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Transcriptomic approaches to modelling long term changes in human cardiac electrophysiology

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Abstract. Slow changes in the activity of the heart occur with time scales from days through to decades, and may in part result from changes in cardiomyocyte properties. The cellular mechanisms of the cardiomyocyte action potential have time scales from < ms to hundreds of ms. Although the quantitative dynamic relations between mRNA transcription, protein synthesis, trafficking, recycling, and membrane protein activity are unclear , mRNA-Seq can be used to inform parameters in cell excitation equations. We use such transcriptomic data from a non-human primate to scale maximal conductances in the O'Hara-Rudy (2011) family of human ventricular cell models, and to predict diurnal changes in human ventricular action potential durations. These are related to circadian changes in the incidence of sudden cardiac deaths. Transcriptomic analysis of human fetal hearts between 9 and 16 weeks gestational age is beginning to be used to inform ventricular cell and tissue models of the electrophysiology of the developing fetal heart.

Keywords: transcriptomics, circadian, fetal.

1 Introduction

1.1 Cardiac Excitation Systems

Normal sinus rhythm in the human is robust, and persists from 21 days after fertilization until death, maybe 100 years later During the lifetime there can be~10^9 beats, each triggered by a propagating excitation wave, at a rate from 40 to 180 beats /min. Noninvasive monitoring of activity of the heart, say by an ECG, show beat to beat fluctuations in the intervals of the cardiac cycles, of the order of tens of ms; accelerations and decelerations of heart rate, lasting 10^1 - 10^2 s; and longer diurnal, monthly, developmental and ageing changes.

A simple computational model of propagating activity in the heart is a nonlinear diffusion system that represents cardiac tissue as mono-domain excitable medium. The nonlinearity is produced by the total membrane ionic current density I_{tot} (μA/μF), given by appropriate excitation equations of the form

$$dV/dt = -I_{tot}/C_m, \qquad I_{tot} = I_{ion} + I_{pump} + I_{exchanger} = \boldsymbol{f}(\boldsymbol{v}(t),\boldsymbol{p}), \tag{1}$$

where V is the membrane potential (mV), t is the time (ms), $I_{ion} + I_{pump} + I_{exchanger}$ are the ionic, pump and exchanger current densities and C_m the specific membrane capacitance (μF). The equations for these currents have been obtained from extensive series of voltage clamp experiments on isolated myocytes, and patch clamp experiments on recombinant ionic channels expressed in cells. The $\boldsymbol{v}(t)$ are dynamic variables that change during the solution of the equation, *e.g.* membrane potential, activation and inactivation variables, and some intracellular ionic concentrations. The $\boldsymbol{p}$ are parameters, some are constants that define the cell type. Some parameters can be changed, to model changes in experimental conditions (*e.g.* temperature, extracellular ionic concentrations).

We assume that changes in cardiac electrical activity over 10^4 – 10^9 s in an individual lifetime may be produced by changes in protein expression and can be modelled by changes in the cell population and spatial distributions

of these parameters over time. For a human cardiac cell models, the parameters are estimated from experiments on human cardiac cells and tissue, obtained *ex vivo* during surgery, or from healthy human donor hearts [1].

1.2 Channel expression

In an individual cell, a given specific ion channel density corresponds to a number of functioning channels, each of which may be composed of several protein subunits and associated regulatory proteins. The number of functioning channels is in a dynamic balance with silent channels, and the total channel number increased by trafficking and insertion to the membrane, and decreased by internalization, recycling and degradation. Proteins newly synthesized by ribosomes on the endoplasmic reticulum can be transported by endosomes to the membrane. The dynamics for the alpha subunit of the I_{Ks} channel are schematized in Fig. 1, with α, β the rates of formation and degradation of the mRNA and protein $K_{v7.1}$. The overall amount of protein depends on the rate of translation and degradation [2]: any given steady state level of protein ($\alpha_{mRNA}.\alpha_{Kv7.1}$/ $\beta_{mRNA}.\beta_{Kv7.1}$) could be produced by differing combinations of transcription [mRNA h^{-1}] and translation rates [protein $mRNA^{-1}$ h^{-1})]. As well as synthesis and degradation reactions, there are intracellular transport processes. A compartmental model would be a system of ordinary differential equations with largely unknown rate coefficients. It is clear that the total amount of mRNA for the protein subunit, the total amount of the protein subunit, and the ionic maximal conductance G_{Ks} need not be linearly or even simply related, that there will delays between mRNA, protein and changes in maximal conductance, and that there could be oscillatory or complex dynamics.

