Heart rate reduction after exercise is associated with arrhythmic events in patients with catecholaminergic polymorphic ventricular tachycardia

SHORT TITLE: Post-Exercise Heart Rate Reduction in CPVT

Krystien V.V. Lieve, MD, MSc[1,2]*; Veronica Dusi, MD[2,3]*; Christian van der Werf, MD, PhD[1,2]; J. Martijn Bos, MD, PhD[4]; Conor M. Lane, MD[4]; Mathis Korseberg Stokke, MD, PhD[5]; Thomas M. Roston, MD[6]; Aurora Djupsjöbacka, MD[2,7]; Yuko Wada, MD[8]; Isabelle Denjoy, MD[2,9]; Henning Bundgaard, MD, DMSc[2,10]; Ferran Roses I Noguer, MD[2,11]; Christopher Semsarian, MBBS, PhD, MPH[12]; Tomas Robyns, MD, PhD[2,13]; Nynke Hofman, MSc, PhD[1,2]; Michael W. Tanck, MSc, PhD[14]; Maarten P. van den Berg, MD, PhD[15]; Janneke A.E. Kammeraad, MD, PhD[16]; Andrew D. Krahn, MD, FRCPC, FHRS[17]; Sally-Ann B. Clur, MBBCh, MSc(Med), FCP(SA)Paed, PhD[1,2]; Frederic Sacher, MD[18]; Jan Till, MD[2,11]; Jonathan R. Skinner, MD[19]; Jacob Tfelt-Hansen, MD, DMSc[2,20]; Vincent Probst, MD, PhD[2,21]; Antoine Leenhardt, MD[2,9]; Minoru Horie, MD, PhD[8]; Heikki Swan, MD[2,7]; Jason D. Roberts, MD, MAS[22]; Shubhayan Sanatani, MD[6]; Kristina H. Haugaa, MD, PhD[5]; Peter J. Schwartz, MD[2,23]; Michael J. Ackerman, MD, PhD[4]; Arthur A.M. Wilde, MD, PhD[1, 2, 24]

* These authors contributed equally to the study

[1] AMC Heart Center, Department of Clinical and Experimental Cardiology, Academic Medical Center, Amsterdam, the Netherlands

[2] European Reference Network 'ERN GUARD-heart'

[3] Department of Molecular Medicine, University of Pavia, Department of Cardiology and Cardiovascular Clinical Research Center, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

[4] Departments of Cardiovascular Medicine (Division of Heart Rhythm Services), Pediatric and Adolescent Medicine (Division of Pediatric Cardiology), and Molecular Pharmacology & Experimental Therapeutics (Windland Smith Rice Sudden Death Genomics Laboratory), Mayo Clinic, Rochester, MN, United States

[5] Department of Cardiology, Center for Cardiological Innovation, Oslo University Hospital, Rikshospitalet, Oslo, Norway and University of Oslo, Oslo, Norway

[6] Department of Pediatrics, Children's Heart Centre, Division of Cardiology, British Columbia Children's Hospital, Vancouver, British Columbia, Canada

[7] Heart and Lung Center, Helsinki University Hospital and Helsinki University, Helsinki, Finland.

[8] Department of Cardiovascular Medicine, Shiga University of Medical Science, Otsu, Japan

[9] CNMR Maladies Cardiaques Héréditaires Rares, Hôpital Bichat; Université Paris Diderot, Sorbonne Paris Cité, F-75018, Paris, France; AP-HP, Service de Cardiologie, Hôpital Bichat, F-75018, Paris, France

[10] The Capital Regions Unit for Inherited Cardiac Diseases, Department of Cardiology, Rigshospitalet, Copenhagen University Hospital, Blegdamsvej 9, 2100, Copenhagen Ø, Denmark

[11] Department of Cardiology, Royal Brompton Hospital, London, United Kingdom

[12] Agnes Ginges Centre for Molecular Cardiology, Centenary Institute and University of Sydney; Department of Cardiology, Royal Prince Alfred Hospital

[13] Department of Cardiovascular Diseases, University Hospitals Leuven, Leuven, Belgium

[14] Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, Amsterdam, The Netherlands

[15] Department of Cardiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

[16] Department of Pediatric Cardiology, Sophia Children's Hospital, Erasmus Medical Center, Rotterdam, the Netherlands

[17] Heart Rhythm Research, Division of Cardiology, University of British Columbia, Vancouver, BC, Canada

[18] Bordeaux University Hospital, LIRYC institute, Pessac, France

[19] The Green Lane Paediatric and Congenital Cardiac Services, Starship Children's Hospital, Auckland, New Zealand and Department of Paediatrics Child and Youth Health, University of Auckland, New Zealand.

