Making sense in a nonsense reading frame: suppression of cardiac sodium channel dysfunction

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This editorial refers to ‘Readthrough of nonsense mutation W822X in the SCN5A gene can effectively restore expression of cardiac Na⁺ channels’ by S. Teng et al., 12 pp. 473–480, this issue.

In the past few years, cardiologists, geneticists, physicians, and also patients have begun to realize the unexpected complexity underlying inherited forms of arrhythmias or cardiomyopathies. The number of disease-causing genes for congenital long-QT syndrome, hypertrophic cardiomyopathy, and other conditions has now reached the double-digit range (locus heterogeneity). As a rule of the thumb, most mutations are family-specific (‘private’) events and localize without any preference within the main regions of a gene (allelic heterogeneity). The identification of inherited cardiac ion channel dysfunction as the cause of familial arrhythmias stimulated many research teams all over the world to specifically target and modulate ion channels in order to restore function and reduce arrhythmogenesis.

For ion channel gene alterations that are not associated with a gain-of-function (where channel function can be reduced by drug-directed inhibition) but a loss-of-function, such a targeted approach is rather more complex and logically would encompass increasing expression of the wild-type gene or restoring intracellular trafficking deficiency of mutant ion channel proteins, e.g. by the use of chaperones. Indeed, loss-of-function mutations are the predominant features for cardiac conduction disorders such as Brugada syndrome, for long-QT syndromes of subtype 2 and probably also other subtypes. Importantly, there is increasing evidence that not only nonsense mutations (either by direct creation of stop codons or frame shifts) but also missense mutations are associated with a loss-of-function (so-called ‘hypomorphic’ mutations) due to impairment of intracellular protein trafficking and retention of mutant protein in endoplasmic reticulum.

This mechanism involving a reduction of the number of functional channel subunits is probably more common than originally thought and may influence future treatment strategies directed to recompense the molecular defect.

Teng et al. analyse the functional consequences of an SCN5A nonsense mutation (p. W822X) that leads—as expected—to a loss-of-function and hNa current reduction (HEK293 cells). The prematurely truncated protein (full-length: 2016 amino acids) not only lacks important functional domains of the cardiac sodium channel (Nav1.5), but was also not detectable with an N-terminal anti-Nav1.5 antibody. This latter observation suggested nonsense-mediated mRNA decay (NMD), a post-transcriptional translation-dependent surveillance mechanism that prevents the synthesis of proteins carrying premature truncations by selective degradation of such transcripts. In addition, a variety of physiological transcripts containing NMD-inducing features, such as those with upstream open-reading frame, transcripts containing introns in the 3′-untranslated region, and transcripts derived from alternative splicing, may also undergo NMD.

The potential of aminoglycosides to read-through disease-causing stop codons was first investigated more than a decade ago; Teng et al. followed these experimental observations, in which aminoglycoside antibiotics were shown to suppress nonsense mutations within the defective CFTR gene by disrupting translational fidelity. As a key finding from cystic fibrosis, aminoglycoside-treated cells now permitted translation beyond the premature termination codon and were able to synthesize increased levels of full-length CFTR protein. Subsequently, topical applications of gentamicin to the nasal epithelium of cystic fibrosis patients who were carriers of nonsense mutations restored CFTR function and increased membrane content of CFTR protein, stimulating first clinical trials in patients. Meanwhile, comparable attempts were being made for other inherited disorders. The efficiency to achieve a read-through and to diminish NMD still was variable and reflects a variety of additional influences on the way to enable full-length translation. Concordantly, Teng et al. were able to demonstrate gentamicin-induced increases of full-length Nav1.5 protein in the presence of the SCN5A nonsense mutation and, subsequently, to record significant increases (eight-fold or up to 30% of comparable wild-type level) of hNa in these cells. In another approach, application of siRNA directed against eFR3a, a cofactor...
controlling appropriate recognition of premature truncations, was shown to increase read-through capacity of SCN5A W822X. Taking into account current knowledge, together with a recent report of a KCNA5 E375X mutation in atrial fibrillation,19 these are the first experimental data in the field of cardiac ion channel genes.

Is it already time to draw any conclusions for further clinical trials or applications? Certainly not, although all of these concepts for making ‘sense in a nonsense frame’ are theoretically and mechanistically attractive to follow. So far, these in vitro pilot studies are quite helpful in pharmacologically addressing the functional consequences of stop codons and in understanding partners and factors—such as eFR3α—in intracellular NMD of prematurely truncated proteins. The variability of the gentamicin response—as seen in cystic fibrosis patients—might be related to different pharmacokinetics and -dynamics and variable interactions with the decoding centre of the rRNA during translation, which is believed to cause reduced accuracy of the codon–anticodon pairing.20 Moreover, delivery of read-through-enhancing agents clearly will have tissue-specific barriers and efficacies and, for the human heart, a spatially homogeneous and constant read-through would be preferable in order to avoid inhomogeneous electrical activity and impulse propagation. Finally, as the authors state, there remains a little taste of uncertainty due to unwarranted translational effects such as incorporated sequence errors beyond the previous stop codon in the cardiac sodium channel gene SCN5A. Finally, recorded Ifs currents obtained after heterologous expression of read-through sodium channel subunits in HEK293 cells showed no significant difference compared with wild-type currents, which suggests not only suppression of previous sodium channel loss-of-function but also restoration of normal channel function.

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References