However, simultaneous measurement by quantitative mass spectrometry of absolute mRNA and protein levels and half-lives in mouse fibroblasts by pulse labelling has shown a correlation between absolute mRNA and protein levels in the same samples [3]. This partially justifies approximating the dynamics of Fig.1 by a simple linear filter, that would give a phase lag and a linear relation between mRNA and maximal conductance.

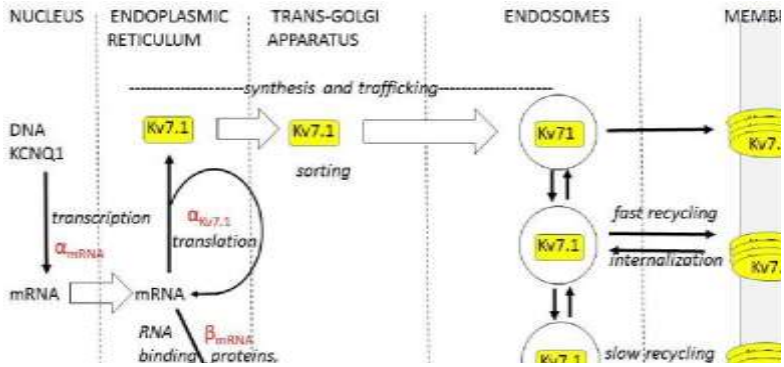

Fig. 1. Schematic of the dynamics of the protein alpha subunit Kv7.1 of the I_{Ks} channel. Each channel is a tetramer and the four pore-forming alpha subunits are associated with modulatory KCNE1 beta subunits.

There are two ways transcriptomics can lead to estimates of the parameters in the cell and tissue excitation equations.

First, where the excitation mechanisms are well described, the transcriptomic data can be taken as quantitative, and used to scale the values of maximal conductances exchanger, release, and uptake processes of a cell model, as in [4, 5] for the human sinoatrial node (SAN). The assumption that mRNA expression can be used to scale maximal conductances was justified by numerical solutions reproducing SAN action potential characteristics.

Second, where there are experimental cell electrophysiology recordings, but not a voltage clamp based mechanistic model for excitation, the transcriptomic data can be treated as qualitative, to indicate what channel systems

are expressed, and the electrophysiological data used to constrain the possible parameter values, as in [6], where the electro-chemical (membrane potential and intracellular [Ca^{+}_{i}) activity of single uterine myocytes is reconstructed. Transcriptomic analysis provides a qualitative list of what mRNAs are expressed [7]. The kinetics of each ion channel or transporter system is modelled separately, usually from data obtained from patch clamp experiments on an expression system. The assumption is that the kinetics of the channel or transporter in the expression system used are approximately the same as in the normal cell, even though accessory subunits and anchoring proteins found in myocytes are not present. Experimentally observed {V(t) , Ca^{++}(t)} constrain the space of conductances/densities that are consistent with the observed electrochemistry, and so the cell is modelled not by a specific set of conductances, but by any member of the space of ionophore densities that is consistent with the observed {V(t) , Ca^{++}(t)}.

1.3 Circadian rhythms

Long term, slow diurnal or developmental changes in cardiac activity over 10^4-10^9 s will have associated changes in cardiac myocyte properties and behavior, but longitudinal or population transcriptomic and cellular electrophysiological studies over these time scales are impractical. Computational modelling of human cell and tissue electrophysiology, informed by the limited available transcriptomic data, may suggest possible cellular electrophysiological changes that could provide the mechanisms for these changes in activity. Here we approach the modeling of circadian (10^4 s) and fetal developmental (10^6 s) from 9 to 16 weeks gestational age (WGA).

Cardiac electrophysiological variables – heart rate, ECG intervals and their variability, and the onset of arrhythmia, all show diurnal rhythms [8]. The timing of sudden cardiac deaths, obtained from out of hospital and in-hospital data [9, 10], and of the onset of ventricular fibrillation, obtained from ICD data downloads [11], both show a diurnal rhythm - see Fig.2a. The time is local clock time. The four-fold change in sudden cardiac death rates has a rise time of ~ 6 hours and a fall time of ~18 hours. The rhythms for sudden cardiac death, onset of ventricular tachycardia and fibrillation are mirrored by the diurnal changes in RR interval [12, 13] – see Fig 2b. These diurnal rhythms in heart rate and SCD could be driven by external factors or autonomic activity [14], or could be generated by circadian changes in ion channel expression within the heart [8] that are entrained, *via* autonomic and humoral factors, by the suprachiasmatic nucleus that provides synchronization to the daylight [15].

Diurnal changes in heart rate will produce rate dependent changes in ventricular action potential duration, dispersion, and variability that provide an obvious possible explanation for diurnal changes in SCD. Any intrinsic diurnal changes in myocyte membrane channel expression could directly modulate the risk of SCD.