[20] Department of Cardiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark and Department of Forensic Medicine, Faculty of Medical Sciences, University of Copenhagen, Denmark

[21] l'institut du thorax, Service de Cardiologie du CHU de Nantes, Hopital Nord, Nantes Cedex, France

[22] Section of Cardiac Electrophysiology, Division of Cardiology, Department of Medicine, Western University, London, Ontario, Canada

[23] Center for Cardiac Arrhythmias of Genetic Origin, IRCCS Istituto Auxologico Italiano, Milan, Italy.

[24] Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders, Jeddah, Kingdom of Saudi Arabia

Address for correspondence

Arthur A.M. Wilde, MD, PhD
AMC Heart Center, Department of Clinical and Experimental Cardiology
Academic Medical Center
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands
Telephone: +31205662904
Fax: +31206971385
E-mail: a.a.wilde@amc.uva.nl

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ABSTRACT

Background

Risk stratification in catecholaminergic polymorphic ventricular tachycardia (CPVT) remains ill defined. Heart rate reduction (HRR) immediately after exercise is regulated by autonomic reflexes, particularly vagal tone, and may be associated with symptoms and ventricular arrhythmias (VAs) in patients with CPVT. Our objective was evaluate whether HRR after maximal exercise on the exercise stress test (EST) is associated with symptoms and VAs.

Methods

In this retrospective observational study, we included patients ≤ 65 years with an EST without antiarrhythmic drugs (AAD) who attained at least 80% of their age- and gender-predicted maximal HR. HRR in the recovery phase was calculated as the difference in heart rate (HR) at maximal exercise and at one minute in the recovery phase (ΔHRR1').

Results

We included 187 patients (median age 36 years, 68 [36%] symptomatic before diagnosis). Pre-EST HR and maximal HR were equal among symptomatic and asymptomatic patients. Patients that were symptomatic prior to diagnosis had a greater ΔHRR1' after maximal exercise (43 [IQR, 25-58] vs. 25 [IQR, 19-34] beats/minute, p<0.001). Corrected for age, gender and relatedness, patients in the upper tertile for ΔHRR1' had an odds ratio of 3.4 (95% confidence interval, 1.6–7.4) of being symptomatic before diagnosis (p<0.001). In addition, ΔHRR1' was higher in patients with complex VAs at EST off AADs (33 [IQR, 22-48] beats/minute vs. 27 [IQR, 20-36], p=0.01. After diagnosis, patients with a ΔHRR1' in the upper tertile of its distribution

had significantly more arrhythmic events as compared to patients in the other tertiles (p=0.045).

Conclusions

CPVT patients with a larger HRR following exercise are more likely to be symptomatic and have complex VAs during first EST off AAD.

KEYWORDS
autonomic nervous system; catecholaminergic polymorphic ventricular tachycardia; exercise stress test; *RYR2*; sudden cardiac death; ventricular arrhythmias

NON-STANDARD ABBREVIATIONS AND ACRONYMS

INTRODUCTION

Inherited arrhythmia disorders such as catecholaminergic polymorphic ventricular tachycardia (CPVT) are an important cause of sudden cardiac death (SCD) among young individuals[1]. Patients with CPVT have a normal 12-lead electrocardiogram and a structurally normal heart [2]. However, in the setting of increased sympathetic activity such as exercise or emotions, these patients display progressive ventricular ectopy of escalating complexity that may include bidirectional or polymorphic ventricular arrhythmias, and may lead to SCD [2]. The exercise stress test (EST) is the gold standard to establish the diagnosis of CPVT[3]. Young age at diagnosis, aborted cardiac arrest (ACA), and the complexity of ventricular arrhythmias have been identified as predictors of risk of arrhythmic events in patients with CPVT[4]. However, in many patients, the risk of future arrhythmic events cannot accurately be estimated.

Heart rate behavior during exercise and recovery from exercise is mediated chiefly by the balance between the two components of the extrinsic autonomic nervous system (ANS): the sympathetic and parasympathetic branches[5]. The ANS has long been known to play a role in arrhythmogenesis and cardiac electrical stability[6]. This was first demonstrated in a study associating SCD in post myocardial infarction patients with reduced heart rate variability and baroreflex sensitivity, both measures of the ANS[7]. A contrary observation was seen in a South African founder population of patients with congenital long-QT syndrome type 1 (LQT1). Here, subjects with strong autonomic reflexes, assessed by baroreflex sensitivity[8] and accentuated heart rate recovery (HRR)[9], had a higher probability of being symptomatic.

