withdraw from the study at any time. Subjects will be unblinded at the time of withdrawal.

## 9.0 Statistical Considerations

### Sample Size Estimation and Power Analysis

The primary endpoint of this randomized controlled 2x2 cross-over trial will be cardiac event defined as appropriate ICD therapy (shock or anti-tachycardia pacing for VT) or death. The objective is to demonstrate decreased event rate in patients treated with flecainide in addition to standard therapy compared to patients treated with standard therapy plus placebo. Previous studies of CPVT patients with ICD's reveal an event rate ranging from 50% over 20 months (Priori et al) to 25% over 3.9 years (Hayashi et al). This wide range results in an estimated event rate during 18 months of treatment with placebo plus standard therapy between 10 and 45%. In our small series of patients receiving open-label flecainide, we observed 1 event in 23 patients (4%), which was likely due to noncompliance. The event rate of the control group is expected to be 15% based on the above data. The sample size estimation was carried out using the Pearson chi-square test for paired proportions. With a sample size of 55, the study will have 80% power to detect a 10% difference in the primary endpoint with a two-sided test at 5% significance level. To compensate for reduced power caused by the noncompliance (the anticipated dropout rate over 36 months is 8% based on our pilot data), 60 patients will be enrolled.

### Statistical Analysis Plan

Descriptive statistics, including means, standard deviations, and ranges for continuous variables, as well as percentages and frequencies for categorical variables, will be provided to describe the study sample. Pearson chi-square test or Fisher's exact test will be used to assess the categorical variables. Differences between group means for continuous variables will be examined using ANOVA or Kruskal-Wallis Test. The Mainland-Gart's test will be used for univariate analysis of the primary outcome, when the assumption of no carry-over effect holds. For multivariate analysis, the Generalized Linear Mixed Model will be used to assess the treatment effect, period effect, and treatment-by-period interaction effect, and to adjust for other risk factors such as gender or age. The analysis of survival data will be carried out if such data are available, using the Kaplan-Meier method with log-rank test to compare time-to-event between the two arms and the proportional hazard Cox model to investigate potential prognostic factors. The alpha-spending function of O'Brien-Fleming will be applied for the interim analysis to maintain an overall significance level at 0.05. Point estimates along with the

corresponding p-values and 95% confidence intervals will be reported. The adjusted p-values and the corresponding 95% confidence interval will be reported for multivariate analyses. Statistical analysis will be done with R for Windows, version 2.9.2 and SAS 9.2.

Secondary analysis will include comparison of the exercise treadmill tests after 3 months of placebo vs. 3 months of flecainide, and quantified as follows for comparison:

Ventricular arrhythmia score:
0 = no ventricular ectopic beats
1 = single PVC's
2 = PVC's in bigeminal pattern
3 = PVC pairs (couplets)
4 = nonsustained VT (≥ 3 beats, but < 30 seconds)
5 = sustained VT (> 30 seconds)

Quantification of arrhythmias:

1. Total number of ventricular ectopic beats during entire exercise test (rest, exercise, and recovery)
2. Number of ectopic beats during worst 10 second period of exercise test (rest, exercise, and recovery)
3. Ratio of ectopic to sinus beats during worst 10 second period of exercise test (rest, exercise, and recovery)

Based on pilot human studies, we anticipate a reduction in the number of ectopic beats during worst 10 second period of exercise from 13 ± 5 beats to 5 ± 5 beats. Assuming an α of 0.05 and 80% power, we would need only 5 subjects to show a statistically significant reduction, as this is a paired analysis.

## 10.0 Privacy/Confidentiality Issues

Only individuals directly involved with the study will have access to data. Information is for research purposes only and will be used for publication purposes. All participants will have their names concealed. Access to identified patient information will be limited to the investigators listed within this IRB application. De-identified information with HIPPA identifiers removed will be available to other investigators following appropriate IRB approval. Confidentiality and security will be maintained for the database. The database is stored behind a firewall (in addition to the institutional firewall) with the highest level of protection, i.e. the same level of protection as the on-line hospital information system at Vanderbilt. This means that users must logon to a web server that sits between the institutional firewall and the firewall to the database, and only this application server is allowed to query the database. Only users approved through our institutional review board will be allowed access to patient identifiers. Other levels of authorization may exist for future approved users following IRB approval, e.g. access to de-identified data.

