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Commentary

Heart failure (HF) has been referred to as the cardiovascular epidemic of our time. Understanding the molecular determinants of HF disease progression and mortality risk is of utmost importance. In this issue of the *JCI*, Zhang et al. uncover an important link between clinical HF mortality risk and a common variant that regulates *SCN5A* expression through microRNA-dependent (miR-dependent) mechanisms. They also demonstrate that haploinsufficiency of *SCN5A* is associated with increased accumulation of reactive oxygen species (ROS) in a genetically engineered murine model. Their data suggest that even modest depression of *SCN5A* expression may promote pathologic cardiac remodeling and progression of HF.

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SCN5A: the greatest HITS collection

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Heart failure (HF) has been referred to as the cardiovascular epidemic of our time. Understanding the molecular determinants of HF disease progression and mortality risk is of utmost importance. In this issue of the *JCI*, Zhang et al. uncover an important link between clinical HF mortality risk and a common variant that regulates *SCN5A* expression through microRNA-dependent (miR-dependent) mechanisms. They also demonstrate that haploinsufficiency of *SCN5A* is associated with increased accumulation of reactive oxygen species (ROS) in a genetically engineered murine model. Their data suggest that even modest depression of *SCN5A* expression may promote pathologic cardiac remodeling and progression of HF.

SCN5A expression in HF

HF represents a final common pathway for a wide range of inherited and acquired cardiac conditions (1). Disease progression consists of a series of maladaptive responses, which include ion channel remodeling (2), neurohormonal dysregulation, metabolic derangement, oxidative stress, and profibrotic signaling (1). HF patients are at an increased risk of death from sudden cardiac arrest (2) and progressive pump failure (1). Risk stratification of HF patients to identify those at highest risk represents an unmet need as advanced therapies, such as implantable cardioverter defibrillators (ICDs) or ventricular assist devices, could be offered more selectively.

Evaluation of clinical factors that confer increased risk of morbidity and mortality in HF patients show clear correlation with QRS duration, a marker of ventricular activation time and myocardial dyssynchrony (3, 4). As the cardiac sodium channel pore-forming subunit Na_v1.5 (encoded by *SCN5A*) is the principal determinant of cardiac excitability and conduction in the subendocardial His-Purkinje network and ventricular chamber myocardium (5–8), tight regulation of this gene ensures optimal cardiac function and stable rhythm

(5, 6). Although mutations in *SCN5A* have been implicated in arrhythmic diseases such as progressive cardiac conduction disease (9), Brugada syndrome (10), and long QT3 (11, 12), and can produce cardiomyopathic changes (13, 14), the vast majority of HF patients are not mutant carriers. Therefore, intense research has focused on identifying genetic modifiers of *SCN5A* expression that may confer increased risk for conduction disease, arrhythmic events (15), or mortality in HF patients.

Several genome-wide association studies (GWAS) of cardiac conduction parameters have identified strong association with single nucleotide polymorphisms (SNPs) in the *SCN5A* locus (16–19). Decoding whether or not these SNPs have functional consequences on *SCN5A* gene expression is essential. Moreover, determining whether the SNPs that alter *SCN5A* expression have impact on clinical syndromes, including heart failure, is of particular interest.

Modulation of *SCN5A* expression

In this latest edition of the *JCI*, Zhang et al. (20) employed a new approach to understand how GWAS-identified SNPs can

modulate *SCN5A* expression by altering miR-dependent regulation. MiRs are short (~19–22 nucleotides), noncoding RNA species that are potent regulators of mRNA transcript stability and translation (21, 22). MiRs are encoded either in intronic regions where they are cotranscribed with protein-coding exons, or in intergenic regions under the control of their own promoters (21, 22). Mature miRs are coassembled with Argonaute (Ago) proteins as part of the RNA-induced silencing complex (RISC) (21). In the classic paradigm, miRs target mRNA via their 3' untranslated region (UTR) through complementary base-pair interaction with the miR “seed” region (5' region of miR; nucleotide positions 2–8). This miR-mRNA interaction targets mRNA for cleavage or reduces translational efficiency (21). The short sequence interactions allow for miRs to broadly regulate an array of mRNA transcripts that collectively control biological processes, such as calcium homeostasis (23) or metabolism (24). Therefore, identification of bona fide miR targets in a given tissue is essential for therapeutic targeting and prevention of off-target effects.