Since patients with LQT1 and CPVT both have arrhythmic events under circumstances of increased sympathetic activity, we hypothesized that CPVT patients with strong autonomic

reflexes may also be at increased risk for arrhythmic events. Here, we studied the association between HRR and arrhythmic events in patients with CPVT.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study design, setting and population

The study population was derived from the International CPVT Registry. This is a retrospective, multicenter cohort study instituted in 2014 by the Academic Medical Center (Amsterdam, The Netherlands) which has included patients with CPVT from 25 international centers to date. The diagnosis of CPVT is based on the clinical phenotype and/or genetically confirmed by the identification of a pathogenic CPVT-associated mutation, primarily in the *RYR2*-encoded ryanodine receptor/calcium release channel (CPVT1), according to the expert consensus guidelines[3]. All centers received institutional review board approval for this type of study.

De-identified clinical and genetic data were recorded on specifically designed web-based forms both at baseline evaluation and during follow-up. Detailed information was required regarding the first available EST without antiarrhythmic drugs (AAD), in particular β-blockers, and the first EST performed on the maximal tolerable dose of β-blockers.

For this study, we selected patients from the International CPVT Registry who had an EST performed without AAD and with available information about the maximum heart rate (HR_{max}) reached during exercise and the heart rate during the first minute of recovery ($HRR_{1'rec}$) (N= 267). Of these, we excluded patients who were over the age of 65 years at the time of EST, because of the known negative correlation between age and vagal reflexes in this age group. In order to guarantee an appropriate chronotropic competence and minimize potential confounding factors due to different EST protocols used across the study population, we

excluded patients who did not reach 80% of their predicted HR_{max} (calculated by age and gender). The available 12-lead electrocardiogram traces of the EST of patients reported to have both complex arrhythmias during the EST and either a maximum HR value in the higher decile or an HRR value in the lower decile were directly inspected. If the arrhythmias were found to affect the maximum HR and/or the recovery HR (impossible to determine the sinus node activity), the patients were excluded from the analysis (one patient with complex ventricular arrhythmias and one patient with atrial arrhythmias were excluded, Figure 1).

Symptomatic patients were defined as patients who had experienced an arrhythmic syncope or ACA before CPVT was diagnosed. Asymptomatic patients had to be at least 15 years old at the time of the EST and off AAD. After the EST without AAD we followed the patients until their first arrhythmic event after diagnosis or date of last contact. Arrhythmic events during follow-up were defined as arrhythmic syncope, ACA, appropriate ICD shock or SCD.

Exercise stress test evaluation

ESTs of the patients included in the present study were performed between April 1995 and March 2017. A multistage fatigue-limited EST was performed according to local protocols. HR decrease during the recovery phase was calculated as the difference (Δ) in HR between the values recorded at peak exercise (HR_{max}) and those recorded one ($HRR_{@1'}$) and two ($HRR_{@2'}$) minutes after termination of exercise and is abbreviated by ΔHRR1'and ΔHRR2'. HR increase was defined as the difference between the HR at peak exercise and the pre-test HR.

Severity of ventricular arrhythmias on the EST was scored according to the worst ventricular arrhythmia noted and was categorized into the following five categories: no ventricular arrhythmias, single ventricular premature beats (VPB) only, bigeminal VPBs, couplets,

nonsustained ventricular tachycardia (NSVT) or sustained ventricular tachycardia (VT). Couplets, NSVT and sustained VT were considered complex ventricular arrhythmias.

Statistical analysis

Data was analyzed using IBM SPSS statistics database (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 24. Armonk, NY: IBM Corp) and with R version 3.4.3 (The R Project for Statistical Computing).

We performed three analyses. The first and second analysis assessed potential correlations between characteristics of the first EST without AAD and the first available EST on the maximal tolerable dose of β-blockers and the presence of symptoms *before* these ESTs. The third analysis assessed potential correlations between characteristics of first EST without AAD and the presence of arrhythmic events *after* this EST.

Clinical parameters are presented for the entire study population as well as for the study population stratified by symptom status. Continuous variables are presented as median (interquartile range [IQR]), were inspected for normality of the distribution and compared by the Student's t test or the Mann-Whitney U test where appropriate. Categorical variables are expressed as absolute and relative frequencies and were compared with the χ^2-test .

ΔHRR had a nonlinear relationship with the occurrence of symptoms before diagnosis. Therefore, we dichotomized this variable at the upper tertile of its distribution to assess the association between ΔHRR and the presence of symptoms. Odds ratios (ORs) with 95% confidence intervals (CI) were estimated by logistic regression. To compensate for possible correlation of characteristics between relatives within a family, generalized estimating

equations with a logit link function and an exchangeable correlation structure were applied. Receiver operating characteristics curves were constructed and the area under the curve was used to determine the performance of the ΔHRR in discriminating between symptomatic and asymptomatic cases. Sensitivity analyses were performed excluding the *RYR2* p.R420W mutation. We used the Kaplan–Meier method to provide survival estimates, which were assessed with the log-rank test. A p-value <0.05 was considered statistically significant.

