

SCN5A: the greatest HITS collection

David S. Park, Glenn I. Fishman

J Clin Invest. 2018;128(3):913-915. <https://doi.org/10.1172/JCI99927>.

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.

Find the latest version:

<https://jci.me/99927/pdf>



SCN5A: the greatest HITS collection

David S. Park and Glenn I. Fishman

Leon H. Charney Division of Cardiology, New York University School of Medicine, New York, New York, USA.

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–

► Related Article: p. 1154

Conflict of interest: The authors have declared that no conflict of interest exists.

Reference information: *J Clin Invest*. 2018;128(3):913–915. <https://doi.org/10.1172/JCI99927>.

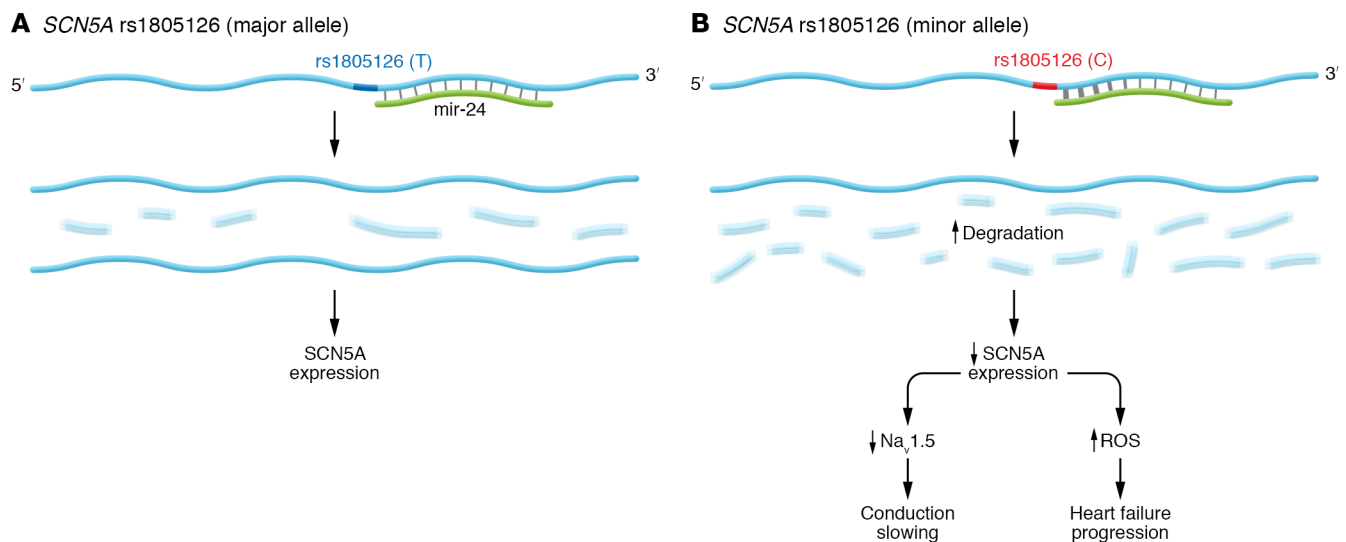


Figure 1. Proposed link between microRNA-dependent regulation of *SCN5A* and disease progression. Genome-wide association studies (GWAS) have linked a synonymous SNP (rs1805126) in the *SCN5A* gene with electrocardiographic measures. (A) Ago2 HITS-CLIP data identify a microRNA-24 (miR-24) binding site 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.

Acknowledgments

This work is supported by grants from the NIH to DSP (R01 HL132073), and GIF (R01 HL105983 and R01 HL92727), an American Heart Association Scientist Development Grant (17SDG33411201) to DSP, and a Fondation LeDucq Transatlantic Network Award to GIF.

Address correspondence to: Glenn I. Fishman, Leon H. Charney Division of Cardiology, NYU School of Medicine, 522 First Avenue, Smilow 801, New York, New York 10016, USA. Phone: 212.263.3967; Email: glenn.fishman@nyumc.org.

