Nonsense-mediated mRNA decay caused by a frameshift mutation in a large kindred of type 2 long QT syndrome

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BACKGROUND Nonsense and frameshift mutations are common in congenital long QT syndrome type 2 (LQT2). We previously demonstrated that hERG nonsense mutations cause degradation of mutant mRNA by nonsense-mediated mRNA decay (NMD) and are associated with mild clinical phenotypes. The impact of NMD on the expression of hERG frameshift mutations and their phenotypic severity is not clear.

OBJECTIVE The purpose of this study was to examine the role of NMD in the pathogenesis of a hERG frameshift mutation, P926AfsX14, identified in a large LQT2 kindred and characterize genotype–phenotype correlations.

METHODS Genetic screening was performed among family members. Phenotyping was performed by assessment of ECGs and LQTS-related cardiac events. The functional effect of P926AfsX14 was studied using hERG cDNA and minigene constructs expressed in HEK293 cells.

RESULTS Significant cardiac events occurred in carriers of the P926AfsX14 mutation. When expressed from cDNA, the P926AfsX14 mutant channel was only mildly defective. However, when expressed from a minigene, the P926AfsX14 mutation caused a significant reduction in mutant mRNA, protein, and hERG current. Inhibition of NMD by RNA interference knockdown of up-frameshift protein 1 partially restored expression of mutant mRNA and protein and led to a significant increase in hERG current in the mutant cells. These results suggest that NMD is involved in the pathogenic mechanism of the P926AfsX14 mutation.

CONCLUSION Our findings suggest that the hERG frameshift mutation P926AfsX14 primarily results in degradation of mutant mRNA by the NMD pathway rather than production of truncated proteins. When combined with environmental triggers and genetic modifiers, LQT2 frameshift mutations associated with NMD can manifest with a severe clinical phenotype.

KEYWORDS Long QT syndrome; Mutation; Nonsense-mediated mRNA decay; Patch clamp; Potassium channel

ABBREVIATIONS **hERG** = human ether-a-go-go-related gene; **HPH** = hygromycin B phosphotransferase; **I_{Kr}** = rapid component of delayed rectifier potassium current; **LQT2** = long QT syndrome type 2; **LQTS** = long QT syndrome; **NMD** = nonsense-mediated mRNA decay; **NOS1AP** = nitric oxide synthase 1 adaptor protein; **PTC** = premature termination codon; **shRNA** = short hairpin RNA; **SNP** = single nucleotide polymorphism; **UPF1** = up-frameshift protein 1; **WT** = wild type

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Introduction

The congenital long QT syndrome (LQTS) is a clinical disorder that affects close to 1:2,500 individuals in the general population.[1] Its dramatic impact stems from its association with ventricular tachyarrhythmias and subsequent syncope or sudden cardiac arrest in otherwise healthy individuals. Several genetic variants of LQTS have now been described, and, thus far, all are related to mutations in genes that encode cardiac ion channel subunits or proteins that modulate ionic currents.[2,3] Long QT syndrome type 2 (LQT2) is the second most prevalent variant, accounting for 35% to 40% of genotyped cases of LQTS.[2] It results from mutations in the human ether-a-go-go-related gene (hERG), also known as KCNH2.[4] This gene encodes the α-subunit of the channel that conducts the rapid component of the delayed rectifier potassium current (I_{Kr}) in the heart.[5–7]

hERG mutations can cause channel dysfunction by a variety of mechanisms, including production of defective I_{Kr} channels and defective protein trafficking.[8,9] Recently, we showed that the nonsense-mediated mRNA decay (NMD) pathway is an important mechanism by which hERG nonsense mutations can influence the phenotypic severity of LQT2.[10] NMD is a surveillance mechanism that selectively degrades defective mRNA transcripts that con-

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tain premature termination codons (PTCs) resulting from nonsense or frameshift mutations.[11,12] Notably, over 30% of the identified LQT2 mutations are nonsense or frameshift mutations that introduce PTCs. Although we previously demonstrated that NMD is a key player in determining the severity of disease in hERG nonsense mutations, the impact of NMD on the expression of hERG frameshift mutations and the phenotypic severity of LQT2 is not clear.[10]