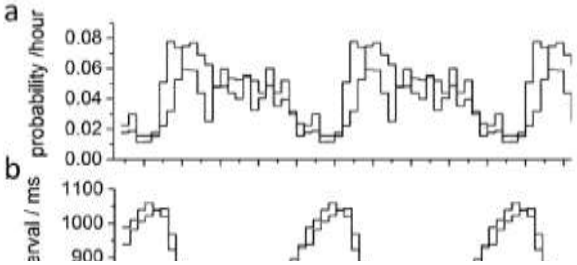

Fig. 2. Circadian changes in cardiac events, averaged over 10^3-10^4 subjects and several years. (a) Replot of SCD data from Berlin, n=2406 [9], ; Massachusetts (n=2203 [10]. (b) Average RR interval for normal adult subjects, replot from [12, 13].

2 Methods

An extensive diurnal transcriptome atlas of the transcriptome of different tissues for the primate *Papio anubis* (baboon) on a 12 hour light-12 hour dark cycle has been published and archived in NCBI's GEO (GSE98965) [16]. 12 animals were used, to give 12 time points every 2 hours *i.e* one animal /time point. 15% of the cardiac transcriptome products shows a diurnal rhythm, with the times of peak expression clustered in a temporal window <6 hrs wide, and periodicity identified by meta2d of Metacycle [17]. Transcriptomics data was extracted for the α-subunits proteins that form part of the membrane ionic channels for the inward currents I_{Na} (SCN5A), $I_{Ca,L}$ (CACNA1C) and outward currents I_{to} (KCND3,KCNA4), I_{Kur} (KCNA5), I_{Kr} (KCNH2), I_{Ks} (KCNQ1) and I_{K1} (KCNJ2/12/4). $I_{K,ACh}$ (KCNJ3/5), $I_{K,ATP}$ (KCNJ11). Only changes in KCNA4 and KCNQ1 are statistically significant (Metacycle integrated $p < 0.05$). The gene level read counts are normalized.

Fetal developmental changes in mRNA for cardiac ion channel protein were extracted from the data [18] deposited in European Genome-Phenome Archive (EGA) which is hosted at the EBI and the CRG, (E-MTAB-7031) for time points 9, 12 and 16 WGA, with each time point being the average from 3 hearts.

The percentage change in mRNA were used to scale the ionic conductances in the O'Hara-Rudy [19] model for human healthy ventricular cells. Equations for isolated cell models were solved using a forward Euler method with a time step of $\Delta t = 0.05$ ms; ion channel gating equations were solved using the Rush-Larsen scheme. The action potential duration at 90% repolarization (APD_{90}) restitution was measured using a standard protocol, with 100 S1 pacing beats at a cycle length of 1,000 ms to establish periodicity, followed by a single premature stimulus (S2) at progressively shorter S1-S2 intervals

3 Results

Circadian changes. The normalized individual gene level read counts are plotted for KCNA4 and KCNQ1 in Fig.3. The Zeitgeber time (ZT) relates to the 24 hour light–dark cycle, with light on at ZT=0. Each 2 hour wide column over 24 hours in the histogram is based on one animal, and the observation that there are compact peaks, at different times for the two mRNAs, suggests that the modulation is related to ZT and not the individual animal. When the G_{to} and G_{Kr} are scaled by the normalized channel expression data of Fig.3 there is a diurnal modulation of APD_{90} and its restitution. This is plotted in Fig. 4 over 48 hours, with S1-S2 intervals from 200 to 1000ms, with APD_{90} colour-coded over for each 2 hour time window of the day. Light on (ZT0) is identified as the average local clock time of dawn over the year (6 *a.m.*), and the diurnal pattern of sudden cardiac death of Fig.2(a) is superimposed. There appears to be a coincidence between the diurnal pattern of APD_{90} and the timing of SCD's.

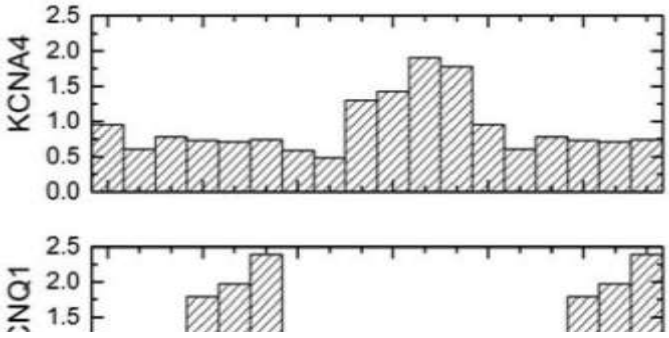

Fig. 3. Diurnal changes over 36 hours in cardiac ion channel expression in baboons that had been entrained on a 24 hours (12 hour light, 12 hour dark) cycle.

