Unexpected $\alpha$–$\alpha$ Interactions With Na$_v$1.5 Genetic Variants in Brugada Syndrome

Hugues Abriel, MD, PhD; Valentin Sottas, MSc

If you do not expect the unexpected, you will not find it, for it is not to be reached by search or trail

–Heraclitus of Ephesus, c.535-c.475 BC

In human genetics, autosomal dominant disorders are characterized by the fact that only 1 mutated copy of a given gene is sufficient to lead to a pathological phenotype. Sophisticated molecular mechanisms underlying dominant disorders have been described and among them, the case of negative dominance is an interesting phenomenon. Dominant-negative mutations have been defined by Herskowitz as mutations encoding mutant polypeptides that when overexpressed disrupt the activity of the wild-type (WT) gene. In its classical description, an inhibitory polypeptide (poison protein) negatively affects the function of a multimeric protein, thus leading to a dominant-negative effect that is in general, more severe than simple haploinsufficiency, where a copy of an allele is not expressed.

Several classes of membrane ion channels are formed by multimers of $\alpha$-subunits to constitute the pore protein and allow for the flux of ions across the membrane. The concept of negative dominance has been convincingly demonstrated with mutants of potassium channel subunits, formed by tetramers of $\alpha$-subunits, in cases of cardiac genetic channelopathies, such as congenital long-QT syndrome. Previous clinical studies have demonstrated that patients carrying missense mutations (which exert a dominant-negative effect) had more severe phenotypes when compared with ones carrying nonsense mutations.

The cardiac voltage-gated sodium channel Na$_v$1.5, encoded by the gene SCN5A, plays an important role in cardiac channelopathies because genetic variants in its gene were found to be linked, to date, to 9 distinct pathological phenotypes. Among the cardiac genetic disorders that are associated with SCN5A variants, Brugada syndrome (BrS) is one of the most prevalent. Briefly, BrS is characterized by malignant arrhythmias and sudden cardiac death and occurs predominantly in adult male individuals. A characteristic coved-type right-precordial ST-segment-elevation of the ECG is observed in patients with BrS. Three main aspects of the pathophysiology mechanisms underlying BrS are still the subject of intense investigations: (1) although rare SCN5A variants have been found in $\approx$20% of patients with BrS, the causality link between these variants and the phenotype has been disputed, a recent genome-wide analysis study demonstrated that $\geq$3 loci contribute to the genetic background of the syndrome, and (3) the origin of the ECG alterations and the arrhythmogenic mechanisms may be explained by $\geq$3 different working models that involve either depolarization or repolarization (or a combination of both) phases of cardiac electric activity. Many of the SCN5A genetic variants have been functionally characterized for the past years. Some of these involve either premature stop codon or frame shifts that cause a lack of expression of the mutant allele, demonstrating that SCN5A haploinsufficiency can cause BrS. However, about two thirds of the SCN5A variants are missense mutations for which the pathogenic mechanisms are different.

A few years ago, our group investigated the BrS SCN5A missense p.L325R mutation. To recapitulate the heterozygous state of the patients, we coexpressed the WT and the p.L325R variants in HEK293 (human embryonic kidney) cells and observed that the sodium current was disproportionately reduced after the expression of the mutant variant (Figure, negative dominance–classical mutants). This phenomenon was not observed with a BrS nonsense mutation. This finding was the first demonstration that a mutant Na$_v$1.5 protein may have a dominant-negative effect, raising the obvious question about the molecular mechanism underlying this unexpected observation because sodium channel multimerization had not been suspected. More recently, 2 other studies confirmed a dominant-negative phenomenon with other BrS mutations and further demonstrated that, in HEK293 cells, Na$_v$1.5 $\alpha$-subunits could be coimmunoprecipitated. These were the first convincing biochemical evidence for an interaction between $\alpha$ subunits of Na$_v$1.5. It is also interesting that a similar negative dominance phenomenon was observed with genetic variants in the gene coding for Ca$_v$2.1 (which has a similar structure to Na$_v$1.5) found in patients with episodic ataxia and epilepsy.