To identify miR targets in the heart, Boudreau and colleagues previously reported a high throughput method to globally profile miR-mRNA target interactions in human cardiac tissue (25). They applied a technique known as high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP), where Ago2 protein was immunoprecipitated to identify bound miRs and mRNAs (25, 26). By overlaying common SNPs on top of the transcriptome-wide map of miR binding sites, they identified a non-amino acid altering (synonymous) SNP (rs1805126, T>C) adjacent to a miR-24 interaction site in the *SCN5A* coding sequence (25). The rs1805126 minor allele was previously shown to associate with cardiac conduction parameters in GWAS (18), but the synonymous nature of the variant led to the presumption that the variant was not causal.

Zhang et al. (20) hypothesized that the rs1805126 minor allele alters miR-24–

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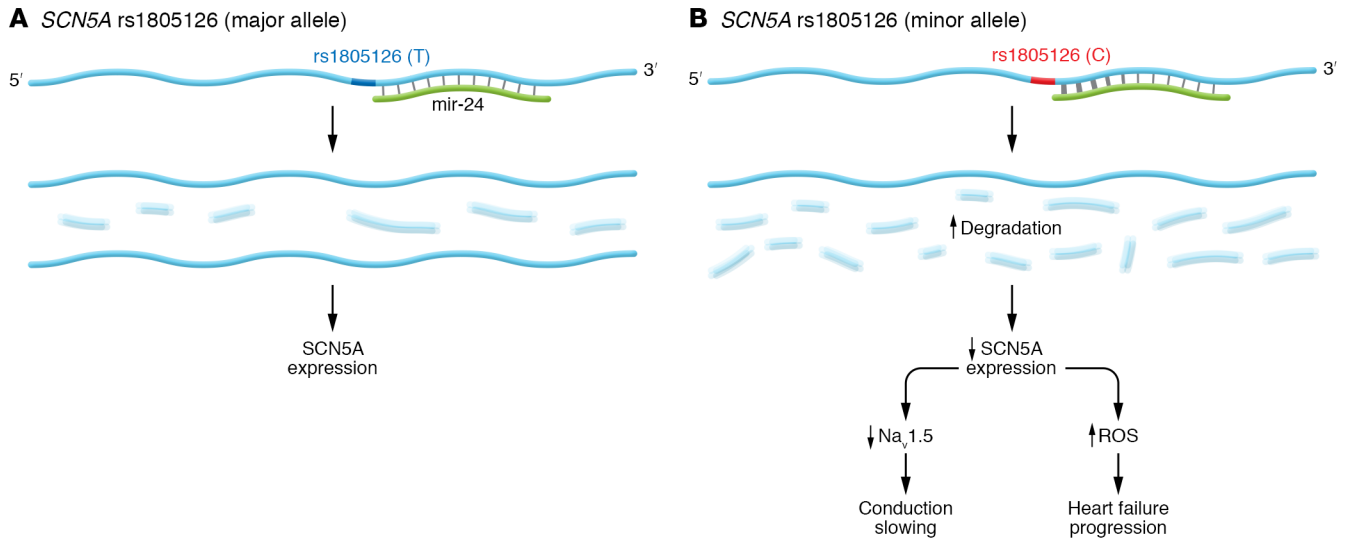


Figure 1. Proposed link between microRNA-dependent regulation of *SCN5A* and disease progression. Genome-wide association studies (GWAS) have linked a synonymous SNP (rs1805126) in the *SCN5A* gene with electrocardiographic measures. (A) Ago2 HITS-CLIP data identify a microRNA-24 (miR-24) binding site immediately adjacent to this SNP. (B) Probability of Interaction by Target Accessibility (PITA) analysis indicates that the rs1805126 minor allele (C) is a thermodynamically more favorable miR-24 target compared to the major allele (T), resulting in greater *SCN5A* degradation and diminished Na_v1.5 expression. While conduction slowing is a predictable consequence of diminished sodium channel expression, Zhang and colleagues (22) show, surprisingly, that reduced *SCN5A* expression is also associated with increased myocardial reactive oxygen species (ROS), and suggest that ROS accumulation promotes heart failure progression and increased nonarrhythmic mortality.