RESULTS

Study population

A total of 187 patients with CPVT from 95 families were included in the study (Table 1 and Figure 1). The median age at the EST without AAD was 36 years (IQR, 19-47). Sixty-eight patients (36%) were symptomatic prior to diagnosis: 52 patients (76%) had an arrhythmic syncope and 16 patients (24%) an ACA as their worst symptom before diagnosis (median age at worst symptom 14 years [IQR, 11-20]). Symptomatic patients were more often the familial proband (49% vs. 8%, p<0.001) and were younger at the EST (23 [IQR, 12-39] vs. 40 [IQR, 27-50] years, p<0.001). The vast majority of the patients (94.7%) had a CPVT1-causative variant in *RYR2* (Supplemental Table 1).

Heart rate recovery during exercise testing and risk of symptoms

Pre-test HR, HR_{max} and HR at first VPB were equal between symptomatic and asymptomatic patients (Table 1 and Figure 2A). However, HRR after the cessation of exercise was different between the groups. Symptomatic patients had a lower $HRR_{@1'}$ (125 [IQR, 110-147] vs. 143 [IQR, 129-154] beats/minute, p<0.001) and $HRR_{@2'}$ (104 [IQR, 88-123] vs. 120 [IQR, 107-134] beats/minute, p<0.001). Significantly higher values of ΔHRR1' (43 [IQR, 25-58] vs. 25 [IQR, 19-34] beats/minute, p<0.001) and ΔHRR2' (66 [IQR, 46-89] vs. 49 [IQR, 36-58] beats/minute, p<0.001) were observed in symptomatic patients (Figure 2B). Expressed as percentages, symptomatic and asymptomatic patients had a ΔHRR1' decrease of 26% and 15% (p<0.001), and a ΔHRR2' decrease of 40% and 28% (p<0.001), respectively.

Within the symptomatic patient group, there were no significant differences in ΔHRR1' or ΔHRR2' between patients who had an arrhythmic syncope and patients who had an ACA as their worst symptom before diagnosis (data not shown).

In order to assess the association between ΔHRR1' and symptoms, we dichotomized the ΔHRR1' value at $\geq$ 36.0 beats/minute which represented the upper tertile of its distribution. Indeed, patients in the upper tertile of ΔHRR1' had an increased risk of being symptomatic (OR 5.0, 95% CI 2.6-9.8, p<0.001). We considered age and gender to be potential confounders in the association between ΔHRR1' and symptoms before diagnosis. In addition, since some patients were related to each other, we also considered relatedness to be a potential confounder. Following adjustment for age, gender and relatedness, patients with a ΔHRR1' $\geq$ 36.0 bpm (representing the upper tertile of its distribution) still had an odds ratio of 3.6 (95% CI 1.9-6.9, p<0.001) of being symptomatic before diagnosis compared to patients with a ΔHRR1' $\leq$ 36.0 bpm (area under the curve, 0.74, Supplemental Figure 1).

Since 39 patients (21%) carried the p.R420W variant in *RYR2,* we performed a sensitivity analysis excluding patients with this mutation to see if the results are not driven by this mutation. Results did not differ on the univariate analysis (OR 4.7; 95% CI 2.3–9.8; p<0.001), or following adjustment for age, gender and relatedness (OR 3.4 95% CI 1.6–7.4; p<0.0014)

Ventricular arrhythmia burden and heart rate recovery

Next, we evaluated the relationship between ventricular arrhythmic burden and HRR. Fifty asymptomatic (42%) and 36 symptomatic (53%) patients had complex ventricular arrhythmias (couplets, NSVT, or VT) as the worst ventricular arrhythmia on the EST (p=0.19, Table 1). Patients with complex ventricular arrhythmias had a greater ΔHRR1' (33 [IQR, 22-48]

beats/minute vs. 27 [IQR, 20-36], p=0.01) and ΔHRR2' (56 [IQR, 40-76] beats/minute vs. 51 [IQR, 38-60], p=0.01, Figure 3), compared to patients with simple ventricular arrhythmias. We then stratified patients with complex ventricular arrhythmias by symptom status. Symptomatic patients with complex ventricular arrhythmias had a greater ΔHRR1' (51 [IQR, 33-63] vs. 26 [IQR, 19-37] beats/minute, p<0.001) and ΔHRR2' (76 [IQR, 57-97] vs 47 [IQR, 34-61] beats/minute, p<0.001) than asymptomatic patients with complex ventricular arrhythmias (Figure 4).

Exercise stress test on maximal tolerable dose of beta blockers

An EST on the maximum tolerated dose of β-blockers was performed in a total of 112 patients (59.9%), and in 71 of these patients (63.4%) information about $HRR_{@1'}$ was available. Median interval between the baseline EST without AAD and the first EST on the maximal tolerable dose of β-blockers was 2.3 years (IQR 0.6-5.5). The most frequently used β-blockers were metoprolol (31.0%) and bisoprolol (29.6%). Supplemental Table 2 shows the β-blocker dosages.

