Short- and Long-Range Correlations in Beat Rate Variability of Human Pluripotent-Stem-Cell-Derived Cardiomyocytes

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Abstract

A healthy heart exhibits fractal, i.e., long-range correlated fluctuations in heart rate variability (HRV). It is recently shown that fractal dynamics is also an intrinsic feature of human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). In this study, we investigate short- and long-range correlations in beat rate variability (BRV) of hiPSC-CMs, obtained from a healthy subject and symptomatic and asymptomatic long QT syndrome patients. It is shown that it is important to distinguish correlation properties in short and long time scales, as the scaling exponents are significantly different and also behave differently in the acute exposure to pharmacological compounds that modulate $\beta 1$-adrenoreceptors and cardiac ion channel generating delayed, outwardly rectifying K^+ current (I_{Ks}). While long-range scaling is sensitive to the drug exposure, short-range scaling is barely affected.

1. Introduction

Self-similar patterns appear in many physiological time series, such as R-R intervals, characterized by a power-decaying relation with a non-integer scaling exponent. Such properties are referred loosely as *fractals*, or having long-range correlation.

In physiological systems, nonlinear complexity is often related to the system's autonomic regulation and robustness against external perturbation. Many studies in the past have confirmed the presence of fractal scaling in heart rate variability (HRV) and its alteration due to aging [1] and cardiac disorders, e.g., [2]. It was shown that isolated heart cells, lacking any neural inputs, also exhibit power-law scaling, suggesting that fractal-like beat rate fluctuation is an intrinsic feature of heart cells [3].

Dynamics of spontaneously beating human heart cells, or cardiomyocytes (CMs), has not been widely explored until recently, when in vitro measurements of isolated human heart cells were made possible with newly developed stem cell technologies. According to the revolutionary

scheme that led to the Nobel Prize in Medicine in 2012, human-induced pluripotent stem cells (hiPSCs) can be obtained from adult tissues such as skin and give rise to any cell type in the body, including CMs [4].

In our previous work [5], we measured and analyzed the beat-to-beat interval series of hiPSC-derived CMs (hiPSC-CMs) and R-R interval of electrocardiogram (ECG), obtained from a healthy individual and symptomatic and asymptomatic long QT syndrome (LQTS, type 1) patients. LQTS is a cardiac disease, characterized by prolonged QT intervals in ECG. The inherited forms of LQTS are a result of mutations in the cardiac ion channel coding genes [5]. We also tested the effect of pharmacological compounds, applied directly to the cultured hiPSC-CMs. In this study, we extend the work further to consider short- and long-range correlation in different scale regimes, rather than computing one global scaling exponent over all scales, and assess the crossover behavior.

2. Data and Methods

2.1. hiPSC-CM preparation

The hiPSCs are generated as described in [6]. The LQT1-specific hiPSCs are derived from LQTS patients' skin fibroblasts carrying G589D missense mutation in KCNQ1 [7, 8]. The skin biopsies are obtained from a 55-year-old female healthy individual, a symptomatic 41-year-old female LQTS patient, and an asymptomatic 28-year-old LQT-mutation carrier. Both symptomatic and asymptomatic LQTS patients are on bisoprolol medication. The hiPSCs are cultured and differentiated into CMs as described in [8]. In this study, 30-40 days old hiPSC-CMs are used. The study is approved by the ethical committee of Pirkanmaa Hospital District (R08070).

2.2. hiPSC-CM and ECG data acquisition

Spontaneously beating hiPSC-CM clusters are measured with 6-well multielectrode array (MEA), as described in [5]. After 30 minutes of baseline measurements,

Bisoprolol (β-blocker), ML277 (I_{Ks} activator), and JNJ303 (I_{Ks} blocker) are applied. They are dissolved in either dimethyl sulfoxide (DMSO) or H_2O. Five minutes after each addition of a drug, field potential data is recorded for 30 minutes. The data is then analyzed by Cardiomyocyte MEA Data Analysis (CardioMDA) [9] to produce the hiPSC-CM cluster's beat-to-beat interval series. Typical length of the hiPSC-CM data is about 1000-3000 beats. ECGs of the individuals, whose hiPSC-CMs are studied, are recorded using MARS-Holter. The ECG recordings used here contain about 100000 beats.

