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Commentary

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Na$_v$-igating through a complex landscape: SCN10A and cardiac conduction

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Genome-wide association studies (GWAS) have implicated *SCN10A*, which encodes a nociceptor-associated voltage-gated sodium channel subunit, as a modulator of cardiac conduction; however, this role has traditionally been ascribed to *SCN5A*, which is highly expressed in cardiac muscle. *SCN10A* is believed to affect cardiac conduction either directly through cardiomyocytes or indirectly via intracardiac neurons. In this issue of the *JCI*, van den Boogaard and colleagues introduce a third possibility: that the *SCN10A* locus acts as an enhancer of *SCN5A* gene expression. The authors demonstrate that *SCN10A* expression is negligible within human and murine hearts, and that a T-box enhancer within the *SCN10A* locus drives *SCN5A* expression within cardiomyocytes. This work reasserts *SCN5A* as the key determinant of cardiac conduction and highlights the importance of deciphering the functionality of coding versus noncoding regions when interpreting GWAS data.

The unexpected finding: *SCN10A*

Since the first identification of a significant association between *SCN10A* and ECG conduction parameters in genome-wide association studies (GWAS), a debate has emerged regarding how this gene and/or its product affect myocardial conduction (1–4). *SCN10A* encodes the α subunit of the voltage-gated sodium channel, Na$_v$1.8, which was previously demonstrated to be expressed in nociceptive fibers of the dorsal root ganglia (DRG), but had never been implicated in heart physiology (5). Traditionally, rapid conduction properties of myocardial tissues have been ascribed to *SCN5A*, which encodes the pore-forming subunit of the major cardiac voltage-gated sodium channel, Na$_v$1.5. Mutations in *SCN5A* have been identified in patients with isolated cardiac conduction disease

and with the arrhythmic disorder Brugada syndrome (6, 7). In addition, mice with *Scn5a* haploinsufficiency exhibit slowed conduction parameters during cardiac electrophysiology testing (8). It was therefore unexpected that GWAS would more strongly correlate ECG parameters with *SCN10A* over *SCN5A*. This surprising finding raises the question of how *SCN10A* fits into a landscape dominated by *SCN5A*. Furthermore, another GWAS has recently identified significant associations between Brugada syndrome and the *SCN10A* locus, making the link between *SCN10A* and myocardial conduction slowing even more compelling (9).

The cardiomyocyte hypothesis: cell-autonomous role of Na$_v$1.8

With the goal of establishing how *SCN10A* contributes to overall sodium current (I$_{Na}$) in the heart, a combination of in vivo cardiac electrophysiology testing and cell culture–based biophysical analyses have been reported. Ambulatory telemetry

monitoring was performed in wild-type mice treated with the Na$_v$1.8-selective inhibitor, A-803467, as well as in *Scn10a* knockout mice, but the results were contradictory. Wild-type mice treated with A-803467 showed marked prolongation of PR and QRS intervals (1); however, *Scn10a* knockout mice exhibited shorter PR intervals with no change in QRS duration (4). These inconsistent results may represent differences between acute and chronic loss of Na$_v$1.8, off-target effects of A-803467 on Na$_v$1.5, or differential responses of cardiomyocytes and intracardiac neurons to A-803467. To elucidate the role of *Scn10a* specifically in cardiomyocytes, Yang et al. studied the effects of Na$_v$1.8 inhibition or deletion in ventricular myocytes (10). Treatment of isolated mouse ventricular myocytes with A-803467 had no effect on peak I$_{Na}$, but blocked a component of the late sodium current, I$_{Na,L}$, resulting in shortening of the action potential duration (APD) at slow stimulation frequencies. Consistent with the inhibitor studies, *Scn10a$^{-/-}$* ventricular myocytes had reduced I$_{Na,L}$, exhibited shorter APD at baseline, and were not responsive to A-803467 (10). Although these results suggest a role for *Scn10a* in APD prolongation and triggered arrhythmias, peak I$_{Na}$ and cardiac action potential upstroke velocity were unperturbed in ventricular myocytes with inhibition or deletion of Na$_v$1.8 (10, 11). Therefore, *Scn10a* does indeed appear to have a cell-autonomous effect on cardiomyocyte electrophysiology, but the mechanism by which Na$_v$1.8 modulates cardiac conduction remained unclear.

