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 Ca2+ 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+1.5 (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 Ca2+ handling. The histidine-rich calcium binding protein (HRC) is a low-affinity high-capacity sarco-plasmic reticulum (SR) Ca2+ buffer that interacts directly with SERCA2a and Triadin, and regulates indirectly the function of the SR Ca2+ release channel (RyR2).5,6 Overexpression of HRC in transgenic mice led to impaired SR Ca2+ uptake and slower Ca2+ 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 Ca2+ uptake, larger Ca2+ transients, and increased contractility at baseline associated with increased spontaneous SR Ca2+ release (sparks, afterdepolarizations, aftercontractions) and poor tolerance to thoracic aortic banding.

In a recent report, Arvanitis et al7 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 JAH, Singh et al8 explore the mechanism by which this polymorphism
altered 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 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 study highlights 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 arrhythmical 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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References
1. Goldberger JJ, Buxton AE, Cain M, Costantini O, Exner DV, Knight BP, Lloyd-Jones D, Kadish AH, Lee B, Moss A, Myerburg R, Ogin J, Passman R, Rosenbaum D, Stevenson W, Zareba W, Zipes DP. Risk stratification for arrhythmic sudden cardiac death: identifying the roadblocks. Circulation. 2011;123:2423–2430.
2. Huijerti H, Castellanos A, Myerburg RJ. Sudden death due to cardiac arrhythmias. N Engl J Med. 2001;345:1473–1482.
3. Havmoller R, Chugh SS. Plasma biomarkers for prediction of sudden cardiac death: another piece of the risk stratification puzzle? Circ Arrhythm Electrophysiol. 2012;5:237–243.
4. London B. Arrhythmias. In: Ginsburg GS, Willard HF, eds. Genomic and Personalized Medicine. San Diego, Calif: Academic Press Inc; 2013:587–601.
5. Jaehnigt EJ, Heidt AB, Greene SB, Cornelissen I, Black BL. Increased susceptibility to isoproterenol-induced cardiac hypertrophy and impaired weight gain in mice lacking the histidine-rich calcium-binding protein. Mol Cell Biol. 2006;26:9315–9326.
6. Park CS, Chen S, Lee H, Cha H, Oh JG, Hong S, Han P, Ginsburg KS, Jin S, Park I, Singh VP, Wang H-S, Franzeni-Armstrong C, Park WJ, Bers DM, Kranias EG, Cho C, Kim DH. Targeted ablation of the histidine-rich Ca\(^{2+}\)-binding protein (HRC) gene is associated with abnormal SR Ca\(^{2+}\)-cycling and severe pathology under pressure-overload stress. Basic Res Cardiol. 2013;108:344.
7. Arvanitis DA, Panoudou D, Kolokathis F, Vafiadaki E, Papalouka V, Kontogianakis Konstantopolous A, Theodorakis GN, Paraskevaidis IA, Adamopoulos S, Dorn GW, Kremastinos DT, Kranias EG. The Ser96A variant in histidine-rich calcium binding protein is associated with life-threatening ventricular arrhythmias in idiopathic dilated cardiomyopathy. Eur Heart J. 2006;29:2514–2525.
8. Singh VP, Rubinstein J, Arvanitis DA, Ren X, Gao X, Haghigi K, Gilbert M, Iyer VR, Kim DH, Cho C, Jones K, Lorenz JN, Armstrong CF, Wang H-S, Gyorke S, Kranias EG. Abnormal calcium cycling and cardiac arrhythmias associated with the human S96A genetic variant of histidine-rich calcium binding protein. J Am Heart Assoc. 2013;2:e000460 doi:10.1161/JAHA.113.000460.
9. London B. Cardiac arrhythmias: from (transgenic) mice to men. J Cardiovasc Electrophysiol. 2001;12:1089–1091.
10. de Meues A, Stephan E, Debrus S, Jean M-K, Loiselet J, Weinstein J, Demaillie J, Bouvagnet P. An isolated cardiac conduction disease maps to chromosome 19q. Circ Res. 1995;77:735–740.
11. Refaat MM, Lubitz SA, Makino S, Islam Z, Frangiskakis JM, Mehdi H, Gutmann R, Zhang ML, Bloom HL, MacRae CA, Dudley SC, Shalaby AA, Weiss R, McNamara DM, London B, Ellinor PT. Genetic variation in the alternative splicing regulator, RBM20, is associated with dilated cardiomyopathy. Heart Rhythm. 2012;9:390–396.

12. Cheng A, Dalal D, Butcher B, Norgard S, Zhang Y, Dickfeld T, Eldadah ZA, Ellenbogen KA, Guallar E, Tomaselli GF, Zayd A. Prospective observational study of implantable cardioverter-defibrillators in primary prevention of sudden cardiac death: study design and cohort description. J Am Heart Assoc. 2013;2:e000083 doi: 10.1161/JAHA.112.000083.

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