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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_v1.8, 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_v1.5. 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_v1.8

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_v1.8-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_v1.8, off-target effects of A-803467 on Na_v1.5, 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_v1.8 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_v1.8 (10, 11). Therefore, Scn10a does indeed appear to have a cell-autonomous effect on cardiomyocyte electrophysiology, but the mechanism by which Na_v1.8 modulates cardiac conduction remained unclear.

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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 highresolution 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_v1.8 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_v1.8 (11). Similarly, Na_v1.8 was undetectable in dog Purkinje fibers, but was present in neighboring intracardiac neurons (15). In humans, Na_v1.8 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_v1.8 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_v1.5 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_v1.8 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_v1.8-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 Nav1.8-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

commentaries



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-associ-

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ated 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 vasoocclusion. SCD models have been developed