The enhancer hypothesis: linking a SNP to *SCN5A* expression

In this issue of the *JCI*, van den Boogaard et al. introduce a completely different mechanism for how the *SCN10A* locus modulates cardiac conduction: as an enhancer of *SCN5A* expression (12). This elegant work adds yet another chapter to the story of how T-box (TBX) transcription factors function as master regulators of fast conduction in the heart. TBX5 activates *SCN5A* expression, whereas TBX3 represses it (13, 14). In their previous report, the Christoffels group used a genome-wide occupancy screen for TBX3 binding elements, which identified two functionally conserved TBX-regulated enhancers within the *Scn5a-Scn10a* locus (14). One of the enhancers was located downstream of *Scn5a*, and the other was located within an intron of *Scn10a*. By serendipity, the *SCN10A* SNP (rs6801957), previously associated with QRS prolongation, happened to be in the TBX-binding element within the intronic *SCN10A* enhancer. SNP rs6801957 not only affected TBX3 and TBX5 binding, but also diminished enhancer function in a zebrafish model (14). In the current report, van den Boogaard and colleagues used high-resolution chromatin conformation capture (4C) to demonstrate that both enhancer regions interact with *Scn5a* and *Scn10a* promoters; however, RNA-seq analysis of adult mouse and human hearts, including the murine atrioventricular (AV) bundle, demonstrated trivial amounts of *SCN10A* expression (12). Given this result, the ability of these enhancers to modulate *Scn5a* expression was exclusively studied using transgenic mice expressing a *Scn5a-LacZ* BAC reporter construct. Elimination of the *Scn10a* enhancer was sufficient to disrupt *Scn5a* reporter gene expression within the myocardium and the proximal ventricular conduction system. Introduction of the SNP rs6801957 variant allele into the BAC reporter construct also resulted in severe depression of *Scn5a* in the compact myocardium and the cardiac conduction system. Furthermore, a direct association was identified between the presence of the SNP rs6801957 variant allele and expression of *SCN5A* in human hearts (12). These results construct a plausible and coherent mechanism whereby genetic variants in the *SCN10A* locus influence cardiac conduction parameters.

In agreement with these observations, data to support Na$_v$1.8 protein expression in cardiomyocytes is scant.

Verkerk et al. found robust Na$_v$1.8 staining in dorsal root ganglia and intracardiac neurons, while the myocardium was only weakly stained (11). Immunohistochemistry of isolated ventricular myocyte preparations failed to detect Na$_v$1.8 (11). Similarly, Na$_v$1.8 was undetectable in dog Purkinje fibers, but was present in neighboring intracardiac neurons (15). In humans, Na$_v$1.8 immunoreactivity was present in both intracardiac neurons and atrial myocytes (16). Whether the discrepancies among these studies constitute species-specific differences or limitations of current antibodies will need to be addressed. Studies evaluating the detection of *SCN10A* mRNA have been more fruitful. Chambers et al. reported *SCN10A* expression in both human and mouse atrial and ventricular tissue (4). Yang et al. reported similar results with differential expression levels of *Scn10a* between murine cardiac chambers, with the lowest levels detected in the left ventricle (10). However, one cannot exclude the possibility of intracardiac neuronal contamination in the RNA preparations in the reported results. Our group and the Christoffels group have previously noted enrichment of *Scn10a* in the cardiac conduction system compared with working myocytes during development (1, 14), but a detailed evaluation of the adult heart has not been performed. In addition, none of the aforementioned RNA techniques were quantitative.

Despite the lack of evidence for robust *Scn10a* expression in cardiomyocytes, the ability of Na$_v$1.8 to modulate cardiac APD in some ventricular myocytes suggests that the expression of *Scn10a*, albeit small, is sufficient to modulate cardiomyocyte electrophysiology (10). Nonsynonymous sequence variants of *SCN10A* identified in GWAS appear to have biophysical consequences, at least in heterologous expression systems (17). Even more tantalizing is that screening of Brugada patients for mutations in *SCN10A* has identified nonsynonymous sequence variants at a frequency approaching that of *SCN5A*, and coexpression of several of these variants with *SCN5A* caused loss of function of Na$_v$1.5 currents (18). These results are reminiscent of the dominant-negative effect seen with trafficking-defective *SCN5A* mutations that sequester wild-type channels in the endoplasmic reticulum through α subunit interaction (19).

