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Searching for Sudden Death SNPs in Calcium Handling Genes

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Cardiac arrhythmias are a major clinical problem and lead to more than 250 000 sudden cardiac deaths (SCDs) each year in the United States alone.[1] Our ability to predict those at risk for sudden death remains poor. The majority of patients who die suddenly are not identified as being at high risk prior to death, and the majority of patients with implantable cardioverter defibrillators (ICDs) will never receive an appropriate shock for ventricular tachycardia (VT) or ventricular fibrillation (VF).[2] An assortment of electrocardiographic (eg, QRS dispersion; T-wave alternans), imaging (eg, fibrosis on MRI), electrophysiological (eg, programmed electrical stimulation), and serum biomarker (eg, inflammatory, CRP; hemodynamic, NT-proBNP) techniques have been or are being explored for use in the risk stratification of sudden death.[3] At present, however, ejection fraction (EF) remains the only approved clinical variable that is used to determine suitability for ICD placement in subjects with ischemic and nonischemic dilated cardiomyopathies.

During the last 2 decades, much has been learned about the mechanisms of sudden death from the rare inherited arrhythmia syndromes that result from ion channel mutations affecting repolarization or depolarization (eg, long QT syndrome, Brugada syndrome) and Ca^{2+} handling (eg, catecholaminergic polymorphic ventricular tachycardia [CPVT]).[4] Both case-control and prospective studies also support the presence of a significant heritable component to the more common forms of sudden cardiac death that are seen in the setting of myocardial infarction and cardiomyopathy. The S1103Y single nucleotide polymorphism (SNP) of the cardiac Na^+ channel $Na_v1.5$ (SCN5A) that is present in ≈13% of African Americans appears to cause mild action potential prolongation and is associated with both a history of arrhythmias and appropriate ICD shocks in subjects with cardiomyopathies. Homozygotes for the glutamine allele of the common β2-adrenergic receptor (β2-AR) Q27E polymorphism (≈20% of whites) have a reproducible ≈1.4-fold increased risk of SCD. In addition, genome-wide association studies (GWAS) focusing on sudden death and/or intermediate phenotypes such as QTc interval prolongation have identified associations with a number of loci and genes, the strongest being several SNPs in the nitric oxide synthase 1 adaptor protein (NOS1AP) responsible for 3 to 5 ms of QTc prolongation per allele. Of note, the mechanisms by which most of the identified SNPs predispose to SCD are not fully understood.

Most of the known nonsynonymous SNPs in cardiac Na^+ and K^+ channels have not been identified by GWAS and are not reproducibly associated with arrhythmias or sudden death in candidate gene studies. This has increased the search for other genes that modify arrhythmias, including genes involved in Ca^{2+} handling. The histidine-rich calcium binding protein (HRC) is a low-affinity high-capacity sarcoplasmic reticulum (SR) Ca^{2+} buffer that interacts directly with SERCA2a and Triadin, and regulates indirectly the function of the SR Ca^{2+} release channel (RYR2).[5,6] Overexpression of HRC in transgenic mice led to impaired SR Ca^{2+} uptake and slower Ca^{2+} transient decay in cardiac myocytes from young mice, and to hypertrophy and failure in older mice. Cardiac myocytes from HRC knockout mice, on the other hand, had enhanced SR Ca^{2+} uptake, larger Ca^{2+} transients, and increased contractility at baseline associated with increased spontaneous SR Ca^{2+} release (sparks, afterdepolarizations, aftercontractions) and poor tolerance to thoracic aortic banding.

In a recent report, Arvanitis et al[7] studied 6 polymorphisms in HRC in 123 Greek subjects with idiopathic dilated cardiomyopathy and 96 unmatched European controls. They found that carriers of the alanine allele (allele frequency ≈0.5) of the S96A SNP were more likely to have an ICD on entry and more likely to have an arrhythmic event (sudden death, VT/VF, appropriate ICD shock) during follow-up in a dose-dependent manner. In the current issue of the *JAHA*, Singh et al[8] explore the mechanism by which this polymorphism

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alters Ca^{2+} handling using cardiac-specific transgenic overexpression of the human HRC alleles on the background of Hrc null (knockout) mice. They reported that compared to the HRC-S96 mice, the Hrc-A96 mice have (1) lower basal but similar isoproterenol-stimulated SR Ca^{2+} release and contractility, (2) decreased SR Ca^{2+} load, (3) greater SR Ca^{2+} leak leading to more frequent Ca^{2+} sparks, waves, and delayed afterdepolarizations (DADs), and (4) increased ventricular arrhythmias following isoproterenol or myocardial infarction. They also found that the HRC-A96 allele binds less well to triadin and leads to hyperphosphorylation of the Ser2814 CaMKII site of RyR2, which was partially reversed by the CaMKII inhibitor KN93. They conclude that HRC acts as a regulator of Ca^{2+} handling similar to calsequestrin, that it inhibits RYR2-mediated Ca^{2+} release, and that the HRC-A96 allele predisposes to arrhythmias through its defective interaction with triadin.

The current study is important in several ways. First, the identification of genetic biomarkers predisposing to sudden death could have great clinical importance. Second, the studies highlight the importance of HRC in Ca^{2+} handling in the heart, and may lead to a new pharmacological target for arrhythmias. Third, the work provides further rationale to search for arrhythmia variants in other Ca^{2+} handling genes. Finally, and perhaps of greatest importance even though additional details remain to be worked out, the studies directly address the mechanism by which a common polymorphism alters cardiac function.

The study does have some potential weaknesses, however. The use of transgenic overexpression on a null background raises some concerns regarding background strain, transgene expression levels, and comparison to the null mouse that would not be as significant if a gene-targeted knock-in strategy had been employed. The mouse heart has substantial electrophysiological differences from the human heart, including a very fast heart rate and short action potential duration.[9] This makes extrapolation of arrhythmic mechanisms to humans problematic, and raises the need for studies in large animal models.

The greatest concern, however, rests with the clinical significance of the HRC S96A SNP. While the finding of more baseline ICDs and arrhythmias during follow-up in carriers of the HRC-A96 allele in the Greek cohort is intriguing, the study is small and subject to multiple potential confounders. The *HRC* gene is located on chromosome 19q13.3: inherited conduction disease in a Lebanese family has been mapped to that locus, but to date no mutations that cause cardiac disease have been identified in HRC.[10] In addition, GWAS studies have not identified an association between chromosome 19 and either arrhythmias or sudden death, as might be expected for a common SNP with a high hazard ratio. Clearly there are potential explanations for this finding, including that the arrhythmic predisposition is limited to a subgroup of idiopathic dilated cardiomyopathy patients. In any event, however, the clinical role of this SNP needs to be confirmed in additional larger cohorts of cardiomyopathy patients. Two ongoing prospective trials in heart failure patients using ICD shocks as a surrogate for sudden death may provide a venue for such confirmation.[11,12]

In summary, the study by Singh et al takes an important step towards identifying the potential mechanism by which a genetic variant in a recently identified Ca^{2+} handling gene may predispose to arrhythmias in heart failure patients. If the clinical association is robust and verified, this could become part of a cohort of genetic biomarkers that would be useful for arrhythmia risk stratification.

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Disclosures

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