Data is initially collected in the medical record for each individual study participant. The information will be extracted from the patient's medical record and then transferred into the Case Report Form (CRF).

The CRFs will include personal identifiers for participant. However, this data will not be accessible as numbers and initials are assigned for each participant and these will become the identifying information for each study participant. A master list with patient demographics will only be accessible to the principle investigator and his senior co-investigator. This data will not be available to others.

**Reference List**

Bokenkamp, R., et al. "Flecainide for recurrent malignant ventricular arrhythmias in two siblings with Andersen-Tawil syndrome." Heart Rhythm. 4.4 (2007): 508-11.

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Watanabe H, Chopra N, Laver D, et al. Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. Nat Med 2009;15:380-3.

Wilde AA, Bhuiyan ZA, Crotti L, et al. Left cardiac sympathetic denervation for catecholaminergic polymorphic ventricular tachycardia. N Engl J Med 2008;358:2024-9

# A Prospective Randomized Crossover trial of Oral Flecainide for Catecholaminergic Polymorphic Ventricular Tachycardia

**Version 5/29/2015**

**Table of Contents:**

## 1.0 Background

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a genetic arrhythmia syndrome characterized by frequent ventricular ectopy and polymorphic, classically bidirectional ventricular tachycardia with physical or emotional stress, which also carries a risk of ventricular fibrillation and sudden death, despite no structural heart abnormality.(Liu et al.) The disease is caused by mutations in the cardiac ryanodine receptor gene (RYR2) or cardiac calsequestrin gene (CASQ2).(Priori et al.;Postma et al.) Exercise can elicit ectopy or VT in the majority of CPVT patients. Treatment consists of beta-blockers and/or calcium channel blockers, but up to 30% of patients require implantable cardioverter-defibrillators (ICDs) due to recurrent symptoms on medical therapy(Priori et al.).

## 2.0 Rationale and Specific Aim

Additional forms of therapy for CPVT are needed, as current medications are not completely effective in all patients. By studying mouse models of CPVT (CASQ null mice), we have observed beneficial effects of flecainide, a class IC sodium channel blocker. In a retrospective clinical study in patients with CPVT we have also shown improvement of ventricular ectopy on exercise tests when flecainide is added to standard therapy.

The Specific Aim of this protocol is to test the hypothesis that ventricular ectopy and/or VT on treadmill exercise test in patients with CPVT on standard therapy is reduced by flecainide, but not by placebo.

This will be a single-blind (blinded subjects) randomized cross-over study, in which each patient will receive treatment A (flecainide or placebo), undergo an exercise test, crossover to treatment B (placebo or flecainide) and undergo another exercise treadmill test.

## 3.0 Animal Studies and Previous Human Studies

Since the causal association between stress and arrhythmic symptoms of CPVT was recognized, beta--blockers have been the mainstay of therapy. Moreover, since polymorphic VT is reproducibly induced with exercise in the majority of patients with CPVT, it is common practice to use repeated exercise testing to evaluate the efficacy of β-blocker therapy. While beta-blockers are very effective for preventing exercise-induced sustained polymorphic VT, the majority of patients with CPVT continue to have different degrees of ventricular ectopy during exercise despite maximally tolerated

dosages. In addition, studies report high mortality rates and a high incidence of recurrent polymorphic VT despite beta-blocker therapy.

The addition of calcium channel blockers to beta-blockers was reported in 6 children with CPVT, with an improvement in exercise-induced ectopy in all, and a marked clinical improvement in 1 (reduction in ICD shocks).(Rosso et al.) Left cardiac sympathetic denervation has also been reported as an effective alternative in 5 patients when symptoms persist despite the maximum tolerable dose of β-blocker. (Wilde et al., Collura et al.)

