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The Beat Goes On – Driven By A Cardiac Calcium Clock?

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“The beat goes on” – Sonny & Cher (1967)

The regulation of heart rate has been an area of great research interest over the last several years. Since the groundbreaking studies assessing the prognostic value of heart rate variability published by Bigger et al. ¹, it has been recognized that heart rate is not a static parameter, but one that can vary greatly due to breathing, activity ² or the sleep-wake cycle ³. Further, diminished heart rate variability has been shown to predict mortality ¹. In light of this significant physiological variability in heart rate, it seems incredible that a single resting heart rate measurement could predict mortality over the next several years. However, in a series of placebo controlled clinical trials enrolling patients with a depressed left ventricular function (ejection fraction $\leq 40\%$), a higher resting heart rate has been found to be a very strong predictor of all cause mortality ⁴. Yap et al. ⁵ pooled the placebo group data from the European Myocardial Infarct Amiodarone Trial (EMIAT) ⁶, the Canadian Amiodarone Myocardial Infarction Trial (CAMIAT) ⁷, the Trandolapril Cardiac Evaluation (TRACE) ⁸, and the Danish Investigation of Arrhythmias and Mortality on Dofetilide – Myocardial Infarction (DIAMOND-MI) ⁹ and created a Cox regression model to identify demographic risk factors associated with all-cause mortality. A high resting heart rate (> 100 bpm) conferred a much higher risk than did a low resting heart rate (≤ 60 bpm). The magnitude of that increased risk was comparable to that seen with increased New York Heart Association (NYHA) class (class IV vs. class 0/I) or increased age (71–80 years vs. ≤ 50 years). An elevated resting heart rate was more predictive of mortality than blood pressure, diabetes, gender, or a prior myocardial infarction.

In this issue of *Heart Rhythm*, Wilton and coworkers report on their efforts to identify genetic polymorphisms that predict resting heart rate ¹⁰. Using baseline data from a longitudinal study of almost 1500 subjects (almost all male), they probed single nucleotide polymorphisms (SNP) in 311 candidate genes that were felt both to have a prevalent minor allele and were felt to plausibly play a role in heart rate regulation. Somewhat surprisingly, given the large number of genes examined, only 5 SNPs in 5 different genes were associated with changes in baseline heart rate: *ADRB1* (encoding the adrenergic beta-1 receptor), *SCN5a* (encoding the cardiac sodium channel), *eNOS* (encoding endothelial nitric oxide synthase), *CASQ1* and *CASQ2* (encoding skeletal and cardiac muscle calsequestrin involved in Ca^{2+} regulation). Even fewer SNPs (in *ADRB1*, *SCN5a* and *CASQ1*) remained statistically significant after adjustment for clinical covariates associated with heart rate.

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“There are 3 kinds of lies: lies, damned lies, and statistics.” – Benjamin Disraeli

By their very nature, genetic studies involve the analysis of a very large amount of data. It is important to consider the possibility that one or more associations found in a genetic study is a spurious statistical anomaly. For example, 3 separate genome screens performed to determine the genetic causes of Parkinson Disease each found 4–6 loci in linkage disequilibrium^{11–13}, but only the locus on chromosome 5 was common to all 3 studies.

One way to add credibility to a genetic analysis is to establish the biological plausibility that the genetic associations could alter heart rate. Wilton and coworkers found that each of the SNP associations demonstrated a gene dosage effect such that patients who were heterozygotes had a higher heart rate than those who were homozygotes for the bradycardia allele, but a lower heart rate than those subjects who were homozygotes for the tachycardia allele. Further, they found that the heart rate increased in a linear fashion based on the number of tachycardia promoting alleles (from 0–6 alleles). While a gene dosage effect does not prove biological plausibility, it does suggest that the findings of Wilton and coworkers are less likely to be spurious.

“there are known knowns...there are known unknowns...but there are also unknown unknowns” – Donald Rumsfeld

Another criterion that is useful in determining biological plausibility is whether the identified genes are known to regulate heart rate or is known to be expressed in the heart. The *ADRB1* gene codes for the beta-1 adrenergic receptor which has long been recognized to modulate heart rate in response to sympathetic nervous system stimulation, as well as in response to circulating norepinephrine and epinephrine¹⁴. The *SCN5a* gene codes for the cardiac sodium channel. This gene is best known for mutations that can cause either the Brugada Syndrome or a congenital long QT Syndrome (LQT3)¹⁵, but mutations in the *SCN5a* gene have been found to be associated with sinus bradycardia in mice¹⁶, in humans¹⁷ and using in silico models¹⁸. These are “known knowns” and it is clearly biologically plausible that polymorphisms in *ADRB1* and *SCN5a* can modulate resting heart rate.

Calcium and Heart Rate

CASQ1 and *CASQ2* encode the skeletal and cardiac isoforms of calsequestrin, respectively. Calsequestrins are the most abundant Ca^{2+} binding proteins located in the intracellular Ca^{2+} storage organelle (sarcoplasmic reticulum SR) of both cardiac and skeletal muscle^{19;20}. Cardiac calsequestrin also importantly regulates ryanodine receptor Ca^{2+} release channels in the SR^{21;22}. Loss of function mutations in *CASQ2* can cause catecholaminergic polymorphic ventricular tachycardia²³, likely because of dysfunctional regulation of Ca^{2+} release from the SR²⁴. The same *CASQ2* mutations also cause significant sinus bradycardia in both humans^{23;25} and mice²⁴. Thus, *CASQ2* represents a “known unknown”: While the exact molecular mechanism how *CASQ2* regulates heart rate is unknown, a role of *CASQ2* is highly biologically plausible based on what we know about the physiology of the sinus node. Experimental work has convincingly demonstrated that the beating of the sinus node is initiated, sustained, and regulated by oscillatory Ca^{2+} releases from the sarcoplasmic reticulum^{26–28}. In fact, the rhythmical depolarization of sinoatrial node cells is so dependent on intracellular Ca^{2+} release that a cardiac “ Ca^{2+} clock” has been proposed²⁹. Thus, even though it did not reach statistical significance in multivariate analysis, it appears plausible that the *CASQ2-T66A* SNP has physiological significance. Consistent with this hypothesis, *CASQ2-T66A* was first identified in a cohort of patients with catecholaminergic polymorphic ventricular tachycardia³⁰.

The link to the *CASQ1* gene appears spurious at first glance, since CASQ1 protein is not found in heart muscle, at least not working myocardium²⁴. Whether CASQ1 protein is present in sinoatrial nodal cells is unknown. Hence, *CASQ1* clearly represents an “unknown unknown”. However, given the biological plausibility based on what we know about *CASQ2* and the regulation of heart rate by intracellular Ca^{2+} , the genetic work by Wilton and coworkers suggests that CASQ1’s function in sinus node physiology should be investigated. Fortunately, with the recent advent of the *CASQ1* knock-out mouse model³¹, this is readily attainable.

Wilton and coworkers are to be congratulated for trying to better understand the underpinnings of resting heart rate. Four of the 5 genes identified by Wilton and coworkers are directly involved in the regulation of intracellular Ca^{2+} homeostasis, a result which emphasizes the only recently recognized importance of the cardiac Ca^{2+} clock²⁹ for heart rate control. It will be important to see if future analyses with different cohorts can replicate their findings³². While genetic factors may not be the primary explanation for resting heart rate (the genetic model accounted for only 1.6% of the variance compared with 13.3% of the variance for environmental factors), the significance of the work by Wilton and coworkers lies in the generation of novel hypotheses on the molecules involved in heart rate regulation. The function of calsequestrins in sinoatrial node cells should be explored.

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