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EDITORIAL

Unexpected dominance: Brugada syndrome *SCN5A* variants exert negative dominance via α-subunit interaction

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This editorial refers to 'Dominant-negative effect of *SCN5A* N-terminal mutations through the interaction of $Na_v1.5$ α-subunits' by J. Clatot *et al.*, pp. 53–63, this issue.

The field of cardiac channelopathies, i.e. dysfunction of ion channels caused by genetic defects, is one of the most fascinating areas of research for modern biomedical scientists. Indeed, these studies enable us to understand how a single genetic and molecular defect that can be recorded in one molecule, such as the bursting behaviour of one $Na_v1.5$ long QT syndrome (LQTS) mutant channel,[1] may lead to sudden cardiac death.[2] Since 1995, this field has benefited from a prolific, interdisciplinary community of clinicians, geneticists, biophysicists, and biologists,[3] as is the case in the study by Clatot *et al.*[4] published in this issue of *Cardiovascular Research*. Among the most striking examples is congenital LQTS, with hundreds of mutations found in a total of 13 genes. The LQTS genes *KCNQ1* and *KCNH2* code for the α-subunits of the K^+ ion channels that are responsible for the I_{Ks} and I_{Kr} repolarizing cardiac currents.[3] Genetic investigations have shown that most LQTS patients are carriers of a single mutated allele consistent with dominant transmission of the pathological trait.[5]

Early studies on LQTS missense mutations of *KCNQ1*[6] have demonstrated a dominant negative effect of the mutant α-subunits, which is consistent with the multimeric nature of voltage-gated K^+ channels. Epidemiological studies[7] subsequently demonstrated the worse outcome of patients carrying missense *KCNQ1* mutations, exerting a dominant negative effect, when compared with individuals having nonsense mutations that lead to haploinsufficiency.

In the case of mutations in the gene *SCN5A*, which codes for the pore-forming α-subunit of the voltage-gated cardiac Na^+ channel $Na_v1.5$, hundreds of genetic variants have been found in patients with a long list of cardiac manifestations.[8] Among them, the Brugada syndrome (BrS) is an interesting case. This syndrome was first described in 1992[9] in patients suffering from syncope and sudden cardiac death caused by ventricular arrhythmias with a peculiar elevation of the ST-segment in the right precordial leads of the electrocardiogram. Mutations in *SCN5A* were found in BrS patients in 1998,[10] and since then, the description of hundreds of *SCN5A* mutations has followed. Note that the question of the direct causality of these *SCN5A* mutants is still under debate.[11] Nevertheless, it is clear that a large number of the *SCN5A* variants found in BrS lead to loss-of-function of $Na_v1.5$ when expressed in cellular expression systems.[12] The mutations found in BrS are mostly missense, nonsense, and frame-shift mutations. Variants with missense mutations often show evidence of misfolding of the mutant proteins and altered trafficking with retention in the endoplasmic reticulum of expressing cells. Here, one should add a word of caution, since most of these findings were obtained using cellular expression systems and not native tissues.

In 2005, our group studied two *SCN5A* BrS mutations.[13] One was a nonsense mutation, p.R535X, and the other was a missense mutation, p.L325R. Both variants, expressed in HEK293 cells, displayed a marked decrease in Na^+ current. With the aim of mimicking the heterozygous state of the patients, we then co-expressed (using similar amounts of cDNA) the wild-type (WT) and the p.L325R variants in HEK293 cells and observed that the Na^+ current was disproportionately reduced (i.e. a larger reduction than if only the same amount of WT allele was expressed). This phenomenon was not observed with the nonsense p.R535X mutation. This was the first demonstration that a mutant $Na_v1.5$ protein may have a dominant-negative effect on the function of the WT subunit, and it raised the obvious question about the molecular mechanism underlying this surprising observation. Indeed, unlike K^+ channels which function as multimers, Na^+ channel multimerization had so far never been suspected, since the pore-forming α-subunit of $Na_v1.5$ is found in a single polypeptide. This notion was first put into question with the provocative results from the Deschênes group, suggesting that $Na_v1.5$ channels, encoded by different alleles, may biochemically and functionally interact.[14]

In this issue of *Cardiovascular Research*, the groups of Neyroud and Deschênes convincingly confirm these early observations and provide further molecular insights into the interaction between the α-subunits of the cardiac Na^+ channels.[4] First, they investigated two BrS mutations found in the N-terminus of $Na_v1.5$ (p.R104W and p.R121W)