Symptomatic patients were younger at the EST on β-blockers (33 [IQR, 16-45] vs. 43 [IQR, 28-50] years, p=0.02, Supplemental Table 3). We did not observe any differences in heart rate profile during the EST or at one minute (ΔHRR1'33 [IQR, 24-44]] vs. 27 [IQR, 21-36 beats/minute, p=0.16) or two minutes (ΔHRR2' 50 [IQR, 43-63] vs. 45 [IQR, 35-52]beats/minute, p=0.098) in the recovery phase (Supplemental Table 3). Thirty-five patients (49.3%) achieved at least 80% of the predicted maximal heart rate, including 12 symptomatic patients (33.3%). In this subset, symptomatic patients had a a significantly greater ΔHRR1' (40

[IQR, 30-44] vs. 25 [IQR, 21-35] beats/minute, p=0.037) and a significantly greater ΔHRR2' (58 [IQR, 46-68] vs. 47 [IQR 36-55] beats/minute, p=0.023) (Table 2 and Figure 5).

Arrhythmic events after diagnosis

After the first EST off AAD and during a median follow-up of 3.5 years (IQR, 1.1-7.4), 10 patients (5.3%) experienced an arrhythmic event: six patients (3.2%) experienced an appropriate ICD shock and four (2.1%) had an arrhythmic syncope. Patients with a ΔHRR1' in the upper tertile of its distribution had significantly more arrhythmic events (n=7) after diagnosis as compared with patients in the combined lower and middle tertile (n=3, p=0.045, Figure 6).

DISCUSSION

Our findings, obtained in a relatively large multicenter cohort of patients with CPVT, indicate that the magnitude of HRR both at one and two minutes after the cessation of exercise to at least 80% of maximum predicted heart rate identified CPVT patients more likely to have been symptomatic prior to diagnosis (and thus prior to the initiation of anti-arrhythmic drugs). Patients with a large ΔHRR1' were five times more likely to have been symptomatic prior to diagnosis. Furthermore, patients with a larger ΔHRR1' and ΔHRR2' were also more likely to have complex ventricular arrhythmias during the first EST off AAD. These results could also be confirmed in the EST on a maximal tolerable dose of β-blockers. Finally, in an analysis that was unadjusted for potential confounders, patients with a ΔHRR1' in the upper tertile of its distribution (i.e. ≥ 36.0 beats/minute) had significantly more arrhythmic events as compared with patients in the other tertiles after diagnosis.

Autonomic reflexes in the general population

The heart rate changes observed during exercise and the recovery from exercise are mediated by the interplay between the sympathetic and the parasympathetic limbs of the ANS. Therefore, the EST can be considered as a simple and economic tool to indirectly assess cardiac autonomic reflexes. Based on studies in animals and healthy humans, the reactivation of the vagal nerve is considered the main force of these heart rate changes during the first four minutes of the recovery from exercise[10].

In stark contrast to the observations in this CPVT cohort in this present study, multiple associations between an increased risk of SCD and **reduced** vagal activity or increased sympathetic activity have been identified over the years. Clinical studies in different settings

consistently showed that a **lower** ΔHRR1' was an independent predictor of both cardiovascular as well as all-cause mortality in middle-aged individuals[11,12]. The Paris Prospective Study was the largest study to assess the relationship between heart rate profile during exercise and recovery from exercise and a risk for lethal arrhythmias[13]. During a 23-year follow-up period, the authors found a two-fold increase in risk of sudden death among those with an ΔHRR1' in the lowest quintile (less than 25 beats/minute) as compared with the highest quintile (more than 40 beats/minute). The underlying mechanism of this association remains largely elusive.

Autonomic reflexes in inherited arrhythmia disorders

Due to the well documented influences of the ANS on arrhythmogenesis and cardiac electrical stability, there is a strong rationale to assess autonomic reflexes in inherited arrhythmia disorders such as congenital LQTS and CPVT.

In 2008, Schwartz et al. assessed the impact of the ANS in a South African founder population harboring the KCNQ1-A341V mutation causing LQT1[8]. LQT1 is caused by loss-of-function mutations in the *KCNQ1*-encoded Kv7.1 potassium channel which provides the slowly activating delayed rectifier current (I_{Ks})[14]. The authors suggested that strong autonomic reflexes, assessed through baroreflex sensitivity, may be detrimental in the setting of an intrinsically increased cardiac susceptibility to both catecholamines and abrupt heart rate changes. In a subsequent study, the authors were able to replicate this finding using a simpler clinical tool: HRR[9]. In this study, ΔHRR1' was also assessed in LQTS patients with preserved I_{Ks} (LQT2 and LQT3) and no differences between symptomatic and asymptomatic patients were observed. Finally, they found a good correlation between baroreflex sensitivity, determined

by the phenylephrine method, and ΔHRR1' with a similar ability to predict the risk of life-threatening arrhythmias (AUC >0.70)[9].

