Introduction
The cardiac voltage sensitive potassium ion channel hERG plays a fundamental role in cardiac action potential repolarisation, with numerous missense variants resulting in Long QT Syndrome (LQTS). A high proportion of pathogenic variants in hERG exhibit mistrafficking and reduced expression at the cell membrane surface, most likely as a result of misfolding and retention in the endoplasmic reticulum. We explored the use of evolutionary analysis [1] to identify structural domains likely to be susceptible to misfolding in missense variants and tested our predictions by assessing trafficking efficiency in a series of hERG channel variants.
Methods
Evolutionary analysis highlighted a region on the cytoplasmic side of the hERG Voltage Sensor Domain (VSD) as likely to be susceptible to misfolding. Ten natural hERG variants selected from the ClinVar database that contain missense mutations within this region were prepared using mutagenesis, and a quantitative LI-COR^{TM} based On-cell assay [2] (where details of the assay can be found), was used to measure the expression of the channel variants at the cell membrane surface. Two well-characterised variants, A614V and L615F [3], were used as positive controls for a strongly mistrafficked phenotype. The trafficking assay was performed 48h after transient transfection of a HEK293 cell line, and each condition was repeated in triplicate; the entire assay was repeated on 4 separate occasions for all conditions. Data are presented as mean ± standard error of the mean (SEM) and statistical analysis was performed using 1-way ANOVA with Bonferroni post-hoc test as appropriate.
Results
In our initial analysis, the positive controls A614V and L615F showed dramatic reduction of cell surface membrane expression by 85±1% and 89±1% of wild type (WT), respectively ($p<0.0001$ for each). Our data showed that the trafficking efficiency of 9 out of 10 natural variants in the VSD matched expectations from evolutionary analysis: 8 out of 10 tested natural variants in ClinVar (at positions ranging from I400 to H492) showed reductions in surface expression by 80% or more, consistent with defective trafficking. R488C (an S3 domain Variant of Unknown Significance in ClinVar), showed a comparatively modest, though significant reduction (by 30±5%; $p<0.005$ compared with WT level) in cell surface expression. A variant with a conservative substitution (V476I) showed cell surface expression levels that were not significantly reduced compared to WT ($p>0.05$).
Conclusion
Our results show that evolutionary analysis is an effective means of identifying structural regions of ion channel proteins that are likely to harbour mistrafficking variants and for selecting variants for experimental phenotyping. The trafficking data identify the bottom (cytoplasmic side) of the hERG VSD as an important element in the structural integrity of the hERG channel.
References
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A 08-35
A novel method for estimating the loss of physiological complexity in heart rate time series.
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Introduction
The heart rate time series is a useful tool for assessing the dynamic nature of brain-heart interactions with advanced mathematical methods. The reduced fractal complexity of the heart rate series is defined as an indicator of various pathologies including aging processes (1). Detrended Fluctuation Analysis (DFA) is a mono-fractal scaling exponent analysis widely used for complexity measurement based on the Hurst method (2). DFA can evaluate the self-similarity and long-range memory of a time series with mono-fractal scaling exponents. This method includes the detrending technique to examine the fluctuations of time series more accurately. However, quantitative calculation of the "loss of physiological complexity" in heart rate time series during pathological or physiological conditions is not introduced yet while several methods are presented to measure complexity (3). This study aims to propose a novel method and new parameters derived from DFA log-log graphs to quantitively calculate fractal complexity loss.
Methods
Three study groups were formed to analyze the data obtained from Physionet Database (4) and a synthetically generally dataset: 1- Normal Sinus Rhythm (NSR) (n=18), 2- Congestive Heart Failure (CHF) (n=29), 3- White noise signal (WNS) (n=20) (Figure 1). RR interval time series derived from 60 minutes long electrocardiogram (ECG) recordings were used for HRV analyses. Approximate Entropy (ApEn), Sample Entropy (SampEn), and Detrended Fluctuation Analysis parameters (short-term; $DFA\alpha_1$, long-term; $DFA\alpha_2$) were calculated for all groups. DFA log-log graph and lines were reconstructed using scaling exponents (Figure 2). The newly developed parameter "Relative total logarithmic fluctuations" was determined for each sample and the new absolute area difference parameters dS1, dS2, and TdS were calculated. The mean values of nonlinear parameters for all groups were compared using independent samples Kruskal Wallis test. The statistical analyses were performed using GraphPad Prism (version 9.0.1) and $p<0,05$ was accepted as statistically significant.
Results
$DFA\alpha_1$ was lower in CHF and WNS groups compared to the NSR group. However, $DFA\alpha_2$ was only lower in CHF but not in the WNS group. The dS1, dS2, and TdS parameters were significantly higher compared to others. ApEn and SampEn did not differ between the groups (Figure 3).
Conclusion
In this study, a new method for estimating fractal complexity loss was evaluated. The novel parameters produced from the DFA log-log graphs are highly differentiating for congestive heart failure and white noise signals. Parameters such as dS1, dS2, and TdS may reflect fractal physiological complexity loss in heart rate time series. Overall the proposed approach may contribute to designing better methods to calculate physiological complexity loss.