Oral flecainide has also been reported to reduce ventricular tachycardia in another genetic arrhythmia syndrome, Andersen-Tawil syndrome (ATS). (Bokenkamp et al.) Two siblings with ATS who failed therapy with beta-blockers and calcium channel blockers had marked improvement with oral flecainide. ATS shares some features with CPVT, in that the characteristic bidirectional ventricular tachycardia (only seen in CPVT, ATS, and digoxin toxicity) is frequently observed, again with minimal symptoms, yet patients are at risk for ventricular fibrillation.

Recently, we discovered that the class 1C antiarrhythmic agent flecainide directly blocked *RyR2* channels, prevented *RyR2*-mediated premature $Ca^{2+}$ release, and suppressed triggered beats in myocytes isolated from mouse hearts lacking calsequestrin, an animal model of CPVT.(Watanabe, et al.) Flecainide treatment completely suppressed ventricular arrhythmias during exercise in this mouse model (Figure1). Flecainide's mechanism of action can be attributed to an open state block of *RyR2* channels, thereby directly targeting the molecular defect responsible for the arrhythmogenic $Ca^{2+}$ waves that trigger CPVT *in vivo*. (Hilliard et al.) Flecainide also appeared to be effective during short-term treatment in two highly symptomatic

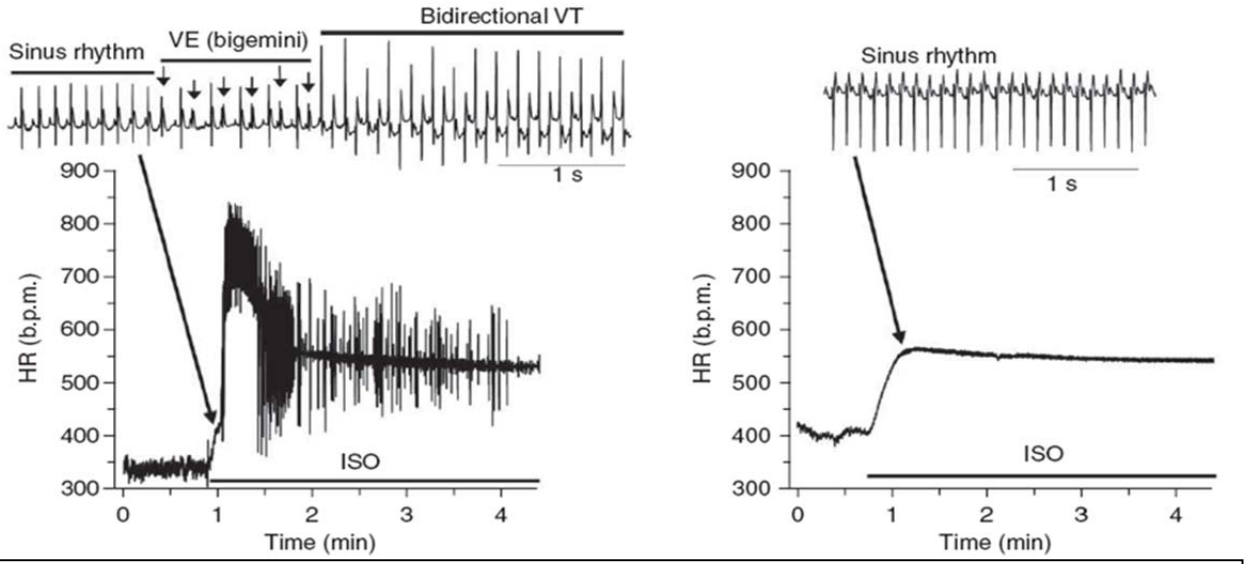


Figure 1. Ventricular arrhythmias during exercise in CASQ null mice (left) are completely abolished with flecainide (right)

CPVT patients.

Based on these animal studies, we have collaborated with international centers to perform am open-label, nonrandomized therapeutic trial of oral flecainide for patients with CPVT and persistent ventricular ectopy on exercise testing on standard therapy. In this trial, 86% of patients had improvement of ventricular ectopy on exercise, and there was no worsening of ectopy with flecainide added to standard therapy.

