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EDITORIAL

The yin and yang of *Tbx5* variant effects on sodium channel function

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This editorial refers to 'Tbx5 variants disrupt Nav1.5 function differently in patients diagnosed with Brugada or long QT syndrome' by P. Nieto-Marín *et al.*, pp. 1046–1060.

Congenital long QT syndrome (LQTS) and Brugada syndrome, two of the most common inherited arrhythmia syndromes, increase the risk of sudden cardiac death in children and young adults. Long QT syndrome is a disorder of cardiac repolarization whereas Brugada syndrome is a disorder of cardiac depolarization. While these disorders account for a small proportion of the population disease burden of sudden cardiac death, they have served as important models tying molecular defects in ion channel function to human disease and have greatly informed our understanding of cardiac electrophysiology more broadly.

The voltage-gated sodium channel $Na_V1.5$, encoded by the *SCN5A* gene, carries the main depolarizing current responsible for initiating the cardiac action potential and so plays a central role in excitability and conduction in the heart. Within a few milliseconds of opening, sodium channels quickly inactivate. However, a small proportion of channels fail to inactivate, or undergo later reactivation, causing a persistent inward current known as late sodium current ($I_{Na\text{-}L}$). *SCN5A* mutations in Brugada syndrome result in a reduced amplitude of the peak inward sodium current that flows at the start of the action potential. Conversely, *SCN5A* mutations in LQTS disrupt channel inactivation causing an increase in the magnitude of $I_{Na\text{-}L}$ and delayed ventricular repolarization.[1]

Rare subtypes of LQTS and Brugada syndrome have been attributed to mutations in the genes encoding proteins that interact with the sodium channel alpha subunit. While the strength of evidence for these minor genes has recently been called into question,[2,3] they have nevertheless helped establish the concept that sodium channels are located in and regulated by sophisticated macromolecular complexes. The accessory proteins making up these complexes include single transmembrane domain β-subunits, anchoring-adaptor proteins (e.g. ankyrin-G and β_{IV}-spectrin), as well as enzymes (e.g. Ca^{2+}/calmodulin dependent protein kinase IIδ, and ubiquitin ligases) that can modulate channel function in response to a range of physiological stimuli.[4,5] In addition, the expression and function of $Na_V1.5$ are modulated by multiple other processes including transcription, alternative splicing, intracellular trafficking, and protein degradation.

Nieto-Marín *et al.*[6] provide evidence that Brugada syndrome and LQTS may both result from dysregulated $Na_V1.5$ function caused by mutations in the transcription factor Tbx5. Tbx5 is one of the core regulatory transcription factors that drives cardiac development.[7] Severe Tbx5 mutations result in Holt-Oram syndrome, characterized by upper limb abnormalities, cardiac septal defects, and conduction disease. Single nucleotide polymorphisms in Tbx5 are also associated with electrocardiographic markers of conduction, such as the PR interval and QRS duration.[8] The latter is consistent with Tbx5's known role in driving the expression of genes for fast conduction, including *SCN5A*, in the developing conduction system. Studies in model organisms suggest that there is very little if any Tbx5 expression in the working myocardium and none in the right ventricular outflow tract.[7] However, analysis of the genotype-tissue expression dataset indicates that Tbx5 is expressed in human atrial appendage and left ventricle[6] thus raising the possibility that Tbx5 may also influence human ventricular myocardial electrophysiology.

To build a case for how variants in Tbx5 could have divergent effects on sodium channel function, Nieto-Marín *et al.* used a combination of molecular and electrophysiological techniques to examine the effect of viral overexpression of wild type and two Tbx5 variants (p.F206L and p. D111Y) on $Na_V1.5$ function in three different model systems, induced pluripotent stem cell-derived cardiomyocytes, HL-1 cells, and mice. Both variants are located in highly conserved sites in the T-box domain that mediates sequence-specific DNA binding; however, the p. F206L variant was found in a patient with Brugada syndrome whilst the p. D111Y variant was found in a patient with LQTS.

The Brugada-associated variant p. F206L was still able to bind to the SCN5A minimal promoter yet was not able to drive SCN5A expression, as confirmed by a markedly reduced NaV1.5 current density in all three model systems. Furthermore, it caused QRS widening in the mouse model consistent with loss of $Na_V1.5$ function *in vivo*. In contrast, the long QT-associated variant p. D111Y, was able to drive expression of *SCN5A* as effectively as wild-type Tbx5. Transcription factors, like Tbx5, however, can regulate the expression of multiple genes. They therefore looked to see if the p. D111Y variant affected the expression of two of the proteins that form part of the $Na_V1.5$ macromolecular complex (see *Figure 1*). Wild-type Tbx5 represses expression of *CAMK2D* and *SPTBN4*. Conversely, overexpression of Tbx5 p. D111Y, did not suppress *CAMK2D* or *SPTBN4*. Beta-4-spectrin acts as a

