Effects of Adrenaline on Beat-to-Beat Variability in hiPSC-CMs with Hypertrophic Cardiomyopathy

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Abstract

Hypertrophic cardiomyopathy (HCM) is a major cause of sudden cardiac death in young individuals, often triggered by adrenergic stress. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) offer a platform for investigating patient-specific electrophysiology in vitro. Here, we investigated the effects of adrenaline on beat-to-beat variability (B2BV) in spontaneously beating HCM hiPSC-CMs using measures derived from heart rate variability (HRV).

The dataset comprised seven hiPSC-CM lines: two control lines, two carrying the MYBPC3-Gln1061X mutation (HCMM), and three carrying the TPM1-Asp175Asn mutation (HCMT). Previous studies have shown that hiPSC-CMs carrying these mutations exhibit an increased incidence of arrhythmias. Interbeat interval (IBI) time series were extracted from patch-clamp recordings, preprocessed, and analyzed across baseline, adrenaline, and washout phases using time-domain and nonlinear HRV-derived measures.

Beat-to-beat dynamics differed between the genotypes both at baseline and in their response to adrenaline. HCMT cells generally showed greater variability than control cells, whereas HCMM cells showed shorter mean IBIs. Several adrenaline-induced changes persisted during washout, indicating incomplete recovery within the experimental time window. Overall, the findings demonstrate genotype-specific modulation of B2BV under adrenergic stimulation, suggesting that HRV-derived analysis of hiPSC-CMs may provide useful insights into arrhythmia-related cellular phenotypes in HCM.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is a heritable cardiac disease characterized by increased ventricular wall thickness, myocyte disarray, and often diastolic dysfunction and myocardial ischemia. HCM is an important cause of sudden cardiac death in young individuals, with adrenergic stress and physical exertion potentially contributing to arrhythmic events. HCM is caused by mutations that are typically inherited as autosomal dominant mutations in sarcomere genes [1]. The phenotypic expression varies between individual patients, and the disease is still underrecognized clinically [2].

There are multiple distinct mutations associated with HCM in genes encoding the cardiac sarcomere proteins. The mutations in α-tropomyosin (*TPM1-Asp175Asn*) and in myosin-binding protein C (*MYBPC3-Gln1061X*) are known founder mutations in the Finnish population. Patient-specific induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a platform to study HCM *in vitro*. Previous studies have shown that hiPSC-CMs carrying *TPM1-Asp175Asn* or *MYBPC3-Gln1061X* mutations exhibit altered cell morphology, a higher incidence of arrhythmias, and abnormal calcium handling [3]. To simulate physiological adrenergic stimulation, adrenaline has been applied to hiPSC-CMs, revealing that the occurrence of ventricular arrhythmias depends on both adrenaline concentration and the specific HCM mutation [4].

However, the effects of adrenergic stimulation on the detailed beat-to-beat dynamics of these cells have not been systematically characterized. In this study, we characterize beat-to-beat variability in HCM hiPSC-CMs using HRV-derived measures [5]. We investigate genotype-specific differences at baseline, changes during adrenaline exposure, and the extent to which beat-to-beat dynamics return toward baseline during washout.

2. Materials and Methods

2.1. Data

The dataset was obtained from the adrenaline-response experiments of Prajapati *et al.* [4] and comprised seven previously characterized hiPSC-CM lines. Two lines (WT04511 and WT04602) served as controls. Two lines (HCMM06108 and HCMM07801) carried the MYBPC3-Gln1061X mutation and formed the HCMM group, while three lines (HCMT02912, HCMT02913, and HCMT13602) carried the α-tropomyosin TPM1-

Asp175Asn mutation and formed the HCMT group [3, 4].

The dataset included measurements at adrenaline concentrations of 0.05 nM, 0.1 nM, 0.5 nM, 1 nM, 10 nM, 100 nM, 1 μM, and 10 μM. For the present analysis, only concentrations of 10 nM, 100 nM, 1 μM, and 10 μM were included, as lower concentrations did not produce a significant change in beating rate. Prajapati *et al.* [4] similarly reported no significant effect on beating rate at 0.05 nM and 0.1 nM adrenaline.

Each sample was divided into three segments: baseline (BL), adrenaline exposure (ADR), and washout (WO). Segmentation was performed using a custom-written function based on timestamp markers indicating the addition of adrenaline and the initiation of washout. A 30-s interval immediately following adrenaline addition was excluded to account for transient instability in cellular behavior.

The dataset included both ventricular-like and atrial-like hiPSC-CMs, classified according to the action potential duration ratio ($\mathrm{APD90}/\mathrm{APD50}$). Cells with ratios below 1.3 were classified as ventricular-like, whereas those with ratios above 1.35 were classified as atrial-like. Atrial-like cells represented only a small minority of the dataset. Moreover, Prajapati *et al.* [4] assessed the effects of adrenaline exclusively in ventricular-like cells, while atrial-like cells were characterized only at baseline. Therefore, the present analysis was restricted to ventricular-like cells.