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Inclusion criteria:

- Clinical diagnosis of CPVT, based on:
  - reproducible polymorphic or bidirectional ventricular tachycardia with exercise OR
  - Ventricular ectopy on exercise test with *RYR2* or *CASQ2* mutation
- Functioning ICD in place
- On stable dose of standard therapy defined as the maximal tolerated dose of beta-blocker and may include a calcium channel blocker. On stable doses of CYP2D6 inhibitors (quinidine, fluoxetine, paroxetine, bupropion, cimetidine) or inducers (rifampin, carbamazepine, phenytoin, Phenobarbital). If CYP2D6 inhibitor/inducer doses require changes during the course of the subject's treatment with active flecainide, serum flecainide levels will be monitored
  - Patients on flecainide are also eligible for enrollment after a 1 week "washout" period during which flecainide is discontinued, and standard therapy alone is used.

Exclusion criteria:

- Pregnant females
- Children < 5 years of age
- Patients unable to perform treadmill exercise
- Patients with significant structural heart disease
- Patients with features consistent with Andersen-Tawil syndrome
  - Periodic paralysis or unexplained weakness
  - Dysmorphic facies
  - Known *KCNJ2* mutation
- Known hypersensitivity to flecainide
- Inability to comply with follow-up

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- Prior ECG and echocardiogram reports

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There will be a 50:50 randomization across all centers, with half the subjects randomized to flecainide then placebo, and half randomized to placebo then flecainide.

Patients previously on flecainide with a serum level > 0.5 mcg/ml will receive a study dose equal to their previous dose. Patients previously on flecainide with a serum level < 0.5 mcg/ml will receive a starting dose equal to their previous dose, with uptitration as below. For all subjects not previously on flecainide, the starting dose will be based on age and weight. Children < 12 years of age will be dosed 3 times per day (every 8 hours) and patients > 12 years of age will be dosed 2 times per day (every 12 hours), based on plasma elimination half-life (Perry et al.) For children < 12 years of age an oral suspension will be used, the starting dose will be 2 mg/kg/day and the maximum dose will be 8 mg/kg/day. For patients > 12 years of age the starting dose will be 100 mg per day (50 mg every 12 hours) and the maximum dose will be 400 mg/day. Study drugs will be prepared and distributed by a central investigational pharmacy; flecainide and placebo will be similar in appearance.

After enrollment (and after a 1 week washout for subjects previously on flecainide), patients will undergo a baseline exercise test on standard therapy (Exercise test 0). Subjects with baseline ECG or exercise test evidence of ischemia must be excluded, unless stress imaging studies are performed and are not suggestive of ischemia. Patients will then initiate treatment A (flecainide or placebo) in a blinded fashion. After 1 week, blood samples for DNA isolation and storage, and determination of a flecainide level will be drawn. Each time a serum sample is obtained for a flecainide level, and ECG will be obtained. For patients on flecainide, the target serum level will be 0.5-0.8 mcg/ml. Patients with levels < 0.35 mcg/ml will have the dose doubled, and those with levels between 0.35 and 0.5 will increase the dose by 50%, unless the maximum dose has been achieved. Patients requiring a dose adjustment (and an equal number of randomly chosen subjects on placebo) will have a second serum sample drawn at 1 month. Any further dose adjustments and serum samples will be made prior to the 3 month visit. Serum levels will be obtained locally and dose adjustments will be done by the central pharmacy, after confirmation with the treating physician that there are no side effects or significant QRS widening.

The dose escalation will continue until either:

1. The trough flecainide level is > 0.5 mcg/ml
2. The QRS width is > 120 ms or prolonged by > 50% of the baseline QRS
3. The maximum dose is achieved.

At 3 months, all subjects undergo a repeat exercise test (exercise test A) and a serum sample drawn for a flecainide level. Subjects will then immediately discontinue the Treatment A study drug, and after 1 week of standard therapy alone, start treatment B. Determination of flecainide levels will be done as above. After at least 3 months of treatment B, another exercise test (exercise test B) will be performed. Treatment B study drug will be discontinued at the time of exercise test B, and the subject's participation in the study will be complete.
Patients who receive therapy (shock or anti-tachycardia pacing) from their ICD during the course of their participation in the study will be carefully assessed and the ICD data downloaded. Therapies will be categorized as "appropriate" if delivered for ventricular tachyarrhythmias, or "inappropriate" if delivered for other reasons. In the event of an appropriate ICD therapy, a serum sample will be drawn for a flecainide level. Subjects that have events during treatment A will discontinue treatment A, start the 1 week washout period, and crossover to treatment B. Subjects with events during treatment B will be removed from the study and unblinded. Further treatment will be determined by the treating physician.