2.3. Detrended fluctuation analysis

We apply detrended fluctuation analysis (DFA), originally developed in Ref. [10]. DFA is a reliable method to quantify long-range correlation in a time series that may be non-stationary.

In DFA, integrated time series (profile) is divided into non-overlapping segments, from which a polynomial trend is estimated by least-square fitting and subtracted, removing any monotonous trend. The fluctuation function is defined by the root-mean-square of the variance of the residuals of the profile. Since $F(s) \sim s^{\alpha}$ in the presence of power-law scaling, plotting $F(s)$ against s in log-log scale and calculating the slope of the linear fit yields the *scaling exponent* α. The algorithm used here is described more thoroughly in Ref. [11].

The scaling exponent α describes the nature of the correlation present in the data. The white noise with no correlation and Brownian noise are characterized by $\alpha = 0.5$ and $\alpha = 1.5$, respectively. $0.5 < \alpha < 1.5$ indicates long-range correlation, i.e., fractal correlation, while $\alpha < 0.5$ corresponds to long-range anti-correlation [11]. It is important to note here that time series may require more than one scaling exponent to describe different behaviors at different time scales. This *crossover* can be detected as change in slope in the log-log plot of $F(s)$ against s.

In order to determine the statistical significance in the changes caused by the acute application of the drugs and differences between the cell groups and short- and long-range scaling exponents, paired sample t-test and independent (unpaired) t-test are used, respectively. The levels of significance are represented as asterisks: (*) p<0.05, (**) p< 0.01, and (***) p<0.001.

3. Results and Discussion

3.1. Beat rate variability of hiPSC-CMs

Beat rate variability (BRV) of hiPSC-CMs , derived from three individuals with different health conditions are examined: healthy control, also called wild type (WT), symptomatic LQTS patient, and asymptomatic LQT-mutation carrier.

Applying DFA, we compute the scaling exponents α_1 for time scales less than 16 beats, and α_2 for scale longer than 40 beats. They represent short-range and long-range correlation respectively. In the extant literature on crossover phenomena (e.g., [2]), α_1 is usually defined by scales less than 10 beats, but we choose 16 beats as our threshold because crossover points occur after 16 beats in most cases. DFA exponents are averaged over 38 data sets of WT-CMs, 34 sets of symptomatic LQT-CMs, and 58 sets of asymptomatic LQT-CMs. The results are summarized in Table 1.

Scaling behavior of the healthy control, also called wild type (WT), and asymptomatic LQT-CMs are almost the same. On the other hand, symptomatic LQT-CMs has significantly smaller α_1 (p<0.01, compared to asymptomatic LQT-CMs), while the α_2 is comparable to those of WT- and asymptomatic LQT-CMs. The difference between mean α_1 and mean α_2 is statistically significant in all cell groups (p<0.001). The significant change in the scaling behavior at short and long time scales indicates the existence of crossover. Thus, it is appropriate to describe the BRV of the hiPSC-CMs with two and possibly more scaling exponents.

Table 1. Average of DFA α_1 and α_2 values describing short- and long-range correlation in BRV of hiPSC WT- and LQT-CMs.

Cell group	mean α_1	mean α_2
Wild type (healthy)	0.93 ± 0.19	1.10 ± 0.15
LQT (symptomatic)	0.84 ± 0.15	1.05 ± 0.23
LQT (asymptomatic)	0.94 ± 0.17	1.06 ± 0.20

Table 2. DFA α_1 and α_2 and their 95% confidence intervals, calculated from R-R intervals of ECG, recorded from the healthy control, symptomatic LQTS patient, and asymptomatic LQT-mutation carrier.