dependent regulation of *SCN5A* (Figure 1). As the rs1805126 variant does not alter the “seed” interaction sequence, the authors speculated and tested through computational means that the C allele produced a more favorable miR-24 interaction with the *SCN5A* transcript. In human heterologous expression systems, miR-24 suppressed Na_v1.5 expression more significantly with the *SCN5A* C allele versus T allele. MiR-24 mimics also reduced sodium current density in neonatal rat cardiomyocytes (NRCMs). In human heart samples, presence of the rs1805126 CC genotype was associated with lower levels of *SCN5A* mRNA and Na_v1.5 channel protein compared to the TT genotype, whereas miR-24 expression was similar between groups. Similar results were seen in expression quantitative trait loci (eQTL) studies (27, 28) and analysis of hearts heterozygous for rs1805126, where the C allele was associated with lower *SCN5A* mRNA levels.

Clinical consequences of *SCN5A* modulation

To explore the clinical consequences of the rs1805126 variant in HF patients, Zhang et al. (20) examined the effect of rs1805126 genotypes in cardiomyopathy patients with ICDs from the Genetic Risk Assessment of Defibrillator Events (GRADE) study

(29) and found a higher mortality rate in patients homozygous for the C allele. Surprisingly, the rs1805126 genotype did not significantly associate with appropriate ICD therapies (a surrogate marker of arrhythmic death). In addition, the authors did not find a significant association with electrocardiographic parameters, although the study was underpowered to do so. As previously stated, prolonged QRS duration is associated with increased morbidity and mortality in HF patients due to dyssynchronous myocardial contraction; therefore, it will be important to reanalyze this association with increased patient enrollment or in another dataset, especially given the known association of rs1805126 with cardiac conduction parameters (18). Similarly, these data should be analyzed for associations between the homozygous rs1805126 genotype and cardiac resynchronization therapy, indicated for patients with significant QRS prolongation and HF (30). Lastly, it would be of interest to note whether the CC genotype is associated with increased right ventricular pacing percentage, as this type of pacing produces myocardial dyssynchrony and has been shown to reduce left ventricular ejection fraction and increase morbidity and mortality (31).

To explore the potential mechanism whereby reduced *SCN5A* expression is

associated with a more severe cardiomyopathic phenotype, Zhang et al. (20) studied *Scn5a* heterozygous knockout mice, which develop increased fibrosis at advanced age (32). *Scn5a* heterozygous knockout hearts had significantly increased reactive oxygen species (ROS) as evidenced by an approximately 2.5-fold increase in oxidation of dihydroethidium (DHE, a measure of steady-state levels of superoxide), which was evident before fibrotic changes. Although association between rs1805126 genotype and increased oxidative stress was not examined, the finding that reduced *Scn5a* expression is sufficient to increase ROS is intriguing and needs further investigation. Accumulation of ROS can result from either overproduction or impaired clearance (33). Increased ROS production in HF is primarily due to functional uncoupling of the mitochondrial electron transport chain (34–37); however, other sources include xanthine oxidase (38), nitric oxide synthase (39), cyclooxygenase (40), and NAD(P)H oxidases (41). Mechanisms of impaired clearance by antioxidants include reduced activity of superoxide dismutase (42) and catalase (43). Identifying which of these pathways contributes to ROS accumulation due to reduced Na_v1.5 expression will be an important first step

in deciphering the mechanisms underlying induction of profibrotic pathways and the development of cardiomyopathy.

Concluding remarks

The work of Zhang et al. (20) adds to the growing body of evidence that sequence variants that regulate *SCN5A* expression can have significant consequences on HF disease progression and mortality. Although the mechanism of worsening HF associated with rs1805126 will need further evaluation, these findings bring us one step closer to creating a genetic HF risk score, which can be used to personalize therapies for this complex and growing patient population.

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