The primary endpoint will be reduction in ventricular ectopy at exercise test compared to baseline during treatment with flecainide but not placebo.

Exercise tests will be scored using the following scale:

Exercise test scoring system:
0= no ventricular ectopy
1=PVC's, < 1 in 2 beats, and < 10/min
2= PVC's in bigeminal pattern or > 10/minute
3= ventricular couplets
4= nonsustained VT (3 or more consecutive beats)

## 7.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Any adverse events (AEs) will be recorded on the adverse event form (see attached) and sent to the Data Coordinating Center within 72 hours of the event. AEs will be reported to the IRB according to the IRB policies and procedures. The data coordinating center will notify the DSMB of any major adverse events. Any unanticipated problems involving risk to the participants or others will be discussed with the PI and DSMB.

Administration of flecainide is associated with the potential for serious side effects. In our preliminary studies, 8% of CPVT patients were unable to take flecainide due to bradycardia or fatigue and dizziness. Patients who discontinue the study drug (flecainide or placebo) due to side-effects will continue to be followed, with an intent-to-treat analysis. Adverse events related to the administration of flecainide will be reported. All unanticipated problems/events such as breach of confidentiality will be reported.

**Serious Adverse Events (SAEs) will have to be reported according to the following special procedure:**

The occurrence of serious adverse events will be reported to the Investigator by telephone or fax; they must be reported to him/her within 24 hours after becoming aware of their occurrence. The Investigator will report SAEs to the Vanderbilt Institutional Review Board per policy.

## 8.0 Study Withdrawal/Discontinuation

Subjects may withdraw from the study at any time. Subjects will be unblinded at the time of withdrawal.