ECG group	α_1	α_2
Control (healthy)	1.12 ± 0.03	1.33 ± 0.03
LQT (symptomatic)	1.21 ± 0.01	1.08 ± 0.02
LQT (asymptomatic)	0.90 ± 0.01	1.00 ± 0.02

3.2. Comparison with ECG

R-R intervals, extracted from ECGs, are used to study heart rate variability (HRV). Corresponding DFA results are shown in Table 2 and illustrated in Fig. 1, presenting short-range and long-range αs and their 95% confidence intervals. The scaling properties are comparable between
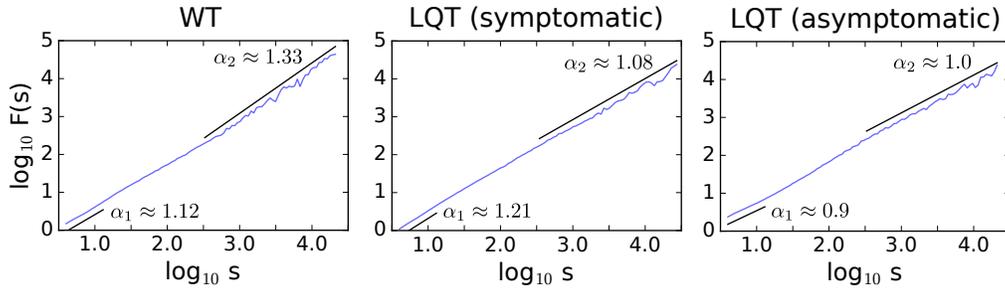
Figure 1. Fluctuation as a function of scale in log10 scales, computed by DFA algorithm, for the healthy control (WT) and LQTS patients. α_1 and α_2 are calculated from least-square fitting on small scale (<14 beats) and large scale (>300 beats).

cellular and heart levels, especially for asymptomatic LQT-CMs. Healthy control has higher α_1 and α_2 in ECG than at the cellular level. The significantly reduced α_1 of symptomatic LQT-CMs is not observed in ECG data.

The findings confirm that hiPSC-CMs exhibit intrinsic BRV with fractal scaling, and there are other internal and external inputs affecting the fractal dynamics beyond the cellular level: at the heart's in situ level, sympathetic and parasympathetic neural inputs regulate the heart rate, thus affecting the fractal behavior.

3.3. Effects of pharmacological compounds

Three different pharmacological compounds are applied to the hiPSC-CMs. Results are summarized in Fig. 2.

β-blocker Bisoprolol is a $\beta1$-adrenoreceptor selective β-blocker that is the standard treatment of choice for LQTS patients. Acute application of bisoprolol leads to a statistically significant increase in the long-range correlation in all the cell groups. Above the therapeutic concentration (260nM), the effect attenuates or remains at the same level for WT- and asymptomatic LQT-CMs. α_2 increases further towards Brownian noise ($\alpha = 1.5$) for symptomatic LQT-CMs, but not significantly.

I_{Ks} activator ML277 is an ion-channel activator, known to shorten the action potential duration of CMs. The application of ML277 to the hiPSC-CMs leads to a significant increase in the long-range correlation in WT- and asymptomatic LQT-CMs. In all the cell groups, higher concentration does not increase α_2 any further. There is no significant change in short-range correlation.

I_{Ks} blocker JNJ303 is an effective and specific I_{Ks} blocker. Though JNJ303 has the opposite effect on I_{Ks} channel, its effect on the fractal scaling is similar to that of ML277. Similar to other compound, application of JNJ303 leads to significant increase in α_2. The effect attenuates slightly or remains the same at higher concentration. Again, no significant changes are observed in short-range correlation.

WT- and asymptomatic LQT-CMs seem to be more sensitive than symptomatic LQT-CMs to the drug exposures. In summary, acute application of pharmacological compounds that modulate $\beta1$-adrenoreceptors and cardiac ion channel generating I_{Ks} alter the CM cluster's fractal complexity by increasing long-range correlation, but independently of the applied dose. There is, however, no significant alteration in the short-range scaling due to the compounds.