Interbeat interval (IBI) time series were extracted from the recorded action potentials. The IBI and APD_{90} time series were filtered for outliers using two moving-window filters. The first identified values outside the range $\mathrm{median} \pm \mathrm{a}\eta$, where η is the median, and the second identified values outside $\eta \pm b\sigma$, where σ is the standard deviation. The constants a and b control the strictness of the filters. In this study, values of $a = 0.1$ and $b = 3.5$ were selected empirically based on visual inspection of the resulting time series. Filtering was performed iteratively, with detected outliers removed before repeating the procedure to prevent smaller outliers from being masked by larger ones.

2.2. Heart Rate Variability Measures and Statistical Analysis

Beat-to-beat variability was characterized using a set of HRV-derived measures, summarized in Table 1. These included mean IBI, RMSSD, pRR20, pRR50, the Poincaré-plot ratio SD1/SD2, and the short-scale DFA exponent α_1. In addition to the absolute values, percentage changes from baseline to adrenaline exposure (BL–ADR) and from baseline to washout (BL–WO) were calculated to quantify the response to adrenaline and subsequent recovery.

Two-sided Mann–Whitney U tests with Bonferroni correction were used for pairwise comparisons between genotype groups. For each genotype, percentage changes from baseline were tested against zero using two-sided Wilcoxon signed-rank tests. Statistical significance was defined as $p < 0.05$.

Table 1. HRV-derived measures used in the analysis [5].

Abbreviation	**Description**
Mean IBI	Mean interbeat interval
RMSSD	Root mean square of successive IBI differences
pRR20	Percentage of successive IBIs differing by more than 20 ms
pRR50	Percentage of successive IBIs differing by more than 50 ms
SD1/SD2	Ratio of the standard deviations perpendicular to and along the line of identity of the Poincaré plot
DFA α_1	Short-scale detrended fluctuation analysis exponent (scales 4–16)

3. Results

Figure 1 summarizes the HRV-derived measures across baseline (BL), adrenaline exposure (ADR), and washout (WO) for the Control, HCMM, and HCMT groups. At baseline, clear genotype-dependent differences were observed. HCMT cells showed longer mean IBIs than Control cells, whereas HCMM cells showed shorter mean IBIs. The variability measures RMSSD, pRR20, and pRR50 were higher in HCMT than in Control, while SD1/SD2 differed between HCMM and Control during baseline and washout.

During adrenaline exposure, genotype-specific differences remained evident. HCMM cells showed shorter mean IBIs than HCMT cells, while HCMT showed higher RMSSD, pRR20, and pRR50 than Control. During washout, HCMM continued to show the shortest mean IBIs, and the patterns in RMSSD, pRR20, and pRR50 remained broadly similar to those observed during adrenaline exposure. SD1/SD2 also remained different between HCMM and Control. Overall, these results demonstrate genotype-dependent differences in beat-to-beat dynamics that persist across the experimental phases.

To assess the acute response to adrenaline and subsequent recovery, percentage changes relative to baseline were also examined (Fig. 1). The baseline-to-adrenaline change in pRR20 differed significantly between Control and HCMT, with the HCMT response closer to zero. In addition, the baseline-to-washout change in pRR50 differed significantly between Control and HCMT.

Complete recovery during washout would result in changes approaching zero relative to baseline. However,

