Nonsense Mutations in hERG Cause a Decrease in Mutant mRNA Transcripts by Nonsense-Mediated mRNA Decay in Human Long-QT Syndrome

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Background—Long-QT syndrome type 2 (LQT2) is caused by mutations in the human ether-a-go-go-related gene (hERG). More than 30% of the LQT2 mutations result in premature termination codons. Degradation of premature termination codon–containing mRNA transcripts by nonsense-mediated mRNA decay is increasingly recognized as a mechanism for reducing mRNA levels in a variety of human diseases. However, the role of nonsense-mediated mRNA decay in LQT2 mutations has not been explored.

Methods and Results—We examined the expression of hERG mRNA in lymphocytes from patients carrying the R1014X mutation using a technique of allele-specific transcript quantification. The R1014X mutation led to a reduced level of mutant mRNA compared with that of the wild-type allele. The decrease in mutant mRNA also was observed in the LQT2 nonsense mutations W1001X and R1014X using hERG minigenes expressed in HEK293 cells or neonatal rat ventricular myocytes. Treatment with the protein synthesis inhibitor cycloheximide or RNA interference–mediated knockdown of the Upf1 protein resulted in the restoration of mutant mRNA to levels comparable to that of the wild-type minigene, suggesting that hERG nonsense mutations are subject to nonsense-mediated mRNA decay.

Conclusions—These results indicate that LQT2 nonsense mutations cause a decrease in mutant mRNA levels by nonsense-mediated mRNA decay rather than production of truncated proteins. Our findings suggest that the degradation of hERG mutant mRNA by nonsense-mediated mRNA decay is an important mechanism in LQT2 patients with nonsense or frameshift mutations. (Circulation. 2007;116:17-24.)

Key Words: arrhythmia • ion channels • long-QT syndrome • myocytes

Long-QT syndrome is a disease associated with delayed cardiac repolarization and prolonged QT intervals on the ECG, which can lead to ventricular arrhythmias and sudden death.1 The inherited long-QT syndrome type 2 (LQT2) is caused by mutations in the human ether-a-go-go-related gene (hERG), which encodes the pore-forming subunit of the rapidly activating delayed rectifier K\(^+\) channel (I\(_{\text{Kr}}\)) in the heart.2,3 More than 250 hERG mutations have been identified in patients with LQT2.4–7 The mechanisms of hERG channel dysfunction in LQT2 mutations have been studied extensively in the last 10 years.8–11 Most previous studies, however, have focused on the analysis of mutant proteins and channel function. More than 30% of LQT2 mutations are nonsense or frameshift mutations that introduce premature termination codons (PTCs).4–7 These PTC mutations generally are assumed to result in truncated dysfunctional channel proteins, and several nonsense and frameshift mutations have been studied at the protein level.8,12–18 However, it is now becoming clear that nonsense and frameshift mutations bearing PTCs can destabilize mRNA transcripts via a mechanism known as nonsense-mediated mRNA decay (NMD) in many human diseases, resulting in decreased abundance of mutant mRNA transcripts rather than in production of truncated proteins.19,20

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NMD is an RNA surveillance mechanism that selectively degrades mRNA transcripts containing PTCs resulting from nonsense or frameshift mutations. The role of NMD as a disease-causing mechanism of PTC mutations is becoming increasingly evident.19,20 According to the proposed rule, NMD occurs when translation terminates >50 to 55 nt upstream of the 3’-most exon-exon junction.21,22 The molecular mechanisms of NMD have been studied extensively. These studies have shown that pre-mRNA splicing deposits the exon junction complex \(\sim 20\) to \(40\) nt upstream of the exon-exon junction in spliced mRNA. The exon junction

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complex can recruit Upf proteins, which are required for NMD. Several Upf proteins (Upf1, Upf2, Upf3a, Upf3b) have been identified. The Upf1 protein appears to play a key role in the distinction between proper and improper translation termination. Upf1 is a group 1 helicase that has RNA-dependent ATPase and ATP-dependent 5'-to-3' helicase activities. Knockdown of Upf1 by RNA interference (RNAi) has been shown to inhibit NMD. The objective of this work was to determine whether NMD occurs in hERG mutations that contain PTCs. We investigated 2 nonsense mutations, W1001X and R1014X, in the C-terminal region of the hERG channel. The W1001X and R1014X mutations have previously been studied at the protein level using hERG cDNAs. It was found that both mutations produced truncated hERG channel proteins and reduced hERG current amplitude. The R1014X mutation also caused a dominant-negative effect on the wild-type (WT) hERG current, which is expected to result in a severe clinical phenotype. However, the R1014X carriers have presented with a mild phenotype. In the present study, we demonstrate that rather than the production of truncated proteins, the primary defect of the W1001X and R1014X mutations is the degradation of mutant mRNA by NMD.