The neuronal hypothesis: Na$_v$1.8 in neuronal cells modulates cardiac electrophysiology

Another possibility is that *SCN10A* indirectly exerts an effect on cardiac conduction through intracardiac neurons. Immunohistochemistry-based studies have shown that most Na$_v$1.8-positive neurons are cholinergic in origin (11). Patch-clamp studies on isolated intracardiac neurons revealed that A-803467 treatment reduces I$_{Na}$ density, accelerates the slow component of current decay, and causes a negative shift in voltage dependence of inactivation. Functionally, A-803467 treatment reduced the firing frequency of intracardiac neurons (11). In mongrel dogs, direct injection of A-803467 into right ganglionated plexi resulted in reduced levels of sinus bradycardia, ventricular rate slowing, PR interval prolongation, and atrial fibrillation (AF) inducibility in response to vagus nerve stimulation (20). *Scn10a* likely affects AF inducibility by modulating atrial refractoriness in response to vagal stimulation (20). On the other hand, a hypermorphic mutation in murine *Scn10a*, termed *Possum*, increases Na$_v$1.8-mediated currents and enhances excitability of DRG sensory neurons. *Possum* mice exhibit marked sinus bradycardia and R-R variability in response to "scruffing," which is abrogated by atropine infusion (21). Taken together, these results suggest that *Scn10a* functions in cholinergic neurons to exert negative chronotropic and dromotropic effects on sinus and AV nodal tissues and modulates myocyte refractoriness. These results are consistent with the PR interval shortening observed in *Scn10a* knockout animals (4). Cardiac- and neuronal-restricted, inducible *Cre* systems may help parse out the tissue-specific and time-dependent contribution of *Scn10a* to conduction parameters and arrhythmia susceptibility.

The Christoffels, Nobrega, Barnett, and Moskowitz groups should be applauded for their present work. Their contribution has helped fulfill the promise of GWAS in accelerating the discovery of coding and noncoding sequence variants that together create phenotypic manifestation of disease. Whether *SCN10A* is acting cell autonomously in cardiomyocytes, indirectly through intracardiac neurons, or purely as a *cis*-acting enhancer element in the transcriptional regulation of *SCN5A*, no one will disagree that the introduction of *SCN10A* into the debate has been both unexpected and exhilarating. Regardless of what combination of these hypotheses turns out to be correct, the

discovery of new disease mechanisms will undoubtedly accelerate the development of therapies in the treatment of cardiac conduction disorders.

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Not simply misshapen red cells: multimolecular and cellular events in sickle vaso-occlusion

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Thromboinflammatory diseases result from the interactions of vascular endothelial cells, inflammatory cells, and platelets with cellular adhesion molecules, plasma proteins, and lipids. Tipping the balance toward a prothrombotic, proinflammatory phenotype results from multicellular activation signals. In this issue of the *JCI*, Li et al. explore the regulation of heterotypic neutrophil-platelet contacts in response to TNF-α–induced venular inflammation with relevance to sickle cell disease (SCD).

Not just misshapen red cells

In a 1910 report of an anemic West Indian man, Herrick first described the "peculiar elongated and sickle-shaped" rbcs produced by individuals with sickle cell anemia (1). Over the next century, evaluation of sickle cell anemia–associated molecular and cellular pathobiology revealed that the polymerization of hemoglobin S and cellular shape change upon deoxygenation were due to a single nucleotide mutation (A to T) in the gene encoding β-globin. The pain crises and organ infarctions that manifest in patients were attributed to a mechanical obstruction of blood flow due to the rigid crescent-shaped cells; however, in 1980 Hebbel and others demonstrated that sickle erythrocytes were excessively adherent to vascular endothelial cells (2).

As a result of this seminal observation, researchers in the 1980s and 1990s were able to define the red cell characteristics, endothelial adhesion molecules, and plasma factors responsible for these phenomena. Patients with sickle cell disease (SCD) have marked leukocytosis, thrombocytosis, markers of inflammation, oxidative stress, and a procoagulant phenotype. Other cells have been implicated in sickle-associated vaso-occlusive events, including neutrophils (3, 4), monocytes (5, 6), platelets (4, 7), invariant NKT lymphocytes (8), and the endothelium itself (2, 9–11).

Of mice and men: murine SCD models tell a hot story

Transgenic mouse models of SCD have redefined the pathophysiology of vaso-occlusion. SCD models have been developed