In this study, we examined the role of NMD in the pathogenesis of a hERG frameshift mutation, P926AfsX14, which was identified in a large LQT2 kindred, and conducted genotype–phenotype correlation studies within the family. The P926AfsX14 mutation, which has also been referred to as G925fs/13, results from the insertion of guanine at nucleotide position 2775 of the hERG cDNA. The resulting alteration in the reading frame leads to the replacement of proline by an alanine residue in position 926 of the hERG protein and a PTC at position 14 in the shifted reading frame. A recent study by Nof et al[13] indicated that hERG channels expressed from a hERG cDNA construct containing this mutation were only mildly defective. However, coexpression of P926AfsX14 with a common hERG polymorphism, K897T, led to a significant loss of hERG current, much greater than what was seen with expression of K897T or P926AfsX14 alone. Therefore it was concluded that the K897T polymorphism can markedly accentuate the loss of function of mildly defective hERG channels, and this mechanism was used to explain the LQTS-mediated arrhythmias and sudden infant death observed in that study.[13] We, on the other hand, hypothesized that the P926AfsX14 mutation is subject to NMD, leading to degradation of the mutant mRNAs before they can produce partially functional hERG channels. This effect of NMD could, however, still lead to a severe clinical phenotype if it occurs in the presence of environmental triggers and genetic modifiers that increase the susceptibility to cardiac events.

Methods

Clinical data

The study was approved by the institutional review board and performed upon receipt of informed consent from subjects. The participants were blood-related members of a large family, many of whom had been previously identified as harboring the P926AfsX14 mutation.[14] Phenotyping was performed based on the history of LQTS-related cardiac events and assessment of QT intervals and T-wave morphology.[15] Genotyping for the P926AfsX14 mutation, hERG K897T polymorphism, and nitric oxide synthase 1 adaptor protein (NOS1AP) polymorphisms was conducted by sequencing of genomic DNAs collected from blood or buccal swab samples.

Plasmid constructs and transfection

A minigene composed of hERG cDNA exons 1 to 10 and hERG genomic DNA from intron 10 to the poly(A) site was constructed by replacing the hERG cDNA C-terminal fragment with an intron-containing hERG genomic DNA fragment obtained from a human BAC clone, which consisted of the entire hERG gene (RP11-166D23) (Figure 1). The minigene was subcloned into a modified pcDNA5 vector in which the BGH poly(A) signal was deleted. Thus, the native poly(A) signal of the hERG gene was used for the formation of the poly(A) tail of hERG mRNA. The P926AfsX14 mutation was introduced into the hERG cDNA and minigene structures by site-directed mutagenesis using the pAlter *in vitro* mutagenesis system (Promega, Madison, WI, USA) (Figure 1). Flp-In HEK293 cells (Invitrogen, Carlsbad, CA, USA) were stably transfected with pcDNA5 hERG cDNA or minigene constructs and selected with 100 μg/mL hygromycin B. Flp-In HEK293 cells contain the Flp recombination target sequence at a single genomic locus, allowing stable integration and expression of a single copy of the cDNA or minigene construct at a specific genomic location in all cell clones.

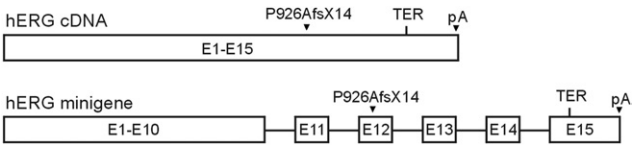

Figure 1 Diagram of the hERG cDNA and minigene structures. The positions of the wild-type termination codon (TER) and the P926AfsX14 mutation are indicated.

RNase protection assay

RNA isolation and the RNase protection assay were performed as previously described.[10] Briefly, antisense RNA riboprobes were transcribed *in vitro* in the presence of biotin-14-CTP. The probe designed to detect the mRNA levels of the hERG minigene contained 278 nucleotides spanning the region of exons 13 and 14. The total length of the probe was 408 nucleotides and contained sequences from the pCRII vector at both ends. The probe for the hygromycin B resistance gene contained 158 nucleotides of the gene and 70 nucleotides from the pGEM vector. Yeast RNA was used as a control for the complete digestion of the probes by RNase. The relative intensity of each band was quantified using the Scion Image software (Scion Corp., Frederick, MD, USA).

Immunoblot analysis

Immunoblot analysis was performed as previously described.[16,17] The cell lysates were subjected to SDS-polyacrylamide gel electrophoresis and then electrophoretically transferred onto nitrocellulose membranes. The membranes were probed with an anti-hERG antibody against the N-terminus of hERG protein (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The expression level of hygromycin B phosphotransferase (HPH) encoded by the hygromycin B resistance gene in pcDNA5 vector was used as a loading control.[18] A polyclonal anti-HPH antibody was used at a 1:1,000 dilution as previously described.[19] The intensity of the protein bands was quantified using the Scion Image software.