Methods

Subjects
The study was approved by the institutional review board and carried out on receipt of informed consent. The participants were blood-related members of a large family previously identified as having the R1014X mutation. Phenotyping was performed on the basis of the history of LQTS-related cardiac events, the assessment of QT intervals and T-wave morphology, and pedigree analysis. Genotyping was conducted by sequencing of DNA samples collected from buccal swabs. Normal control subjects were unrelated individuals.

RNA and DNA Preparations From Blood Samples
Total RNA was isolated from peripheral blood lymphocytes using the RiboPure-Blood kit (Ambion, Austin, Tex). The isolated RNA was treated with RNase-free DNase to remove genomic DNA. Genomic DNA was isolated from lymphocytes or Epstein Barr virus–transformed lymphoblastoid cells with the DNeasy tissue kit (Qiagen, Valencia, Calif).

Allele-Specific Quantification of RNA Transcripts and Genomic DNA
The relative abundance of RNA transcripts from WT and R1014X alleles was determined by a modified “hot-stop” polymerase chain reaction (PCR) method. In this assay, the regular reverse-transcription PCR was carried out using the primers in exon 13 (E13-F, forward 5'-GCCTTCCTCAGGAGGATGTC-3') and exon 14 (E14-R, reverse 5'-GAAAGCGAGTCCAAGGGT-3'). After 35 cycles, [32P]-dCTP was added and subjected to a single cycle of PCR. With hot-stop PCR, only homoduplexes incorporated [32P]-labeled and any heteroduplexes formed during previous cycles were unlabeled. Thus, hot-stop PCR will prevent the detection of WT/mutant heteroduplexes, which are resistant to restriction enzyme digestion. Because hot-stop PCR analysis yields a relative measure of transcripts from 2 alleles, normalization to a reference housekeeping gene is unnecessary. The hERG genomic DNA was analyzed by hot-stop PCR with the same forward primer as used in reverse-transcription PCR and a reverse primer in intron 13 (I13-R, 5'-CTCCGCCTTAGGATGTTG-3'). For analysis of allelic variation in hERG mRNA expression in normal subjects, the ratio of a common polymorphism 1692A/G was determined by hot-stop PCR using the primers in exon 6 (E6-F, forward 5'-ATCAACTTCCGCACCCCTA-3') and exon 7 (E7-R, reverse 5'-ATGTTGCTGCCTCATGT-3'). The labeled PCR products were treated with Taq I or Nhe I restriction enzyme and analyzed by 5% PAGE and autoradiography. For quantitative analysis, the intensity of each band was quantified with Scion Image software (Scion Corp, Frederick, Md). The ratio of 2 alleles was calculated, and a correction factor according to the respective GC content of each digested product was applied to the ratio.

Construction of Minigenes
Human genomic DNA was used as a template for PCR amplification of fragments spanning from hERG exons 12 to 15. The PCR products were cloned into pCRII vector with the TA cloning kit (Invitrogen, Carlsbad, Calif) and verified by DNA sequencing. The minigenes were then subcloned into a mammalian expression vector pcDNA5/FRT (Invitrogen). The N-terminus of the minigene was tagged by Myc epitope, which is in frame with the hERG translation sequence. The W1001X and R1014X mutations in the minigenes were introduced by site-directed mutagenesis (Stratagene, La Jolla, Calif).
were generated with the pAlter in vitro site-directed mutagenesis system (Promega, Madison, Wis) and verified by DNA sequencing.