While the suggested pro-arrhythmogenic mechanism in LQT1 is through the effects of sympathetic activation on $I_{Ks,}$[9], in CPVT, the pro-arrhythmogenic diastolic calcium leakage from the sarcoplasmic reticulum becomes more pronounced in the setting of high sympathetic tone, ultimately resulting in delayed after-depolarizations that may lead to triggered arrhythmias. At the end of exercise, there is an instantaneous rebound of vagal reflexes while abundant catecholamines are still in the heart. This increases the heterogeneity of recovery periods which may confer increased susceptibility for reentrant arrhythmias (ventricular tachycardia and fibrillation)[15]. In addition, the higher the sympathetic activation during exercise, the higher the heart rate will be at peak exercise. The amount of heart rate increase at peak, combined with the strength of vagal reflexes will determine the delta and thereby the greatest disparity in the recovery period. Interestingly, and probably related to the concept just expressed, suppression of the vagal activity by atropine had antiarrhythmic effects in Casq2 knockout and $RyR2^{R4496C/+}$ mice[16]. Since patients with inherited arrhythmia disorders are often young and otherwise healthy, the variability in HRR mainly reflects a genetic effect,[17] leaving physical training as the most significant non-genetic confounding factor. Indeed, the genetic traits controlling autonomic reflexes are inherited independently from the mutations causing the inherited arrhythmia disorders and therefore may act as an independent modifier of arrhythmic risk.

In our study, we focused on those patients who reached at least 80% of their predicted HR_{max} during the EST, because the steepness of HRR is influenced by exercise intensity and HR_{max}[18].

The mean HR_{max} at the EST without AAD in our cohort (169 beats/minute) was similar to HR_{max} in previously published CPVT populations,[19–21] but higher than reported by Crotti et al. in their LQT1 population (145 beats/minute)[9]. This may reflect the fact that CPVT patients are exercised maximally in order to try to elicit repetitive ventricular arrhythmias in order to determine the arrhythmic risk of the patient. This may also explain why the mean values of ΔHRR1' that we observed in our cohort are higher than those observed in the LQT1 population,[9] but in line with ΔHRR1' in healthy individuals with comparable age and exercise intensity[22].

We performed a subset analysis in 71 patients in whom data on an EST on β-blocker therapy including information about the $HRR_{@1'}$ was available. When we selected the patients that had achieved at least 80% of the predicted heart rate, we found that symptomatic patients had a significantly larger ΔHRR2' and a trend towards a larger ΔHRR1'. Kannankeril et al. evaluated the parasympathetic effects on HRR in healthy subjects and concluded that the effects are most pronounced in the first four minutes of the recovery phase[10]. In addition, Sundaram et al.[23] evaluated the contribution of the sympathetic withdrawal to $HRR_{@1'}$. In that study, they found that β-adrenergic withdrawal is not a significant factor in the $HRR_{@1'}$. These results suggest that EST in the presence of β-blockers should have no effect on reinstitution of vagal tone and therefore no effect on HRR as compared with HRR in the absence of AAD. In addition, since heart rate declines exponentially after exercise, ΔHRR1' depends on the HR_{max} achieved and is not an optimal marker to assess HRR during submaximal exercise. Therefore, it is most likely that the different protocols used in our study play a role in these findings.

Clinical implications

Our findings have important clinical implications. We were able to demonstrate that HRR in the EST without AAD is associated with the presence of arrhythmic events prior to the diagnosis (and thus before initiation of medical therapy) and the severity of EST-induced VAs in a large cohort of CPVT patients. Considering the fact that approximately 42% of the asymptomatic patients had an EST with complex ventricular arrhythmias despite never having suffered an arrhythmic event, our data may be a useful tool for refined risk stratification. Additionally, at a comparable intensity of exercise, HRR is a reproducible measurement in the same subject[24]. For example, in asymptomatic genotype-positive relatives the presence of strong vagal tone in the absence of ventricular arrhythmias during the first EST could be an argument to be more aggressive with β-blocker therapy than in those without strong vagal activation post-exercise.