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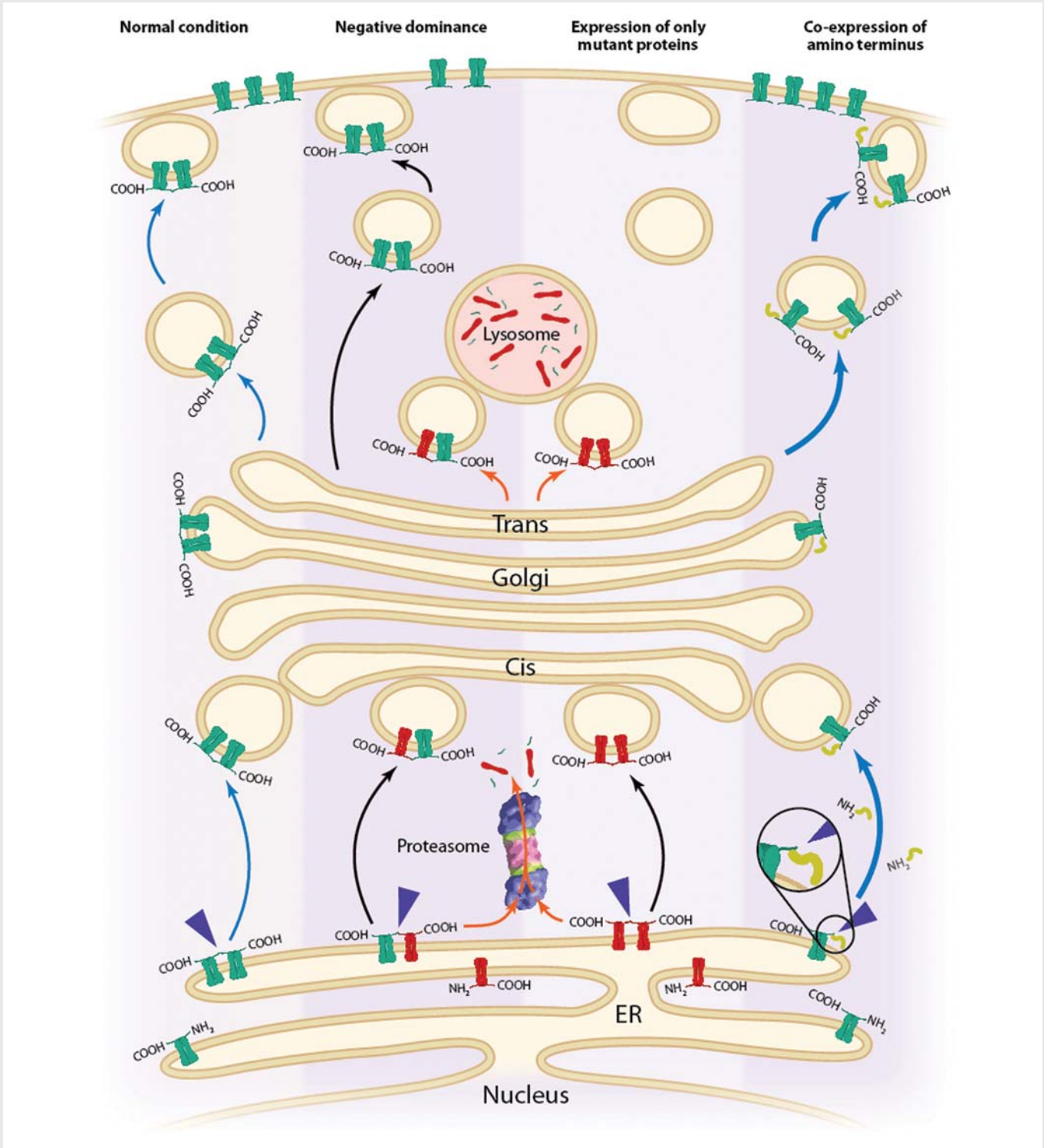

Figure 1 Proposed trafficking processes in HEK293 that depend upon transfection conditions; based on the findings of Clatot *et al.*[4] From left to right: under normal conditions, after the synthesis of the WT $Na_v1.5$ channel (green channel), single channels stay in the endoplasmic reticulum (ER). Interaction between two $Na_v1.5$ channel N-termini (depicted by the blue arrowheads) allows the complex to be targeted to the plasma membrane through the *cis*- and *trans*-Golgi apparatus. Under dominant-negative conditions, the complex of WT and mutant $Na_v1.5$ channels (red channel) is mainly retained in the ER. In contrast to WT channels, mutant channels can be degraded by the proteasomal and/or the lysosomal pathways (orange arrows). Only a few WT channels interacting together reach the plasma membrane compared with the situation under normal conditions. In the case of expression of mutant channels only, the complex, due to the interaction between two N-termini of mutant channels, is mainly degraded by the proteasomal and/or lysosomal pathways (orange arrows). Interaction of an N-terminal part overexpressed in HEK293 cells (yellow) of the WT $Na_v1.5$ channel with full-length WT channels increases the number of channels at the plasma membrane (thick blue arrows) in comparison with normal conditions. The mechanisms underlying this observation may rely on the masking of an ER retention motif in the N-terminus of the channel.

and clearly observed that the mutants, when co-expressed with the WT channel, had a dominant-negative effect on the Na^+ current (*Figure 1*). They then obtained evidence suggesting a crucial role of the N-terminus domain of $Na_v1.5$ in this phenomenon (by studying an N-terminally truncated WT channel) and also showed that overexpression of only the N-terminus of WT channel in HEK293 cells led to a doubling of the Na^+ current. Lastly, they were able to show that $Na_v1.5$ subunits could be co-immunoprecipitated, thus strongly suggesting an interaction in the cells that could be dependent on the N-termini (*Figure 1*).

The main conclusion of these recent studies is that, unexpectedly, a dominant-negative phenomenon can also be observed by co-expressing different cDNAs of $Na_v1.5$ in cellular expression systems. These important findings clearly need to be further investigated since, as is often the case, they raise more questions than we had before knowing them. What are the details of the cellular and biochemical mechanisms leading to the reduced expression of the WT $Na_v1.5$ channels at the cell surface? What is the nature of proposed interactions between the N-termini? Will future examinations reveal that BrS patients, who harbour such dominant-negative variants, tend to display more severe phenotypes than the ones with nonsense mutations leading to haploinsufficiency? And finally, will we be able to translate these findings into clinical management of BrS patients by refining risk stratification and devising new therapeutic strategies?

Lastly, one of the main lessons of these studies is that one should 'be prepared to expect the unexpected'!

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