Stable Expression of Minigene Constructs in HEK293 Cells

The minigenes in pcDNA5/FRT vector were stably transfected into HEK293 cells by using the Flp-In method (Invitrogen). In this approach, an FRT site sequence is integrated into the genome of HEK293 cells and recombined by Flp recombinase with the FRT site of the pcDNA5/FRT vector. The pcDNA5/FRT vector carries the hygromycin resistance gene, which is used for the selection of stable cell lines.

RNase Protection Assay of mRNA Transcripts From Minigene Transfected Cells

RNase isolation and RNase protection assay (RPA) were performed as previously described.29 Briefly, cytoplasmic RNA was isolated from HEK293 cells or neonatal rat ventricular myocytes expressing hERG minigenes with the RNase kit (Qiagen). The antisense RNA riboprobes were transcribed in vitro in the presence of biotin-16-UTP (Roche, Indianapolis, Ind). RNA (30 μg) was analyzed with the riboprobes using the RPAIII and BrightStart BioDetect kits (Ambion). Yeast RNA was used as control for the complete digestion of riboprobes by RNase. The expression level of the hygromycin resistance gene from the pcDNA5/FRT vector or the E2 gene from adenovirus was used as a loading control for normalization. The intensity of each band was quantified with Scion Image software.

RNA Interference

Two plasmids, pSUPERpuro-hUpf1/I and pSUPERpuro-hUpf1/II (kindly provided by Dr Oliver Mühlemann), were used to inhibit expression of Upf1 as described by Paillusson et al.29 These plasmids contain short hairpin RNAs targeting 2 sequences of hUpf1 (5'-GAGAATCGCCTACTTCCTACT-3' for pSUPERpuro-hUpf1/I and 5'-GATGCAGTTCCGCTCCATT-3' for pSUPERpuro-hUpf1/II). The HEK293 cells stably expressing WT or R1014X minigenes were transfected with a mixture of 1 μg pSUPERpuro-hUpf1/I and 1 μg pSUPERpuro-hUpf1/II or 2 μg pSUPERpuro with scrambled sequence of hUpf1/I using LipofectAmine 2000 (Invitrogen). At 24 hours after transfection, puromycin was added to the final concentration of 1.5 μg/mL for 48 hours to eliminate the untransfected cells. Before analysis, the cells were cultured without puromycin for at least 24 hours to avoid potential effects of this translation inhibitor on NMD. The knockdown of the Upf1 protein was analyzed by Western blot as described.29

Construction and Use of Recombinant Adenovirus

The AdEasy vector kit was used to generate WT and R1014X minigene recombinant adenoviruses (Stratagene, La Jolla, Calif). First, the WT and R1014X minigenes were subcloned into pShuttle-CMV vector and recombined with the pAdEasy plasmid in Escherichia coli strain BJ5183. The pAdEasy/minigene plasmids were transfected into HEK293 cells. After 2 days, the transfected cells were cultured in growth medium containing 1.25% Seaplaque agarose to promote the formation of recombinant viral plaques. Approximately 2 to 3 weeks later, individual plaques were picked, amplified in HEK293 cells, and purified over a discontinuous CsCl gradient.

Primary Culture of Neonatal Rat Ventricular Myocytes

Neonatal rat ventricular myocytes were prepared as described.30 Briefly, 1- to 3-day-old Sprague-Dawley rat pups were killed under ether anesthesia by decapitation, and hearts were removed through a sternotomy. The ventricles were trimmed free of atria, fat, and connective tissues. Myocytes were dissociated by several 20-minute cycles of collagenase/pancreatin treatment and serum neutralization. Myocytes were cultured in Dulbecco’s modified Eagle’s medium with 17% Media 199, 10% horse serum, 5% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). After 1 day in culture, myocytes were infected with the recombinant adenoviruses.