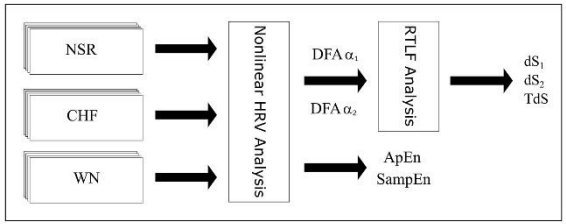

Figure 1
The diagram of analysis methods.

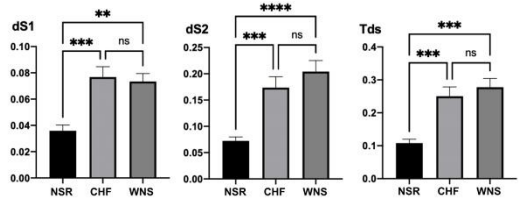

Figure 2
Comparison of mean values (± SE) of dS1, dS2, and TdS in NSR, CHF, and WNS groups. **p<0.01, ***p<0.001 and ****p<0,0001 is compared to the NSR group. (ns: non specific)

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A 08-36

Evaluation of cardiac mitochondria morphological and bioenergetic changes following breast cancer treatment

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Mitochondrial dysfunction is an important determinant of cardiotoxic effects triggered by chemotherapeutic treatments. Anthracyclines (doxorubicin) and trastuzumab are known cardiotoxins that may induce cardiac mitochondrial damage (1,2). This study sought to evaluate cardiac mitochondrial morphological and bioenergetics changes associated with doxorubicin and trastuzumab treatment and potential underlying mechanisms of mitochondrial dysfunction.

Female C57BL/6J mice (12 wks, n=5-6, all groups) were anesthetized (4% v/v isoflurane by inhalation for induction and 1.8% v/v for maintenance) and ovariectomized (OVT). At 5 weeks post-surgery: group 1 received a single dose of doxorubicin (DOX; 4 mg/kg) per week for 3 weeks; group 2 received the same dose of DOX in addition to trastuzumab (10mg/kg; DOX+TRZ) at week 4; group 3 received saline for 3 weeks and the same dose of trastuzumab (TRZ) at week 4. The OVT control (CON) and sham group received saline for 4 weeks. At the end of treatment, left ventricle (LV) tissue homogenate was prepared for measurement of mitochondrial respiration (O2k). LV biopsies were also fixed in PHEM buffer containing 1% glutaraldehyde and 4% formaldehyde and prepared for transmission electron microscopy (TEM) to evaluate mitochondrial morphology. Western blot analysis was used to assess proteins involved in mitochondrial shape and cristae stability.

Compared to CON, maximal coupled mitochondrial respiration was not altered in the DOX group, while TRZ-treatment elevated respiration rates. The combination DOX+TRZ did not lead to additive effects. TEM analysis revealed altered mitochondrial structure and shape following DOX, TRZ and DOX+TRZ-treatment, although abnormal shape was most pronounced in the DOX group. This also included the presence of holes with granular deposits in the mitochondria. In the TRZ-treated group, increased numbers of spherical mitochondria (<500nm) were observed. These data were supported by slight elevation of mitochondrial fractions, citrate synthase activity and a significant increase in TOM20 levels, suggesting more intact mitochondria, though smaller in size following TRZ-treatment. Overall, DOX+TRZ did not show additive effects on mitochondrial structural damage but had the highest cytoplasmic vacuolation with granular deposits and autophagy-like vesicles. Expression of fusion-protein OPA1 was significantly decreased in DOX and DOX+TRZ groups compared to CON and TRZ. No difference was seen between TRZ and CON. Levels of fission-protein DRP1 were significantly upregulated in DOX and DOX+TRZ groups, while FIS1 upregulation was observed in groups treated with either DOX or TRZ.

Despite drastic changes in mitochondrial shape in DOX-treated mice, mitochondrial functional impairments were not observed. In contrast, TRZ increased mitochondrial function that was associated with more, smaller mitochondria and may suggest a transient increase in function in response to cellular stress.