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Alterations in ubiquitin-mediated degradation of $Na_V1.5$ can cause arrhythmia.

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The voltage-gated sodium (Na_V) channel is required for cardiomyocyte function. In heart, its major, pore-forming, α-subunit is $Na_V1.5$, encoded by *SCN5A*, which maps to chromosome 3p21. The Na_V channel plays a key role in myocardial excitability, since it is responsible for generating the rising phase of the cardiac action potential. $Na_V1.5$ often shows alterations in inherited channelopathies causing cardiac arrhythmias. Two well-known arrhythmias in which pathogenic variants of $Na_V1.5$ are implicated are Brugada syndrome (BrS) and Long QT syndrome (LQTS) type 3 (LQT3), which are due, respectively, to loss- and gain-of-function of the channel[1].

In this issue of *Acta Physiologica*, Wang *et al.*[2] address how arrhythmia-associated variants in *SCN5A* can affect $Na_V1.5$ turnover, providing a mechanistic explanation for how certain genetic alterations determine a phenotype, either for loss- or gain-of-function channelopathies. The authors show that a BrS-associated point mutation in $Na_V1.5$, L1239P, causes loss-of-function of the Na_V channel by creating a new PY motif which can be recognized by the WW domain of the E3 ubiquitin ligase Nedd4-2, thereby targeting $Na_V1.5$ to degradation. Conversely, a LQT3-

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associated point mutation disrupting the existing C-terminal PY motif, Y1977N, causes gain-of-function by preventing interaction with Nedd4-2, therefore restricting degradation of $Na_V1.5$ (Figure 1). Cell surface biotinylation, immunofluorescence and patch clamp were performed to demonstrate the effects of these pathogenic variants in transiently transfected HEK293 cells. In a clever way, these researchers also disrupted the newly generated PY motif of the BrS-associated variant, nicely restoring $Na_V1.5$ levels and function as a consequence of decreased ubiquitination by Nedd4-2 and degradation.

The data by Wang and colleagues agree with the view that the ubiquitin-proteasome system is fully responsible for degradation of the BrS-associated L1239P variant, in which a new Nedd4-2 binding site has been created, as well as of the WT itself, which bears the previously described PY motif[3]. Yet, it is often difficult to exclude that the lysosome may be playing a more direct role instead. Thus, inhibiting proteasomal activity could affect stability of components of the endocytic machinery, easily influencing the rate of $Na_V1.5$ endocytosis, its subsequent trafficking to the lysosome and degradation. Alternatively, components of the proteasome themselves can be regulated by the endocytic machinery.

Certainly connected with this mechanistic view, the same research group showed, in a recent article, that downregulation of $Na_V1.5$ in heart failure can be explained by increased intracellular Ca^{2+} and Nedd4-2 activation[4]. In that work, the authors found that elevated Ca^{2+} increases Nedd4-2 expression, probably at the level of transcription, and through an indirect mechanism. Higher Nedd4-2 levels would then lead to increased interaction with $Na_V1.5$, with its subsequent ubiquitination and degradation. Altogether, these data further confirm that $Na_V1.5$ turnover takes place by Nedd4-2-mediated ubiquitination.

However debatable the issue of proteasome- vs. lysosome-mediated degradation may be, for ion channels as for other integral plasma membrane proteins, reporting a novel mechanistic explanation for cardiac arrhythmias is worth to underline. Leaving aside alterations in the channel biophysical properties, this is the first report – to our knowledge – in which BrS can be explained by increased degradation of $Na_V1.5$ already present at the plasma membrane, as opposite to already described decreased transport to the cell surface; indeed, retention of mutants on their way to the plasma membrane, either in the endoplasmic reticulum or in the Golgi complex, likely takes them also to degradation[5].

Regarding LQT3, accounting for just 5 % of all types of genotyped LQTS cases[1], gain-of-function is normally caused by $Na_V1.5$ alterations that affect channel gating and lead to increased intracellular Na^+ concentration. Interestingly, though, mutations in proteins interacting with $Na_V1.5$ have also been found linked to LQT3. This is the case of α1-syntrophin, which is part of the dystrophin multiprotein complex[6]. This complex, connected to the actin cytoskeleton, is however not present in cardiomyocyte intercalated discs, where an important pool of $Na_V1.5$ is found, and instead regulates $Na_V1.5$ targeting to the lateral plasma membrane[7]. Another $Na_V1.5$-interacting protein implicated is caveolin 3, which is an important component of the cholesterol-enriched invaginations at the plasma membrane, known as caveolae, and shown to be enriched in ion channels. Moreover, mutations in α1-syntrophin and caveolin 3 appear to share a common mechanism by which neuronal nitric oxide synthase is upregulated, leading to increased $Na_V1.5$ S-nitrosylation and late sodium current[6].

Intriguingly, Wang *et al.* discuss that the altered residue that generates the new PY motif causative of BrS is within a transmembrane domain. Based on published data on other channel proteins recognized by the ubiquitin-proteasome system in a comparable way, they argue that the point mutation causes abnormal folding of the newly synthesized variant and, on top of that, exposes the new site for binding to Nedd4-2 (Figure 1). A possible way to envisage this scenario may be by considering the consequence of having a new proline ring in this location, which conformation would generate a rigid turn. While in principle facilitating folding, such unique cyclic structure placed here would potentially alter folding, thereby promoting the degradation rate of the BrS-associated variant. Moreover, as a result of misfolding, the presence of two PY motifs may turn into a synergistic, rather than additive, effect on degradation, as compared with the rate seen in the WT. In any case, it is clear that differences in sodium current are not due to altered gating, but rather to different amounts of the channel at the plasma membrane, in fact, as a result of reduced cellular $Na_V1.5$ levels.

In summary, understanding how pathogenic variants exert their effect at a molecular level is of utmost importance, and it has been done here in a remarkable way. No matter how simple the model used by Wang *et al.* may be, their study proposes that turnover of $Na_V1.5$ can be altered by inherited mutations. Such mutations can affect interaction of $Na_V1.5$ with the well-characterized E3 ubiquitin ligase, Nedd4-2, thereby influencing the patient's phenotype. To get further insights into the pathophysiology of Nedd4-2 action in regulating turnover of $Na_V1.5$, as well as of other

channels, a conditional, tissue-specific, *Nedd4-2* knockout model may perhaps be useful for future studies.

Conflict of interest

The authors declare no conflict of interest.

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Figure 1: Internalization and degradation of $Na_V1.5$ are affected by inherited pathogenic variants.

$Na_V1.5$ is synthesized in the endoplasmic reticulum (ER) and exported to the Golgi apparatus to reach the cell surface. Once at the plasma membrane, its C-terminal PY motif can be recognized by the WW domain of the HECT-type, E3 ubiquitin ligase Nedd4-2. Ubiquitination (Ub) of $Na_V1.5$ promotes its internalization and targeting to degradation, likely at the proteasome; the potential role of lysosomal degradation is also considered (question mark). In the BrS-associated L1239P variant, a new Nedd4-2 binding site has been created (in red), which favors its ubiquitination and degradation, turning into channel loss-of-function (thick arrows). On the contrary, in the LQT3-associated Y1977N variant, the usual PY motif is disrupted (in grey), thereby preventing interaction with Nedd4-2, which leads to more channels at the cell surface, and to the consequent gain-of-function (dotted arrow).