Statistical Analysis

Data are presented as mean±SD for QTc intervals or mean±SEM for PCR and RPA analyses. Statistical comparison of QTc intervals between R1014X mutation carriers and noncarriers was performed with a family-based analysis approach using the software package PedGenie, a Monte Carlo simulation–based program.31 ANOVA with Bonferroni correction for multiple pairwise comparisons between mutation/treatment groups was used for statistical analysis of RPA data. Values of P<0.05 were considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Patient Description

A total of 22 family members were tested for the presence of the R1014X mutation. Nine family members were identified as R1014X mutation carriers. Nine family members were identified as R1014X mutation carriers (Figure 1). The ECG data were available for 7 of the mutation carriers, all of which showed a prolonged QTc interval and typical LQT2 ECG pattern with the subtle bifid T waves. The mean initial QTc interval in the mutation carriers was 461±7 ms (n=7) versus 420±13 ms (n=8) in noncarriers (P<0.001). Four mutation carriers had exercise tests, with maximum QTc value of 510±10 ms. In this family, 89% (8 of 9) of the R1014X mutation carriers were asymptomatic. The only person with a history of cardiac events is the 71-year-old proband. From 32 to 42 years of age, she had multiple syncopal episodes and 1 cardiac arrest that were associated with the presence of hypokalemia (serum K+, 2.7 mEq/L) caused by taking a dietary supplement containing potassium-wasting diuretics or taking QT-prolonging antiarrhythmics. Since then, she has remained asymptomatic by stopping the potassium-wasting diet, avoiding QT-prolonging drugs, and taking β-blockers.

Analysis of mRNA Isolated From Blood Samples

The R1014X mutation causes premature termination of the hERG channel protein. This mutation has previously been studied at the protein level.15 However, it has been known that nonsense and frameshift mutations that contain PTCs can lead to the degradation of mRNA transcripts by NMD in many diseases.19,20 To determine the underlying pathogenic mechanism of the R1014X mutation, it is important to study this mutation at the mRNA level. Because the affected heart tissue from the mutation carriers was not available for this study, we analyzed hERG mRNA transcripts isolated from the lymphocytes of patients carrying the R1014X mutation. To distinguish between WT and R1014X alleles, we performed allele-specific quantification analysis using the hot-stop PCR assay. The WT allele contains a TaqI restriction site, which is destroyed by the R1014X mutation. After reverse transcription of mRNA, cDNA was amplified by hot-stop PCR. After digestion of the PCR products with TaqI, the WT allele should yield 2 fragments of 287 and 72 bp, and the R1014X allele should give a fragment of 359 bp. As shown in Figure 2A, cDNA from a normal subject showed a
Figure 2. Hot-stop PCR analysis of mRNA and genomic DNA isolated from lymphocytes. A. Analysis of WT and R1014X mutant alleles in a normal subject (NS) and the proband. Schematic diagrams are shown for hot-stop PCR analysis of cDNA (left) and genomic DNA (right). In cDNA analysis, a forward primer in exon 13 (E13-F) and a reverse primer in exon 14 (E14-R) were used, and in genomic DNA analysis, the same forward primer and a reverse primer in intron 13 (I13-R) were used. The position of TaqI and the size of the fragments from WT and mutant PCR products are shown. After digestion with TaqI, the 32P-labeled hot-stop PCR products were analyzed by PAGE and autoradiography. In cDNA analysis (left), the bands from WT and R1014X alleles are 287 and 359 bp, respectively; in genomic DNA analysis (right), the bands from WT and R1014X alleles are 275 and 347 bp, respectively (the 72-bp band ran off the gel). Similar results were obtained in 2 additional R1014X carriers, and 3 to 5 independent experiments were performed for each patient. B. Analysis of allelic variation of hERG expression by analyzing 1692A/G polymorphism in 5 normal subjects. A schematic diagram is shown for the position of NheI and the size of the fragments from A and G alleles. The 32P-labeled hot-stop PCR products were digested with NheI. The 286- and 332-bp bands represent 1692A and 1692G alleles, respectively (the 46-bp band ran off the gel). Results shown are representative of 2 independent experiments.

The allele-specific quantification analysis depends on the assumption that there is no significant allelic variation in hERG mRNA expression. To rule out possible allelic variation in hERG expression in the general population, we examined the allele-specific expression of hERG mRNA in normal subjects. We analyzed 3 normal subjects who are heterozygous for a common polymorphism, 1692A/G. To distinguish between 1692A and 1692G alleles, the relative levels of mRNA transcripts from 1692A and 1692G alleles were measured by the hot-stop PCR assay. The 1692A allele contains an NheI restriction site, which is absent in the 1692G allele. Thus, digestion with NheI should allow us to determine the relative ratio of the 2 WT alleles. After digestion of the PCR products with NheI, the 1692A allele should be cut into 2 fragments of 286 and 46 bp, and the 1692G alleles should remain uncut (322 bp). As shown in Figure 2B, in subjects 3, 4, and 5 (lanes 3 to 5), there are 2 bands of 268 and 332 bp, suggesting that they are heterozygous for the 1692A/G polymorphism. In these 3 normal subjects, the average ratio of 1692G to 1692A was 0.97 ± 0.07. This result suggests that there is no significant allelic variation in hERG mRNA expression in normal subjects. Subjects 1 and 2 (lanes 1 and 2) are homozygous for 1692A and 1692G, respectively.