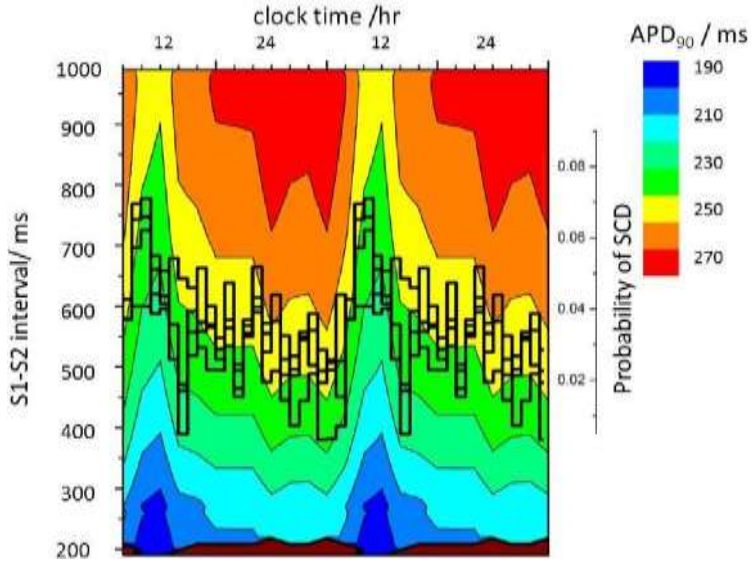

Fig. 4. Circadian changes in ventricular endocardial cell model dynamic APD_{90} restitution curves, computed using data in Fig.3. The sudden cardiac death rates from Fig 2 are superimposed, with ZT0 (light on) taken as 06.00 *a.m.* APD_{90} is colour-coded.

Developmental changes. The development of ventricular structure, intercellular coupling, and transcriptome in the human fetus has been mapped from 9 to 20 WGA by DT-MRI [20, 21], RNA-Seq [18] and qPCR[20], and related to noninvasive electrophysiology [21].

The organization of the ventricular wall, quantified by fractional anisotropy, apparent diffusion coefficients and fiber paths lengths extracted by tractography, increases linearly with gestational age, with the helical organisation, and transmural change in fiber helix angle characteristic of the adult mammalian heart developed by 20 weeks. RNAseq showed no significant differential expression of Cx40 or Cx 43 between 9 and 16 WGA [18] , while both Cx 40 and Cx43 expression relative to GAPDH obtained by Western blotting increased significantly [20]. The increase in organisation correlates with an increase in Connexin expression [20]. In tissue models these increase both propagation velocity and its anisotropy.

Differential expression of genes coding ion channel subunits has been extracted from the data of [18]; of the 806 genes differentially expressed between 9 and 16 WGA) only 20 relate to cardiac excitation with potassium voltage-gated channel (KCNJ2, KCNJ2-AS1,KCTD9,KCNJ8), sodium voltage-gated channel (SCN2B,SCN7A), gap junction protein alpha (GJA1, GJA3) and ryanodine receptor 2 (RYR2), all upregulated; and potassium voltage-gated channel and modifiers KCNAB3, KCNA1, KCNG1, KCNQ2);calcium voltage-gated channels (CACNG4, CACNG7, CACNA1G/H/S), potassium calcium activated channel (KCNN1) and Ryanodine receptor RYR2 all downregulated.

The ORd model is parameterized for the healthy adult, and there are no well-founded, experimentally based models of human fetal myocardial cells whose parameters could be scaled by the transcriptomic data. In [21, 22] conductance parameters of an adult endocardial myocyte model were scaled so their APD_{90} restitution curves reproduce 35 week gestational age fetal QT restitution curve. The transcriptomic data would give an increase in maximal rate of rise of action potential, and increase in its conduction velocity and duration, but quantitative values are speculative in the absence of fetal cell and tissue electrophysiological data. However, transcriptomics of *ex-vivo* or *postmortem* material could be applied to the ORd system to model ageing in the adult.

4 Conclusions

Transcriptomic data provides a route to parameterizing human cardiac excitation equations to reproduce slow developmental changes, seen over time scales of days to decades, time scales that are not accessible for cell and tissue electrophysiology. What is needed is a clearer, quantitative description of the dynamics of transcription and membrane protein synthesis: the first order approximation of a linear relation between mRNA level and functional membrane ionophore activity is oversimplified, even though it leads to plausible behaviors.

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