- Metra M, Teerlink JR. Heart failure. *Lancet*. 2017;390(10106):1981–1995.
- Nattel S, Maguy A, Le Bouter S, Yeh YH. Arrhythmogenic ion-channel remodeling in the heart: cardiac failure, myocardial infarction, and atrial fibrillation. *Physiol Rev*. 2007;87(2):425–456.
- Iuliano S, Fisher SG, Karasik PE, Fletcher RD, Singh SN, Department of Veterans Affairs Survival Trial of Antiarrhythmic Therapy in Congestive Heart Failure. QRS duration and mortality in patients with congestive heart failure. *Am Heart J*. 2002;143(6):1085–1091.
- Kashani A, Barold SS. Significance of QRS complex duration in patients with heart failure. *J Am Coll Cardiol*. 2005;46(12):2183–2192.
- Park DS, et al. Genetically engineered *SCN5A* mutant pig hearts exhibit conduction defects and arrhythmias. *J Clin Invest*. 2015;125(1):403–412.
- Papadatos GA, et al. Slowed conduction and ventricular tachycardia after targeted disruption of the cardiac sodium channel gene *Scn5a*. *Proc Natl Acad Sci U S A*. 2002;99(9):6210–6215.
- Park DS, et al. *Fhf2* gene deletion causes temperature-sensitive cardiac conduction failure. *Nat Commun*. 2016;7:12966.
- Shekhar A, et al. Transcription factor ETV1 is essential for rapid conduction in the heart. *J Clin Invest*. 2016;126(12):4444–4459.
- Schott JJ, et al. Cardiac conduction defects associate with mutations in *SCN5A*. *Nat Genet*. 1999;23(1):20–21.
- Chen Q, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature*. 1998;392(6673):293–296.
- Bennett PB, Yazawa K, Makita N, George AL Jr. Molecular mechanism for an inherited cardiac arrhythmia. *Nature*. 1995;376(6542):683–685.
- Wang Q, et al. *SCN5A* mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell*. 1995;80(5):805–811.
- Olson TM, et al. Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. *JAMA*. 2005;293(4):447–454.
- Wan E, et al. Aberrant sodium influx causes cardiomyopathy and atrial fibrillation in mice. *J Clin Invest*. 2016;126(1):112–122.
- Shang LL, et al. Human heart failure is associated with abnormal C-terminal splicing variants in the cardiac sodium channel. *Circ Res*. 2007;101(11):1146–1154.
- Sotoodehnia N, et al. Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. *Nat Genet*. 2010;42(12):1068–1076.
- Pfeuffer A, et al. Genome-wide association study of PR interval. *Nat Genet*. 2010;42(2):153–159.
- Holm H, et al. Several common variants modulate heart rate, PR interval and QRS duration. *Nat Genet*. 2010;42(2):117–122.
- Chambers JC, et al. Genetic variation in *SCN10A* influences cardiac conduction. *Nat Genet*. 2010;42(2):149–152.
- Zhang X, et al. A common variant alters *SCN5A*-miR-24 interaction and associates with heart failure mortality. *J Clin Invest*. 2018;128(3):1154–1163.
- Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol*. 2014;15(8):509–524.
- Quiat D, Olson EN. MicroRNAs in cardiovascular disease: from pathogenesis to prevention and treatment. *J Clin Invest*. 2013;123(1):11–18.
- Harada M, Luo X, Murohara T, Yang B, Dobrev D, Nattel S. MicroRNA regulation and cardiac calcium signaling: role in cardiac disease and therapeutic potential. *Circ Res*. 2014;114(4):689–705.
- Hathaway QA, Pinti MV, Durr AJ, Waris S, Shepherd DL, Hollander JM. Regulating MicroRNA expression: at the heart of diabetes mellitus and the mitochondrion [published online ahead of print October 6, 2017]. *Am J Physiol Heart Circ Physiol*. https://doi.org/10.1152/ajpheart.00520.2017.
- Spengler RM, et al. Elucidation of transcriptome-wide microRNA binding sites in human cardiac tissues by Ago2 HITS-CLIP. *Nucleic Acids Res*. 2016;44(15):7120–7131.
- Chi SW, Zang JB, Mele A, Darnell RB. Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. *Nature*. 2009;460(7254):479–486.
- Liu Y, et al. RNA-Seq identifies novel myocardial gene expression signatures of heart failure. *Genomics*. 2015;105(2):83–89.
- Koopmann TT, et al. Genome-wide identification of expression quantitative trait loci (eQTLs) in human heart. *PLoS One*. 2014;9(5):e97380.
- AlJaroudi WA, et al. Effect of angiotensin-converting enzyme inhibitors and receptor blockers on appropriate implantable cardiac defibrillator shock in patients with severe systolic heart failure (from the GRADE Multicenter Study). *Am J Cardiol*. 2015;115(7):924–931.
- Russo AM, et al. ACCF/HRS/AHA/ASE/HFSA/SCAI/SCCT/SCMR 2013 appropriate use criteria for implantable cardioverter-defibrillators and cardiac resynchronization therapy: a report of the American College of Cardiology Foundation appropriate use criteria task force, Heart Rhythm Society, American Heart Association, American Society of Echocardiography, Heart Failure Society of America, Society for Cardiovascular Angiography and Interventions, Society of Cardiovascular Computed Tomography, and Society for Cardiovascular Magnetic Resonance. *J Am Coll Cardiol*. 2013;61(12):1318–1368.
- Sharma AD, et al. Percent right ventricular pacing predicts outcomes in the DAVID trial. *Heart Rhythm*. 2005;2(8):830–834.
- Royer A, et al. Mouse model of *SCN5A*-linked hereditary Lenègre's disease: age-related conduction slowing and myocardial fibrosis. *Circulation*. 2005;111(14):1738–1746.
- Moris D, et al. The role of reactive oxygen species in myocardial redox signaling and regulation. *Ann Transl Med*. 2017;5(16):324.
- Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial ROS-induced ROS release: an update and review. *Biochim Biophys Acta*. 2006;1757(5–6):509–517.
- Sawyer DB, Colucci WS. Mitochondrial oxidative stress in heart failure: “oxygen wastage” revisited. *Circ Res*. 2000;86(2):119–120.
- Liu M, Liu H, Dudley SC Jr. Reactive oxygen species originating from mitochondria regulate the cardiac sodium channel. *Circ Res*. 2010;107(8):967–974.
- Ide T, et al. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. *Circ Res*. 2001;88(5):529–535.
- Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *J Physiol*. 2004;555(pt 3):589–606.
- Umar S, van der Laarse A. Nitric oxide and nitric oxide synthase isoforms in the normal, hypertrophic, and failing heart. *Mol Cell Biochem*. 2010;333(1–2):191–201.
- Kim JW, et al. Gene expression of cyclooxygenase in the aging heart. *J Gerontol A Biol Sci Med Sci*. 2001;56(8):B350–B355.
- Kuroda J, Ago T, Matsushima S, Zhai P, Schneider MD, Sadoshima J. NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. *Proc Natl Acad Sci U S A*. 2010;107(35):15565–15570.
- Fukai T, Fölz RJ, Landmesser U, Harrison DG. Extracellular superoxide dismutase and cardiovascular disease. *Cardiovasc Res*. 2002;55(2):239–249.
- Borchi E, et al. Enhanced ROS production by NADPH oxidase is correlated to changes in antioxidant enzyme activity in human heart failure. *Biochim Biophys Acta*. 2010;1802(3):331–338.