Minigene Analysis of the R1014X and W1001X Mutations

To study whether the decrease in the abundance of mRNA levels in the R1014X mutation is due to NMD, we constructed minigenes containing the hERG genomic sequence spanning from exon 12 to 15 and expressed the minigenes in HEK293 cells. In the minigene experiments, the 2 LQT2 nonsense mutations R1014X and W1001X were analyzed by RPA. Figure 3A shows the structure of the minigene and the mRNAs after splicing. The R1014X and W1001X mutations lead to a PTC in exon 13, which is expected to trigger NMD. As shown in Figure 3B, the mRNA level of the R1014X minigene was significantly lower than that of the WT minigene. Because degradation of mRNA by NMD depends on protein synthesis, we examined whether inhibition of protein synthesis by cycloheximide (CHX) abrogates NMD of the mutant mRNA, as has been shown for other PTC-containing transcripts. The cells expressing WT and R1014X minigenes were treated with CHX for 3 hours before RNA isolation. Treatment with CHX had no effect on the level of WT mRNA but significantly increased the level of R1014X minigenes, indicating that the mutant mRNA is degraded by NMD. Similar results were observed in the W1001X minigene (Figure 3C), suggesting that the degradation of PTC-containing mRNAs by NMD may represent a common mechanism in LQT2 patients with nonsense mutations.

Effect of Suppression of Upf1 on NMD of the R1014X Mutation

Recently, the Upf1 protein has been identified as a key factor for NMD. Reducing Upf1 expression by RNAi has been used as a functional assay to assess the NMD sensitivity of PTC-containing mRNA transcripts. To study the role of Upf1 in the reduced mRNA level of the R1014X mutation, we used the RNAi method to knock down Upf1 protein expression. In these experiments, HEK293 cells stably ex-
pressing the WT and R1014X minigenes were transfected with pSUPERpuro-hUpf1/I and pSUPERpuro-hUpf1/II. The Upf1 knockdown in the transfected cells was confirmed by Western blot analysis using anti-Upf1 antibody (a gift from Dr. Jens Lykke-Andersen) (Figure 4A). Detection of tubulin with anti-tubulin antibody served as a loading control. In the RPA analysis of hERG minigene mRNA transcripts, the level of R1014X mutant mRNA was significantly increased in Upf1-siRNA–transfected cells (Figure 4B). These results suggest that mRNA transcripts of the R1014X mutation undergo NMD.

Analysis of NMD in Neonatal Rat Myocytes Using R1014X Adenovirus Minigene

The above experiments indicate that mRNA transcripts of the R1014X mutation are subject to NMD in lymphocytes and HEK293 cells. The noncardiac cells may behave differently from cardiac cells in the degradation of mutant mRNA by NMD. Therefore, it is important to evaluate whether the defects observed in noncardiac systems are present in cardiac myocytes. To test whether NMD of the R1014X mutation occurs in cardiac myocytes, we infected neonatal rat ventricular myocytes with WT or R1014X minigene adenovirus and performed RPA analysis. As shown in Figure 5, the mRNA level of the R1014X mutant was significantly lower than that of WT. Treatment with CHX had no effect on the level of WT mRNA but significantly increased the level of R1014X mutant mRNA, suggesting that the R1014X mutant mRNA is degraded by NMD in cardiac myocytes. No protected bands in the control lane indicate that the riboprobe is specific for exogenous hERG transcripts.

Discussion

The present results demonstrate that the W1001X and R1014X mutations lead to a reduction of mutant mRNA transcripts by NMD. Our findings provide the first evidence that PTC-containing mRNA transcripts in LQT2 are subject to NMD. NMD is an evolutionarily conserved mRNA surveillance pathway that detects and eliminates PTC-containing mRNA transcripts, thereby preventing the synthesis of truncating proteins.