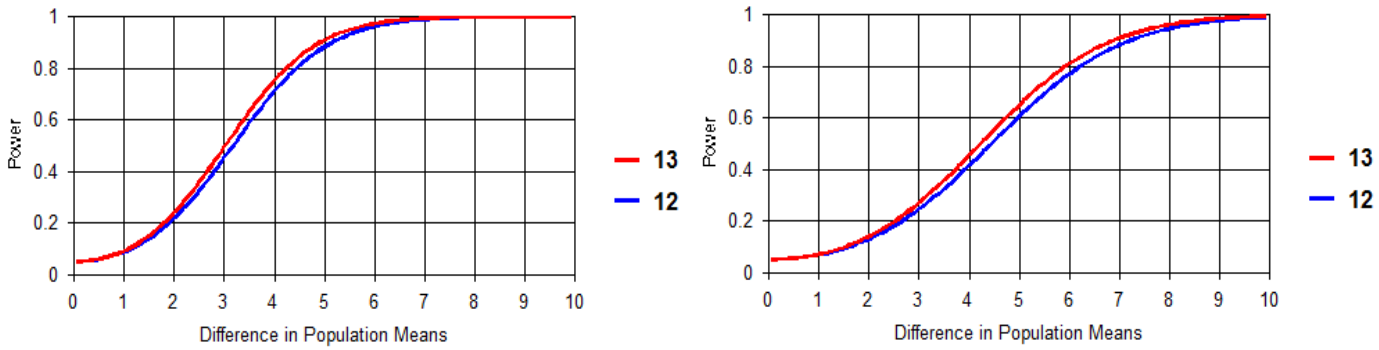
## 9.0 Statistical Considerations

### Statistical Analysis Plan

Our previous open-label comparison of flecainide to standard therapy showed a 7 beat reduction in number of ectopic beats in worst 10 seconds of exercise test (from 12 ± 5 beats to 5 ± 5 beats).[1] A second study in 12 genotype-negative CPVT patients demonstrated a 6 beat reduction in number of ectopic beats in worst 10 seconds of exercise test (from 12 ± 7 beats to 6 ± 7 beats).[2] **A target of 14** enrolled subjects provide adequate statistical power (80% power with an alpha of 0.05) to detect the previously observed differences (6-7) in number of PVC's in worst 10 second period during exercise testing.[3] Graphs of Power (Y-axis) vs observed difference in means (X-axis) are provided below. The 2 panels represent power calculations using different values for the within-group standard deviation (SD). These values are informed from the 2 previous studies in CPVT patients using the same methodology (left, within group SD = 5, observed in reference 1; right within group SD = 7, observed in reference 2). Red lines indicate N=13, and blue lines indicate N=12, allowing for potential subject dropout.

References for statistical analysis plan


1. van der Werf C, Kannankeril PJ, Sacher F, Krahn AD, Viskin S, Leenhardt A, Shimizu W, Sumitomo N, Fish FA, Bhuiyan ZA, Willems AR, van der Veen MJ, Watanabe H, Laborderie J, Haïssaguerre M, Knollmann BC, Wilde AA. Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. J Am Coll Cardiol. 2011 May 31;57(22):2244-54.
2. Watanabe H, van der Werf C, Roses-Noguer F, Adler A, Sumitomo N, Veltmann C, Rosso R, Bhuiyan ZA, Bikker H, Kannankeril PJ, Horie M, Minamino T, Viskin S, Knollmann BC, Till J, Wilde AA. Effects of flecainide on exercise-induced ventricular arrhythmias and recurrences in genotype-negative patients with catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm. 2013 Apr;10(4):542-7.
3. Dupont WD, Plummer WD. Power and Sample Size Calculations: A Review and Computer Program. Controlled Clinical Trials. 1990;11:116-28.




Descriptive statistics, including means, standard deviations, and ranges for continuous variables, as well as percentages and frequencies for categorical variables, will be provided to describe the study sample. Pearson chi-square test or Fisher's exact test will be used to assess the categorical variables. Differences between group means for continuous variables will be examined using ANOVA or Kruskal-Wallis Test. Point estimates along with the corresponding p-values and 95% confidence intervals will be reported. The adjusted p-values and the corresponding 95% confidence interval will be reported for multivariate analyses. Statistical analysis will be done with R for Windows, version 2.9.2 and SAS 9.2.

Analysis will consist of a comparison of the exercise treadmill tests after 3 months of placebo vs. 3 months of flecainide, and quantified as follows for comparison:

Ventricular arrhythmia score:
0 = no ventricular ectopic beats
1 = single PVC's
2 = PVC's in bigeminal pattern
3 = PVC pairs (couplets)
4 = nonsustained VT (≥ 3 beats, but < 30 seconds)
5 = sustained VT (> 30 seconds)

Quantification of arrhythmias:

1. Total number of ventricular ectopic beats during entire exercise test (rest, exercise, and recovery)
2. Number of ectopic beats during worst 10 second period of exercise test (rest, exercise, and recovery)
3. Ratio of ectopic to sinus beats during worst 10 second period of exercise test (rest, exercise, and recovery)

## 10.0 Privacy/Confidentiality Issues

Only individuals directly involved with the study will have access to data. Information is for research purposes only and will be used for publication purposes. All participants will have their names concealed. Access to identified patient information will be limited to the investigators listed within this IRB application. De-identified information with

HIPPA identifiers removed will be available to other investigators following appropriate IRB approval. Confidentiality and security will be maintained for the database. The database is stored behind a firewall (in addition to the institutional firewall) with the highest level of protection, i.e. the same level of protection as the on-line hospital information system at Vanderbilt. This means that users must logon to a web server that sits between the institutional firewall and the firewall to the database, and only this application server is allowed to query the database. Only users approved through our institutional review board will be allowed access to patient identifiers. Other levels of authorization may exist for future approved users following IRB approval, e.g. access to de-identified data.

Data is initially collected in the medical record for each individual study participant. The information will be extracted from the patient's medical record and then transferred into the Case Report Form (CRF).

The CRFs will include personal identifiers for participant. However, this data will not be accessible as numbers and initials are assigned for each participant and these will become the identifying information for each study participant. A master list with patient demographics will only be accessible to the principle investigator and his senior co-investigator. This data will not be available to others.