We have also performed vehicle control experiments, in which CM clusters are measured without any drug exposure, but with vehicle solutions, DMSO or H_2O. We observe no statistically significant changes in α_1 and α_2, when vehicle solutions are added every 30 minutes. Therefore, significant changes in the scaling properties caused by drug exposures reflect the intrinsic effects of the pharmacological compounds themselves.

4. Conclusion

We have confirmed that fractal scaling properties are indeed intrinsic at the cellular level, also for LQT-CMs. In particular, asymptomatic LQT-CMs show scaling behavior very similar to that of healthy CMs, suggesting that the intrinsic mechanism contributing to fractality is not altered by the presence of LQT-specific genetic mutation.

In this study, we have had a particular focus on two scaling exponents to describe BRVs at short and long time scales. The significant difference in the short and long range αs indicates that it is appropriate to use two (or even more) scaling exponents. Our results show that the effects of drug exposure are different for the scaling properties at short and long range: while short-range scaling is unaltered by the modulation of $\beta1$-adrenoreceptors and cardiac ion channels generating I_{Ks}, the long-range scaling is sensitive to the drug exposure. Generally, the long-range scaling exponents increase towards Brownian noise, independently of the drug concentration.
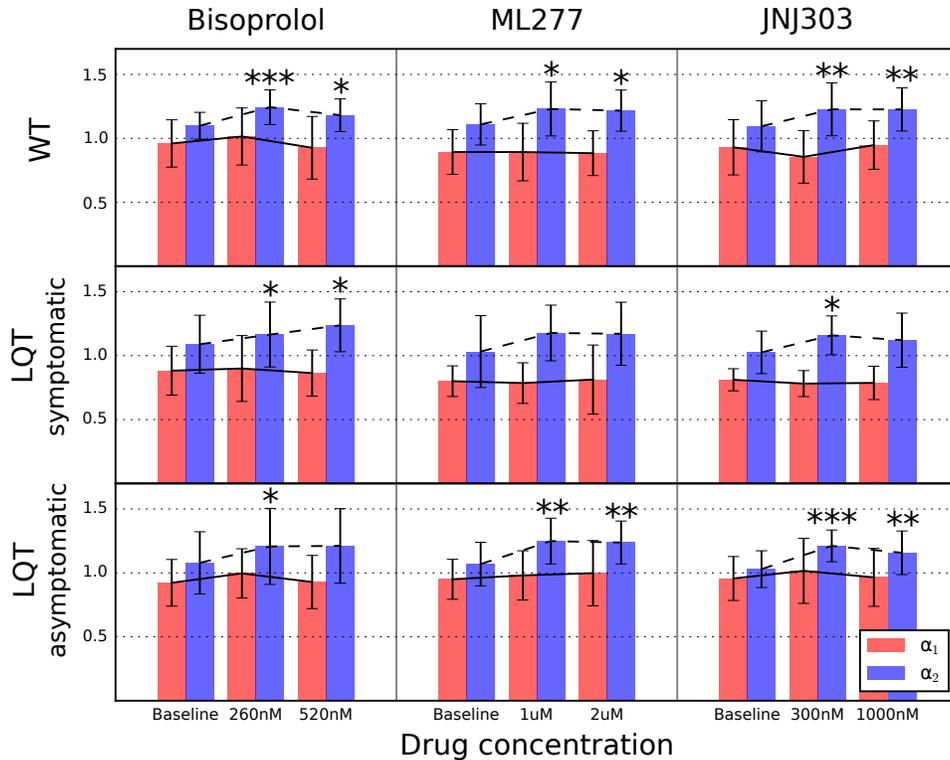
Figure 2. Change in average short-range correlation α_1 and long-range correlation α_2 as a function of drug concentration, by cell groups and compounds. Significance levels are indicated by (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

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