Figure 3. Analysis of the R1014X and W1001X mutations using minigene constructs. A, The structure of the Myc-tagged minigene and spliced mRNAs. The positions of WT termination codon (TER) and mutation-induced PTCs are indicated. B, C, Analysis of mRNA by RPA. HEK293 cells were stably transfected with WT, R1014X (B), or W1001X (C) minigenes, and the expressed mRNA was analyzed by RPA. Cells expressing WT and mutant minigenes were treated (+) or not treated (−) with 100 μg/ml CHX for 3 hours before RNA isolation. The level of hygromycin resistance gene transcripts (Hygro) served as a loading control. The quantitative data after normalization using protected hygromycin resistance gene mRNA are plotted as percentage of WT control from 4 (B) or 3 (C) independent experiments. Probability values are Bonferroni corrected.

Figure 4. Effect of suppression of Upf1 by RNAi on NMD of the R1014X mutation. HEK293 cells stably expressing the WT and R1014X minigenes were transfected with pSUPERpuro-hUpf1/I and pSUPERpuro-hUpf1/II (Upf1) or pSUPERpuro-scrambled (CON) constructs. A, Western blot analysis of Upf1 protein. B, Analysis of mRNA by RPA. The quantitative data after normalization using protected hygromycin-resistant gene mRNA are plotted as percentage of WT control from 4 independent experiments. Probability values are Bonferroni corrected.
translated and potentially harmful proteins. NMD occurs when translation terminates 50 to 55 nt upstream of the 3′-most exon-exon junction. According to this rule, >90 LQT2 nonsense and frameshift mutations are potential targets for NMD. Several LQT2 nonsense and frameshift mutations have been studied at the functional and protein levels with cDNA constructs. All previous studies, however, have been carried out under the assumption that these nonsense and frameshift mutations lead to the production of truncated proteins. Because NMD requires introns, the absence of introns in cDNA constructs would preclude the degradation of PTC-containing transcripts by NMD. As a result, NMD effects could not be observed when cDNAs were used in these studies. In the present study, we used minigene constructs that contain the hERG genomic DNA with both exons and introns and showed that the W1001X and R1014X mutations cause a marked decrease in mutant mRNA transcripts. Inhibition of protein synthesis by CHX or knockdown of Upf1 by RNAi results in the restoration of mutant mRNA to levels comparable to the WT minigene. These results strongly suggest that the degradation of mutant mRNA by NMD is an important mechanism in LQT2 mutations carrying PTCs.

Previous studies have shown that different LQT2 mutations cause hERG channel dysfunction by different mechanisms. This led to a proposed classification of LQT2 mutations according to their underlying mechanisms. The classification scheme (shown in Figure 6) illustrates the mechanisms underlying LQT2 mutations. Class 1 mutations cause abnormal protein synthesis by defective transcription or translation. Class 2 mutations lead to defective protein trafficking. Class 3 mutations result in abnormal gating and/or kinetics, and class 4 mutations result in altered or absent channel selectivity or permeability. In the present study, we show that LQT2 nonsense mutations cause a decrease in mutant mRNAs by NMD, thereby altering the amount of mRNA available for subsequent hERG protein generation. We propose that the degradation of PTC-containing mRNA transcripts by NMD represents a new class of LQT2 pathogenic mechanism (class 5).

The mutations that undergo NMD will result in the degradation of mutant mRNAs before they produce large quantities of truncated proteins. By eliminating abnormal mRNA transcripts carrying PTCs, NMD prevents the production of truncated proteins that could act in a dominant-negative manner, leading to deleterious effects on the cells. One of the physiological roles of NMD is to protect against severe disease phenotypes by converting the dominant-negative effect to haploinsufficiency. NMD as a modifier of phenotypic severity has been reported in many human diseases. For example, in Marfan syndrome, an autosomal-dominant connective tissue disorder caused by mutations in the fibrillin 1 gene, nonsense mutations that result in reduced levels of mutant mRNA are associated with a mild phenotype. In contrast, patients with nonsense alleles that escape NMD develop a severe phenotype as a result of the dominant-negative effect.