**Reference List**

Bokenkamp, R., et al. "Flecainide for recurrent malignant ventricular arrhythmias in two siblings with Andersen-Tawil syndrome." Heart Rhythm. 4.4 (2007): 508-11.

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802

803 **Summary of changes to protocol:**

804 The reasons for changes to the protocol are reflected in the manuscript Methods:

805 "The primary endpoint was appropriate ICD therapy, and the secondary endpoint was degree of
806 ventricular arrhythmias induced on exercise testing. Adequate power for the primary endpoint
807 required enrolling 60 subjects. In June 2015, after only 14 of the desired 60 subjects were able to
808 be enrolled, the investigators, with approval from the funding source and the Data and Safety
809 Monitoring Board (DSMB), modified the study protocol to evaluate only the secondary endpoint
810 (ventricular arrhythmias on exercise test). At that point, subjects who had completed the
811 Treatment A exercise test crossed over, without the 18 month treatment duration. Similarly,
812 subjects discontinued Treatment B once the three month exercise test was complete."

813 **The protocols differ in the following sections:**

814 **Section 2**: Aims 1 and 3 removed. Aim 2 becomes primary aim.

815 **Section 6**: Study procedures: Duration of therapy shortened from 18 months to 3 months.
816 Primary endpoint changed from cardiac event to ventricular ectopy at exercise test.

817 **Section 9**: Statistical considerations are significantly revised, with original and final statistical
818 plans copied below.

819

## Original Statistical Considerations:

### Sample Size Estimation and Power Analysis

The primary endpoint of this randomized controlled 2x2 cross-over trial will be cardiac event defined as appropriate ICD therapy (shock or anti-tachycardia pacing for VT) or death. The objective is to demonstrate decreased event rate in patients treated with flecainide in addition to standard therapy compared to patients treated with standard therapy plus placebo. Previous studies of CPVT patients with ICD's reveal an event rate ranging from 50% over 20 months (Priori et al) to 25% over 3.9 years (Hayashi et al). This wide range results in an estimated event rate during 18 months of treatment with placebo plus standard therapy between 10 and 45%. In our small series of patients receiving open-label flecainide, we observed 1 event in 23 patients (4%), which was likely due to noncompliance. The event rate of the control group is expected to be 15% based on the above data. The sample size estimation was carried out using the Pearson chi-square test for paired proportions. With a sample size of 55, the study will have 80% power to detect a 10% difference in the primary endpoint with a two-sided test at 5% significance level. To compensate for reduced power caused by the noncompliance (the anticipated dropout rate over 36 months is 8% based on our pilot data), 60 patients will be enrolled.

### Statistical Analysis Plan

Descriptive statistics, including means, standard deviations, and ranges for continuous variables, as well as percentages and frequencies for categorical variables, will be provided to describe the study sample. Pearson chi-square test or Fisher's exact test will be used to assess the categorical variables. Differences between group means for continuous variables will be examined using ANOVA or Kruskal-Wallis Test. The Mainland-Gart's test will be used for univariate analysis of the primary outcome, when the assumption of no carry-over effect holds. For multivariate analysis, the Generalized Linear Mixed Model will be used to assess the treatment effect, period effect, and treatment-by-period interaction effect, and to adjust for other risk factors such as gender or age. The analysis of survival data will be carried out if such data are available, using the Kaplan-Meier method with log-rank test to compare time-to-event between the two arms and the proportional hazard Cox model to investigate potential prognostic factors. The alpha-spending function of O'Brien-Fleming will be applied for the interim analysis to maintain an overall significance level at 0.05. Point estimates along with the corresponding p-values and 95% confidence intervals will be reported. The adjusted p-values and the corresponding 95% confidence interval will be reported for multivariate analyses. Statistical analysis will be done with R for Windows, version 2.9.2 and SAS 9.2.

