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EDITORIAL COMMENT

Ion Channel Dysfunction Associated With Arrhythmia, Ventricular Noncompaction, and Mitral Valve Prolapse

A New Overlapping Phenotype*

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In this issue of the *Journal*, 2 elegant papers report the association of arrhythmia, primarily sinus bradycardia, left ventricular noncompaction (LVNC), and mitral valve prolapse (MVP) (1,2), demonstrating that the underlying cause is mutation and dysfunction of the hyperpolarization-activated cyclic nucleotide channel 4 (*HCN4*), a major constituent of the pacemaker current (I_f) in the sinoatrial node (SAN) (3). The investigators

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demonstrate abnormalities in channel function consistent with the arrhythmia phenotype and speculate as to the underlying pathogenesis that leads to LVNC, a heterogeneous myocardial phenotype associated with abnormal trabeculation of the LV (4). Uncertainty, however, belies the question of how do mutations in this ion channel cause the combined phenotype. To develop a plausible hypothesis, understanding the data reported by these 2 studies, as well as a review of prior studies, is required.

Schweizer et al. (1) identify *HCN4* mutations in 2 unrelated families and an additional unrelated proband with sinus node dysfunction (SND)/bradycardia, LVNC, and MVP. Using a candidate gene approach, they identified a novel *HCN4-G482R* loss-of-function mutation, located within the highly conserved GYG motif of the channel pore domain that segregated with all affected members in the 4-generation index family (5). The common W4R variant in the cysteine and glycine-rich protein 3 (*CSRP3*) gene encoding a Z-disk protein, previously reported in patients with dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy, and healthy subjects also was identified (6,7). In addition to the index family, a second unrelated family and the unrelated proband were shown to have truncation (*HCN-695X*) and missense (*HCN-P883R*) *HCN4* mutations with no mutations identified in *CSRP3*. Family members and probands of all 3 families had severe SND with or without atrial or ventricular arrhythmias, syncope, or sudden death and a normal QTc interval. Noninvasive imaging demonstrated biventricular hypertrabeculation/LVNC and MVP. Patch-clamp studies demonstrated no hyperpolarization-activated inward currents in mutant *HCN4-G482R* subunits, consistent with loss of function. Homozygous *HCN4-G482R* channels were nonfunctional, and heteromeric mutant and wild-type *HCN4* channel subunits had 65% current reduction, consistent with a dominant-negative mechanism, resulting in I_f current reduction in heterozygotes and lower current densities.

Milano et al. (2) report on 4 families with SND with or without syncope/cardiac arrest, ventricular arrhythmias, and atrial arrhythmias, with echocardiography demonstrating LVNC with or without MVP. *HCN4* mutations were identified in all families (*Tyr481His* in 2 families, *Gly482Arg* and *Ala414Gly* in 1 family each). All mutations affected conserved residues with 2 mutations (*Tyr481His*, *Gly482Arg*) affecting highly conserved residues

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within the pore domain of *HCN4* and the other (*Ala414Gly*) affecting the cytoplasmic S4-S5 linker of *HCN4*. Heterologous expression studies with the *Tyr481His* and *Gly482Arg* mutations demonstrated a large negative shift of the voltage dependence of activation compared with expression of wild-type channels, indicating the importance of the pore region for the voltage dependence of activation. All mutations resulted in significantly lower HCN4 current density.

Together, these studies demonstrate that *HCN4* mutations result in loss of function and significantly reduced I_f current density associated with bradycardia, arrhythmias, and LVNC with or without MVP. Conceptually, these findings are consistent with the "final common pathway" hypothesis proposed nearly 15 years ago, which suggested that mutations in genes encoding proteins within the same pathway (or secondary disturbance of protein function as a result of binding partner abnormalities, drugs, and so on) leads to a common phenotype (8). This hypothesis enabled successful targeted candidate gene screening for arrhythmias, cardiomyopathies, and congenital heart disease (CHD), leading to the current understanding that arrhythmias are caused by disturbed ion channel function ("ion channelopathies"), hypertrophic cardiomyopathy by disturbed sarcomere function, DCM by disturbed sarcomere and cytoskeleton function, and arrhythmogenic right ventricular cardiomyopathy by disturbed desmosome function (9). For LVNC, the picture is less clear; mutations most commonly occur in sarcomere-encoding genes, but animal and human data suggest a central role of signaling pathways. In the cardiomyopathies, ion channel gene mutations also have been implicated, but the causative mechanism(s) remain unclear.

HCN channels, found in SAN cells and neurons, are responsible for hyperpolarization-activated currents, called I_f in the heart (3,5). The HCN channel characteristic distinguishing them from other currents is its unique ion selectivity and gating properties. The HCN channel family has 4 distinct members, with HCN4 being the prominent cardiac form. Native I_f current, as well as the currents induced by heterologously expressed HCN channels, have 4 hallmark properties: 1) channel activation by membrane hyperpolarization; 2) channel activation by direct interaction with cAMP; 3) Na^+ and K^+ permeability; and 4) a specific pharmacological profile. HCN channels consist of 4 subunits arranged around the centrally located, pore-forming 4 different homotetramers with distinct biophysical properties. Each channel subunit consists of: 1) the transmembrane core harboring the gating machinery and ion-conducting pore; 2) the cytosolic NH_2-terminal domain; and 3) the COOH-terminal domain with the cyclic nucleotide binding domain and the peptide connecting the CNBD with the transmembrane core (the "C-linker") that confers modulation by cyclic nucleotides. The I_f current is important in the initiation and regulation of the heartbeat, which is therefore called the "pacemaker current." Mutation in the *HCN4* gene, located on chromosome 15q24.1, was first reported by Schulze-Bahr et al. (10) in a patient with SND, atrial fibrillation, and chronotropic incompetence. The 1-bp deletion mutation (*HCN4-573X*) resulted in a premature stop codon and a C-terminus lacking the CNBD domain. In vitro heterologous expression revealed a dominant-negative loss of cAMP modulation. Several other publications demonstrating SND with severe bradycardia, with or without atrial fibrillation or ventricular arrhythmias, have now been reported. These findings with *HCN4* mutations would be predicted by the "final common pathway" hypothesis: ion channels cause rhythm disturbance. However, Schweizer et al. (1) and Milano et al. (2) report the additional phenotypes of LVNC and MVP that would not be predicted to result solely from an ion channel mutation. Schweizer et al. (1) reported 1 family with a *CSRP3* variant that is more in line with the causes of myocardial disease, but this was not seen in other gene-positive families. So, how does LVNC occur?

Neither publication presents mechanistic data, but the investigators speculate on how LVNC and MVP develop. Schweizer et al. (1) noted that *HCN4* is involved in early embryonic heart development, helping to form myocardium and the conduction system. During later development, *HCN4* is downregulated in the myocardium, with abundant expression restricted to the SAN and conduction system. They hypothesize that *HCN4* loss of function interferes with molecular mechanisms required during cardiac development, resulting in LVNC. Samsa et al. (11) previously suggested that Notch pathway disturbance causes LVNC with CHD, whereas sarcomere, cytoskeletal, and Z-disk mutations cause myocardial disease-only phenotypes. Based on this, Schweizer et al. (1) suggested signaling pathway involvement in ventricular wall maturation and compaction (e.g., Notch, Neuregulin, Ephrin, or Bone morphogenic protein), could be involved. Milano et al. (2), on the other hand, suggested that because primary channelopathies are associated with myocardial structural abnormalities such as DCM, this also occurs with *HCN4*. Their