Most R1014X mutation carriers in this family have presented with a mild LQT2 phenotype. In contrast to patients with pore-region mutations, who usually present with a longer QT interval and more frequent cardiac events, the QTc interval in the R1014X mutation carriers is only mildly prolonged (461 ± 7 ms), and only the proband experienced arrhythmia-related cardiac events that were always associated with hypokalemia or the use of QT-prolonging drugs. We have previously shown that the R1014X mutation causes hERG channel dysfunction by defective trafficking of the mutant protein. In addition, the truncated mutant protein exhibits a dominant-negative effect on the WT hERG. This implies that a severe phenotype would be expected in the R1014X mutation carriers. However, our present study reveals that the R1014X mutant mRNA transcripts are markedly decreased by NMD, and as a result, the dominant-negative effect caused by the production of truncated proteins would be minimized. Therefore, haploinsufficiency rather than a dominant-negative effect is probably the underlying mechanism for the R1014X mutation, which is consistent with pore-region mutations.
with the observed clinical presentation of this family. It is interesting to note that the W1001X mutation carriers also present with a mild LQT2 phenotype. Moss et al reported that LQT2 patients with mutations in the pore region of hERG have a significantly higher risk of arrhythmia-related cardiac events than patients with nonpore mutations. Although the difference may be explained by in vitro electrophysiological effects of reported hERG mutations, with pore mutations having a greater negative effect on hERG current than nonpore mutations, it also is possible that NMD may play a role. It is noted that only 6% of LQT2 mutations in the pore region are nonsense or frameshift mutations, whereas >40% of the mutations in nonpore regions are nonsense or frameshift mutations. Clearly, further genotype-phenotype correlation studies are required to test whether NMD contributes to the observed differences in clinical presentations of pore and nonpore LQT2 mutations.

There are potential limitations to the present study. Our present experiments analyzed endogenously expressed mRNA from patients carrying the R1014X mutation, but the RNA was isolated from lymphocytes rather than the affected heart tissue. Although we have shown that the R1014X mutant minigene expressed in neonatal rat ventricular myocytes leads to reduced mRNA levels by NMD, further studies are required to determine whether the endogenous PTC-containing mRNA in human heart tissue is subject to NMD. Verification of our findings in human heart would strengthen the conclusion that hERG mutations that contain PTCs can lead to degradation of the mutant mRNA by NMD.

In summary, our findings that nonsense mutations in hERG lead to a reduced level of mutant mRNA by NMD add to our understanding of the disease-causing mechanisms of hERG mutations in LQT2. Thus, in studies of hERG nonsense and frameshift mutations, it is important to first analyze the abundance of mRNA to determine whether these PTC mutations are targeted by NMD. Obviously, this important point had been overlooked in previous studies that analyzed hERG PTC mutations only at the protein and functional levels. Because PTC mutations account for >30% of LQT2 mutations, the RNA surveillance imposed by NMD is of fundamental importance in the pathogenesis of LQT2.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Congenital long-QT syndrome type 2 (LQT2) is caused by mutations in human ether-a-go-go related gene (*hERG*), which encodes a voltage-gated potassium channel (*I_{Kr}* ) in the heart. The present work demonstrates that LQT2 nonsense mutations show a decrease in mutant mRNA transcripts via nonsense-mediated mRNA decay (NMD), an RNA surveillance mechanism that selectively eliminates the mRNA transcripts that contain premature termination codons. These results indicate that, contrary to intuition, the predominant consequence of *hERG* nonsense mutations is not the production of truncated proteins but rather the degradation of mutant mRNA by NMD. Given that nonsense and frameshift mutations account for >30% of LQT2 mutations, the RNA surveillance imposed by NMD is of fundamental importance in the pathogenesis of LQT2. Our findings have important implications for genotype-phenotype correlation investigations in LQT2. By eliminating abnormal mRNA transcripts carrying premature termination codons, NMD prevents the production of truncated proteins that could act in a dominant-negative manner. The clinical significance of NMD is the protection against severe disease phenotypes by converting the dominant-negative effect to haploinsufficiency. Thus, NMD appears to be an important factor in modifying phenotypic severity in LQT2.
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