Limitations of the study

Due to the retrospective nature of the study not all parameters were available for all patients. In a relatively large proportion of patients in the International CPVT registry an EST off AAD and/or HRR parameters at EST off AAD were not available for analysis. Patients with missing HRR parameters at EST off AAD were significantly younger on the EST without AAD and more often symptomatic. Therefore, a selection bias cannot be excluded. Due to the multicenter and retrospective nature of the study, different types of EST and recovery protocols were used. In addition, we cannot fully exclude that in some patient the EST was terminated due to ventricular arrhythmias rather than due to fatigue. However, we only included patients that were exercised to at least 80% of their maximal predicted heart rate to account for the different exercise protocols and the possibility of a submaximal EST. A large proportion of the

patients (77%) include family members of the familial proband. These patients are typically diagnosed through cascade screening and are therefore diagnosed at a relatively 'old' age. This patient group may manifest with a milder phenotype with less arrhythmic risk compared with the proband who often present at a younger age. However, our population likely represents the 'general' CPVT population because disease severity may have been overestimated in the earlier cohorts.2 Finally, due to the low number of arrhythmic events during follow in the subset of patients analyzed, we were unable to build a multivariable model for predictors of arrhythmic events after diagnosis.

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DISCLOSURES

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TABLES

Table 1. Baseline characteristics and results of first EST off AAD.

	All (n=187)		**Asymptomatic (n=119)**		**Symptomatic (n=68)**		**p-value**
Proband	43	(23)	10	(8)	33	(49)	<0.001
Female gender	110	(59)	62	(52)	48	(71)	0.021
Age at exercise test, years	36	(19-47)	40	(27-50)	23	(12-39)	<0.001
Age at worst symptom, years	14	(11-20)		NA	14	(11-20)	
Syncope	52	(28)		NA	52	(76)	
ACA	16	(9)		NA	16	(24)	
Pre-test HR, bpm	76 (N=181)	(66-87)	76 (N=114)	(67-87)	76 (N=75)	(65-86)	0.970

HR at first VPB, bpm (N=135)*	126 (N=99/135)	(110-140)	130(N=56/84)	(112-149)	120 (N=43/51)	(108-135)	0.113
HR_{max}	169	(160-179)	168	(157-178)	173	(160-183)	0.074
% of max predicted HR	94	(89-100)	95	(90-100)	92	(87-99)	0.030
Heart rate increase, bpm	93	(79-106)	91	(79-104)	98	(79-111)	0.116
$HRR_{@1'}$	137	(121-151)	143	(129-154)	125	(110-147)	<0.001
ΔHRR1'	28	(21-43)	25	(19-34)	43	(25-58)	<0.001
%ΔHRR1'	17	(12-25)	15	(11-20)	26	(15-33)	<0.001
$HRR_{@2'}$	115 (N=155)	(97-131)	120 (N=96)	(107-134)	104 (N=59)	(88-123)	<0.001
ΔHRR2'	53 (N=155)	(39-68)	49 (N=96)	(36-58)	66 (N=59)	(46-89)	<0.001
%ΔHRR2'	32 (N=155)	(24-40)	28 (N=96)	(22-34)	40 (N=59)	(28-49)	<0.001

Worst ventricular arrhythmia							0.647
No arrhythmia	52	(28)	35	(29)	17	(25)	
VPB	25	(13)	17	(14)	8	(12)	
Bigeminy	24	(13)	17	(14)	7	(10)	
Couplet	34	(18)	21	(18)	13	(19)	
NSVT/VT	52	(28)	29	(24)	23	(34)	

Categorical variables are expressed as absolute and relative frequencies. Continuous variables are expressed as median with interquartile range. All heart rates are expresses as beats per minute. Mann-Whitney U test and Students t-test were used to calculate p-values where appropriate. Total numbers are included when they differ from those in the overall study group. *52 patients did not have any ventricular arrhythmias on the exercise stress test.

EST= exercise stress test; HR= heart rate; $HR_{max,}$= maximum heart rate; $HRR_{@1'}$= heart rate at the first minute of recovery; ΔHRR1' = absolute difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded one minute after termination of exercise;

%ΔHRR1’ = relative difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded one minute after termination of exercise; $HRR_{@2'}$ = heart rate at the second minute of recovery; ΔHRR2’ = absolute difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded two minutes after termination of exercise; %ΔHRR2’ = relative difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded two minute after termination of exercise; NA = not applicable; VPB = ventricular premature beat.

Table 2. Results of first EST while on maximal β-blocker dose.