In this issue of Circulation: Cardiovascular Genetics, Hoshi et al. from the Deschênes group, present another unexpected twist. The initial motivation for their studies was the observation that several BrS SCN5A variants, for which the pathogenic potential was not demonstrated, presented only minor, if any, biophysical defects when studied in cellular expression systems. The authors named these variants atypical because classical BrS variants are either less expressed at the cell membrane (Figure) or showed loss-of-function biophysical alterations.
They observed that the atypical variants, when coexpressed with the WT channels, showed a surprisingly strong negative effect on the sodium current, ranging from 30% to 70% when compared with variants expressed alone. One variant, p.L567Q, was chosen to demonstrate that the results seen in HEK293 cells were not only a peculiarity of the expression system but also a phenomenon observed in neonatal ventricular myocytes and in human-induced pluripotent stem cell-derived cardiomyocytes. Hoshi et al.\textsuperscript{18} were also able to demonstrate with biotinylation experiments that the sodium current decrease, induced by the dominant-negative effect of p.L567Q channels on WT channels, was the result of reduced surface membrane protein expression of both WT and mutant channels (Figure). In addition, coimmunoprecipitation experiments were performed to show that the interaction between WT and atypical p.L567Q α-subunits was maintained. Finally, they performed functional experiments with MTSET ([2-(Trimethylammonium)ethyl] methanethiosulfonate) inhibitor on HEK293 cells, coexpressed with WT-p.C373Y (resistant to MTSET) and p.L567Q channels, to demonstrate that both channels were not only functional but also expressed at the cellular surface at similar levels (Figure).

These unexpected and intriguing findings by Hoshi et al.\textsuperscript{18} expand the range of possible molecular and pathological mechanisms underlying BrS and demonstrate the surprising fact that atypical, supposedly benign, mutant proteins may have a dominant-negative effect on WT channel proteins. Moreover, this work shows that the same level of mutated proteins and WT proteins are found at the membrane for the condition of negative dominance with atypical Na\textsubscript{v}1.5 variants. No other previous work had investigated the density of mutated and WT channels in the condition of negative dominance with classical Na\textsubscript{v}1.5 mutants. The mechanisms underlying this unusual dominant effect are not yet understood, as mentioned in the thoughtful discussion of Hoshi et al.\textsuperscript{18} Elucidation of these mechanisms is a clear objective for future studies because one can postulate that this knowledge will help us to understand the astonishing diversity in the pathological cardiac phenotypes linked to SCN5A variants.\textsuperscript{6}

In conclusion, we raise a few open questions related to these new findings: What is the stoichiometry of sodium channel α-subunit multimerization? What are the molecular determinants of these α–α interactions? Could other voltage-gated sodium channel isoforms expressed in cardiac cells also interact with Na\textsubscript{v}1.5 and induce a dominant-negative effect?\textsuperscript{19}

Finally, the question of the clinical relevance of these findings is also obvious. Meregalli et al.\textsuperscript{20} observed a few years ago that there is a positive correlation between BrS expressivity (in particular the occurrence of syncope) and the Na\textsubscript{v}1.5 variant-induced

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**Figure.** Hypothetical model of trafficking of Na\textsubscript{v}1.5 depending on the genetic variants. From left to right: under normal conditions, after the biosynthesis of wild-type (WT) Na\textsubscript{v}1.5 (blue) channels in the endoplasmic reticulum (ER), the interaction between Na\textsubscript{v}1.5 α-subunits may allow the complexes to be trafficked to the plasma membrane efficiently through the cis and trans-Golgi apparatus. Note that the stoichiometry of these complexes is not known. With atypical Na\textsubscript{v}1.5 mutant, the atypical mutant (green) channels use the same pathway to reach the plasma membrane. With classical Na\textsubscript{v}1.5 (red) mutants, the proteins, most likely because they are misfolded, are degraded by the ER-associated protein degradation (ERAD) pathway. Here is depicted an extreme condition in which all the mutant channels are degraded and none of them reaches the membrane. Under the condition of negative dominance, the complexes formed by WT channels with either classical or atypical mutants are degraded by the ERAD pathway. Only fewer WT channels and atypical mutant channels reach the plasma membrane.
reduction of sodium current. Could it be that dominant-negative variants in Na,1.5 with either typical or atypical mechanisms lead to varying degrees of severity of the BrS phenotypes?

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Disclosures
None.

References

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