Secondary analysis will include comparison of the exercise treadmill tests after 3 months of placebo vs. 3 months of flecainide, and quantified as follows for comparison:

Ventricular arrhythmia score:
0 = no ventricular ectopic beats
1 = single PVC's

2 = PVC's in bigeminal pattern
3 = PVC pairs (couplets)
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Quantification of arrhythmias:

1. Total number of ventricular ectopic beats during entire exercise test (rest, exercise, and recovery)
2. Number of ectopic beats during worst 10 second period of exercise test (rest, exercise, and recovery)
3. Ratio of ectopic to sinus beats during worst 10 second period of exercise test (rest, exercise, and recovery)

Based on pilot human studies, we anticipate a reduction in the number of ectopic beats during worst 10 second period of exercise from 13 ± 5 beats to 5 ± 5 beats. Assuming an α of 0.05 and 80% power, we would need only 5 subjects to show a statistically significant reduction, as this is a paired analysis.

## Final Statistical Analysis Plan

Our previous open-label comparison of flecainide to standard therapy showed a 7 beat reduction in number of ectopic beats in worst 10 seconds of exercise test (from 12 ± 5 beats to 5 ± 5 beats).[1] A second study in 12 genotype-negative CPVT patients demonstrated a 6 beat reduction in number of ectopic beats in worst 10 seconds of exercise test (from 12 ± 7 beats to 6 ± 7 beats).[2] **A target of 14** enrolled subjects provide adequate statistical power (80% power with an alpha of 0.05) to detect the previously observed differences (6-7) in number of PVC's in worst 10 second period during exercise testing.[3] Graphs of Power (Y-axis) vs observed difference in means (X-axis) are provided below. The 2 panels represent power calculations using different values for the within-group standard deviation (SD). These values are informed from the 2 previous studies in CPVT patients using the same methodology (left, within group SD = 5, observed in reference 1; right within group SD = 7, observed in reference 2). Red lines indicate N=13, and blue lines indicate N=12, allowing for potential subject dropout.

References


1. van der Werf C, Kannankeril PJ, Sacher F, Krahn AD, Viskin S, Leenhardt A, Shimizu W, Sumitomo N, Fish FA, Bhuiyan ZA, Willems AR, van der Veen MJ, Watanabe H, Laborderie J, Haïssaguerre M, Knollmann BC, Wilde AA. Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. J Am Coll Cardiol. 2011 May 31;57(22):2244-54.
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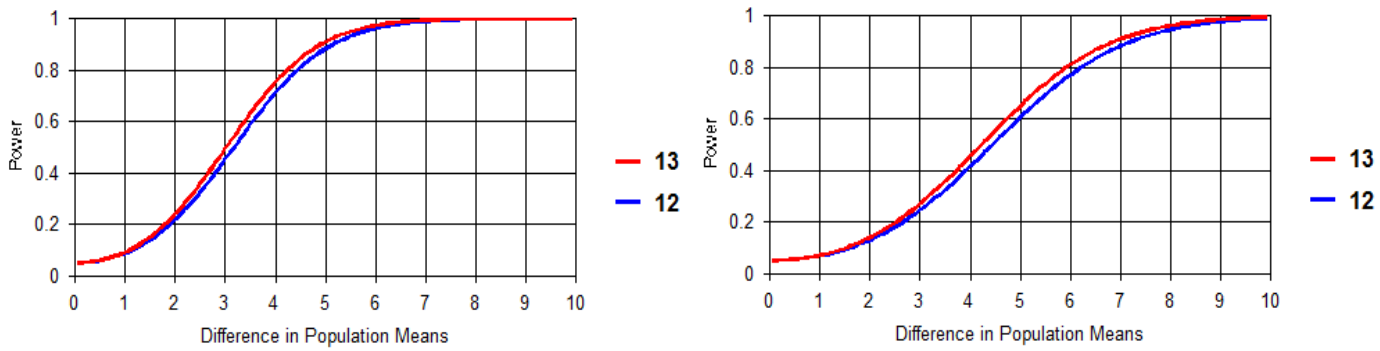

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3. Ratio of ectopic to sinus beats during worst 10 second period of exercise test (rest, exercise, and recovery)

940 **Summary of changes to statistical plan:**

941 The originally proposed secondary analysis becomes the primary analysis, and power
942 calculations are presented for the enrolled subjects, with new references added.

943