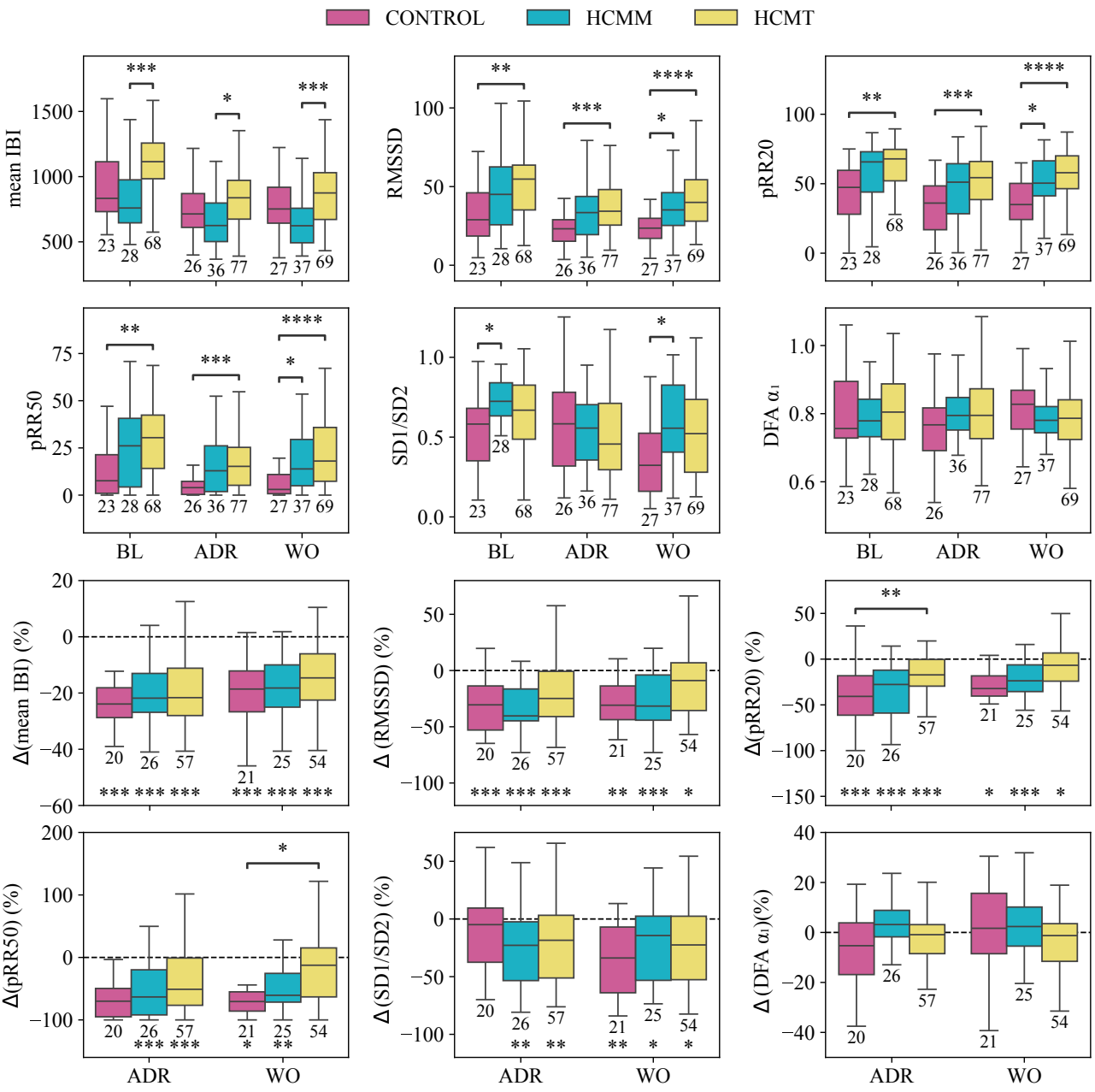

Figure 1. HRV-derived measures and percentage changes from baseline for each genotype and experimental phase. Numbers below the whiskers indicate sample sizes. P-value annotations are defined as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

several measures remained shifted from baseline, indicating that beat-to-beat dynamics had not fully recovered within the washout period. In particular, the change in mean IBI remained approximately -20%. In contrast, the change in RMSSD in HCMT moved closer to zero during washout, suggesting greater recovery of this particular measure. Thus, the response to adrenaline appears to persist beyond the stimulation phase, although the degree of recovery differs between measures and genotypes.

4. Discussion

The present analysis demonstrates genotype-dependent differences in beat-to-beat dynamics of spontaneously

beating hiPSC-CMs, both at baseline and during adrenergic stimulation. HCMT cells generally showed greater variability than Control cells, whereas HCMM cells showed shorter mean IBIs. These findings extend previous mutation-specific electrophysiological observations and suggest that HRV-derived measures can provide complementary information beyond mean beating rate and overt arrhythmic events.

Several measures remained shifted from baseline during washout, indicating incomplete recovery within the experimental time window. One possible explanation is altered energetic reserve in HCM cardiomyocytes: elevated ATP demand together with metabolic and mitochondrial abnormalities may limit the capacity to respond to β-adrenergic stress [6,7]. Previous hiPSC-CM studies have also demonstrated energetic abnormalities [8] and abnormal calcium handling [9] in HCM models. The mechanisms underlying the present beat-to-beat changes cannot, however, be determined from the current data.

Several limitations should be considered. First, the number of independent hiPSC-CM lines was small, although multiple cells were analyzed from each line. Second, several adrenaline concentrations were pooled, so potential dose-dependent differences were not assessed separately. Third, incomplete return toward baseline during washout cannot distinguish prolonged effects of adrenergic stimulation from other time-dependent changes in spontaneous cellular activity. Finally, the HRV-derived measures quantify variability of cellular IBIs rather than autonomically regulated HRV in the intact heart and should therefore be interpreted specifically as measures of cellular beat-to-beat dynamics.

5. Conclusion

The results demonstrate genotype-dependent differences in beat-to-beat dynamics both at baseline and in response to adrenergic stimulation. HCMT cells generally showed greater beat-to-beat variability than Control cells, whereas HCMM cells showed shorter mean interbeat intervals. Several adrenaline-induced changes persisted during washout, indicating that beat-to-beat dynamics did not fully return to baseline within the experimental time window. These findings support beat-to-beat variability analysis as a complementary approach for characterizing mutation-specific electrophysiological phenotypes in hiPSC-CMs.

Acknowledgments

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