	All (n=35)		**Asymptomatic (n=23)**		**Symptomatic (n=12)**		**p-value**
Proband	9	(26)	3	(13)	6	(50)	0.049
Female	22	(63)	12	(52)	10	(83)	0.149
Age at EST, years	40	(26-51)	41	(26-51)	39	(24-45)	0.297
Pre-test HR	71 (N=34)	(61-83)	71 (N=22)	(60-83)	73	(61-82)	0.896
HR at first VPB (N=30)*	132 (N=20/30)	(114-137)	132 (N=12/20)	(119-139)	132 (N=8/10)	(106-136)	0.956
HRmax	153	(148-162)	150	(146-161)	161	(155-164)	0.377
% of max predicted HR	88	(84-93)	87	(84-93)	90	(85-94)	0.322

$HRR_{@1'}$	125	(113-136)	126	(116-140)	119	(106-127)	0.230
ΔHRR1'	30	(23-44)	25	(21-35)	40	(30-44)	0.037
% ΔHRR1'	18	(14-27)	17	(13-20)	25	(19-30)	0.040
$HRR_{@2'}$	108 (N=33)	(93-116)	109 (N=21)	(101-116)	97	(83- 109)	0.092
ΔHRR2'	50 (N=33)	(40-59)	47(N=21)	(36-55)	58	(46-68)	0.023
% ΔHRR2'	30 (N=33)	(26-38)	28(N=21)	(24-34)	37	(30-47)	0.020

Categorical variables are expressed as absolute and relative frequencies. Continuous variables are expressed as median with interquartile range. All heart rates are expresses as beats per minute. Mann-Whitney U test and Students t-test were used to calculate p-values where appropriate. Total numbers are included when they differ from those in the overall study group. *5 patients did not have any ventricular arrhythmias on the exercise stress test.

EST= exercise stress test; HR= heart rate; $HR_{max,}$= maximum heart rate; $HRR_{@1'}$= heart rate at the first minute of recovery; ΔHRR1' = absolute

difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded one minute after termination of exercise; %ΔHRR1’ = relative difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded one minute after termination of exercise; $HRR_{@2'}$ = heart rate at the second minute of recovery; ΔHRR2’ = absolute difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded two minutes after termination of exercise; %ΔHRR2’ = relative difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded two minute after termination of exercise; VPB = ventricular premature beat.

FIGURE LEGENDS

Figure 1. Flowchart of the included patients.

AAD: anti arrhythmic drugs; EST: exercise stress test; ΔHRR1' difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded one minute after termination of exercise.

Figure 2. Comparison of heart rates in symptomatic and asymptomatic patients at different points of exercise testing without anti-arrhythmic drugs (panel A) and ΔHRR1' and ΔHRR2' (Panel B).

bpm= beats per minute; EST= exercise stress test; HR= heart rate; $HRR_{@1'}$= heart rate at the first minute of recovery; ΔHRR1'= difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded one minute after termination of exercise; $HRR_{@2'}$= heart rate at the first minute of recovery; ΔHRR2'= difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded two minutes after termination of exercise. Mann-Whitney U test and Students t-test were used to calculate p-values where appropriate.

Figure 3. Comparison between ΔHRR1' and ΔHRR2' for patients with simple and complex ventricular arrhythmias.

Bpm= beats per minute; ΔHRR1'= difference in heart rate between the values recorded at the peak exercise and those recorded one minute after termination of exercise; ΔHRR2'= difference in heart rate between the values recorded at the peak exercise and those recorded two minutes after termination of exercise. Mann-Whitney U test and Students t-test were used to calculate p-values where appropriate.

Figure 4. The behavior of the ΔHRR1' and ΔHRR2' in patients with complex ventricular

arrhythmias stratified by symptom status.

Bpm= beats per minute; N= number; ΔHRR1'= difference in heart rate between the values recorded at the peak exercise and those recorded one minute after termination of exercise; ΔHRR2'= difference in heart rate between the values recorded at the peak exercise and those recorded two minutes after termination of exercise. Mann-Whitney U test and Students t-test were used to calculate p-values where appropriate.

Figure 5. Comparison of heart rates in symptomatic and asymptomatic patients at different points of exercise testing on the maximum tolerable dose of β-blockers (panel A) and ΔHRR1' and ΔHRR2' (Panel B).

bpm= beats per minute; EST: exercise stress test; HR= heart rate; $HRR_{@1'}$= heart rate at the first minute of recovery; ΔHRR1'= difference in heart rate between the values recorded at the peak exercise and those recorded one minute after termination of exercise; $HRR_{@2'}$= heart rate at the first minute of recovery; ΔHRR2'= difference in heart rate between the values recorded at the peak exercise and those recorded two minutes after termination of exercise. Mann-Whitney U test and Students t-test were used to calculate p-values where appropriate.

Figure 6. Comparison in arrhythmic events after the exercise stress test without anti-arrhythmic drugs of upper tertile ΔHRR1'vs rest group.

Kaplan-Meier curves displaying arrhythmic events after the exercise stress test without anti-arrhythmic. AAD: anti-arrhythmic drugs; EST: exercise stress test ; ΔHRR1'= difference (Δ) in heart rate between the values recorded at the peak exercise and those recorded one minute after termination of exercise.