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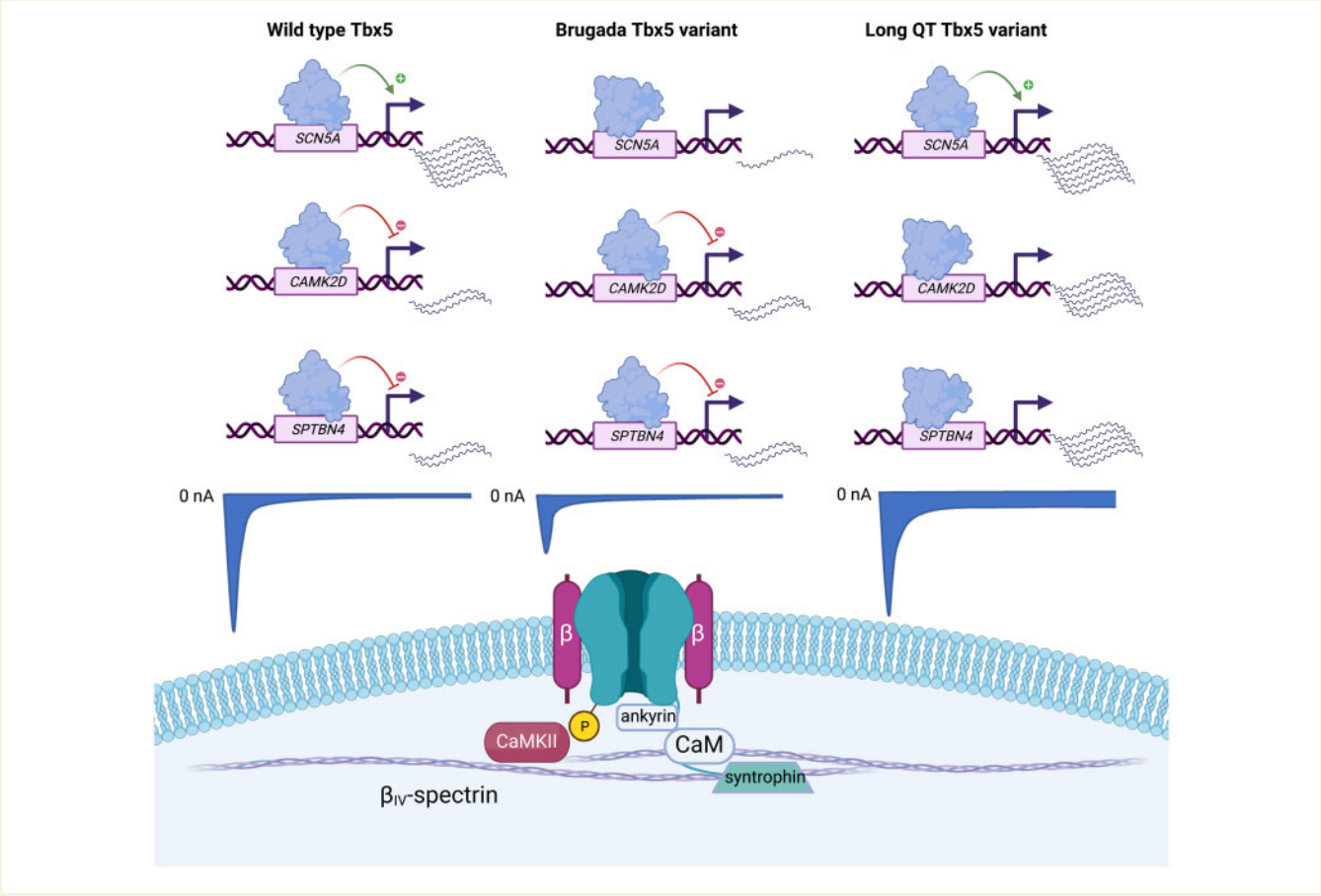

Figure 1 Multiple components of the Nav1.5 macromolecular complex are transcriptionally regulated by Tbx5. Wild type Tbx5 activates transcription of *SCN5a* but represses transcription at the *CAMK2D* and *SPTBN4* promoters. The Brugada syndrome-associated variant p. F206L is unable to drive transcription of *SCN5A* but shows normal repression at *CAMK2D* and *SPTBN4*. The p. D111Y variant associated with long QT syndrome abrogates repression of *CAMK2D* and *SPTBN4* but maintains normal activation of *SCN5A* transcription. The cardiac sodium channel alpha subunit, $Na_V1.5$ is part of a macromolecular complex (only a few of the many known components are shown). The structural protein β_{IV}-spectrin binds to and recruits Ca^{2+}/calmodulin kinase IIδ to the $Na_V1.5$ macromolecular complex. Phosphorylation of $Na_V1.5$ by CaMKIIδ augments late sodium current. *Created with BioRender.com*.

scaffold to anchor CaMKIIδ to $Na_V1.5$ where it can phosphorylate the channel and increase I_{NaL}.[4] The authors confirmed this in the Tbx5p.D111Y expressing cells by showing that augmentation of the late sodium current was inhibited by KN93 or knockdown by siRNA of CaMKIIδ.

It is well established that individual transcription factors influence the expression of multiple genes (up to thousands) both directly and via interactions with other transcription factors. Furthermore, different mutations in the same transcription factor can have a divergent range of effects on gene expression patterns—including both loss of expression as well as increased expression of other genes.[9] One limitation of the present study is that only a very small portion of the hundreds of genes regulated by Tbx5 were investigated. Thus, we cannot assume that the phenotypes observed in individuals with these Tbx5 variants are determined solely by the novel mechanisms revealed by Nieto-Marín *et al.* or whether they may be further modified by as yet unidentified consequences of the Tbx5 variants. It should also be pointed out that under current American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) guidelines for gene variant classification[10] Tbx5 p. D111Y would not be classified as a likely pathogenic variant as it has been found in a single LQTS family and has a relatively high population allele frequency (0.0033). Nevertheless, this does not detract from the insights provided by Nieto-Marín *et al.* into how a transcription factor can have pleiotropic effects on cardiac electrophysiology via altering the expression of different components of the $Na_V1.5$ macromolecular complex.

Conflict of interest: none declared.

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