

# Single Nucleotide Deletion Mutation of *KCNH2* Gene is Responsible for LQT Syndrome in a 3-Generation Korean Family

Jong Keun Park,<sup>1\*</sup> Yong-Seog Oh,<sup>2\*</sup>  
Jee-hyun Choi,<sup>1</sup> and Sungjoo Kim Yoon<sup>1</sup>

<sup>1</sup>Department of Biomedical Sciences, <sup>2</sup>Division of Cardiovascular Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea

\*Jong Keun Park and Yong-Seog Oh contributed equally to this work.

Received: 9 November 2012  
Accepted: 22 April 2013

Address for Correspondence:

Sungjoo Kim Yoon, PhD  
Department of Biomedical Sciences, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 137-701, Korea  
Tel: +82.2-2258-7474, Fax: +82.2-594-2385  
E-mail: sjkyoon@catholic.ac.kr

This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea. (A000385).

## INTRODUCTION

Arrhythmic disorders are responsible for 2,000-3,000 deaths out of 55,000-60,000 cardiovascular disorder related death per year during last decade in Korea. One of the arrhythmic disorders, long QT syndrome (LQTS), is an inherited disorder characterized by the prolongation of the QT interval in the electrocardiogram (ECG), syncope due to ventricular tachyarrhythmia, and sudden cardiac death (1). The normal QT interval is < 430 ms in male and < 450 ms in female. Thus, the longer duration than these intervals (> 450 ms for male and > 470 ms for female) were considered disease status. Prolongation of QT interval is caused by decrease of repolarizing power during cardiac action potential, which can be resulted from either decrease in repolarizing current (late input of potassium ion) or increase in membrane current (sodium/calcium channel) during action potential plateau.

The most common causes of LQTS are mutations in the genes *KCNQ1* (LQT1), *HERG/KCNH2* (LQT2), and *SCN5A* (LQT3), which form potassium channels (*KCNQ1* and *KCNH2*) and sodium channel (*SCN5A*). Mutations in the potassium channel genes, *KCNQ1* and *KCNH2* account for 42%-45% and 35%-45% of all LQTS with genetic mutations of congenital LQTS families, respectively (2). Mutations in *SCN5A* gene are identified in 2%-8% of genetic LQTS. Mutations are also found rarely in *KCNE1*

(LQT5), *KCNE2* (LQT6), *KCNJ2* (LQT7), and *CACNA1c* (LQT8). In addition to these genes which encodes for subunits of the cardiac ion channels thus functions to flow cardiac ion currents,  $I_{Ks}$ ,  $I_{Kr}$ , and  $I_{Na}$ , non-channel associated genes, ankyrin B, caveolin 3, A-kinase anchor protein 9 and syntrophin, alpha 1, were also associated with LQTS (3, 4).

Long QT syndrome (LQTS) is characterized by the prolongation of the QT interval in ECG and manifests predisposition to life threatening arrhythmia which often leads to sudden cardiac death. We encountered a 3-generation family with 5 affected family members in which LQTS was inherited in autosomal dominant manner. The LQTS is considered an ion channel disorder in which the type and location of the genetic mutation determines to a large extent the expression of the clinical syndrome. Upon screening of the genomic sequences of cardiac potassium ion channel genes, we found a single nucleotide C deletion mutation in the exon 3 of *KCNH2* gene that co-segregates with the LQTS in this family. This mutation presumably resulted in a frameshift mutation, P151fs+15X. This study added a new genetic cause to the pool of mutations that lead to defected potassium ion channels in the heart.

**Key Words:** Arrhythmia, Cardiac; Long QT Syndrome; Frameshift Mutation

(LQT5), *KCNE2* (LQT6), *KCNJ2* (LQT7), and *CACNA1c* (LQT8). In addition to these genes which encodes for subunits of the cardiac ion channels thus functions to flow cardiac ion currents,  $I_{Ks}$ ,  $I_{Kr}$ , and  $I_{Na}$ , non-channel associated genes, ankyrin B, caveolin 3, A-kinase anchor protein 9 and syntrophin, alpha 1, were also associated with LQTS (3, 4).

LQT1 (*KCNQ1*) gene is located in the chromosome 11p15.5 and encodes a voltage gated potassium channel KvLQT1 which coassembles with minK beta subunit (*KCNE1*) to form the  $I_{Ks}$  ion channel. This channel is responsible for the slow delayed rectifier potassium channel. LQT2 is caused by mutations of the potassium voltage-gated channel, subfamily H (eag-related), member 2 (*KCNH2/HERG*) on chromosome 7. *KCNH2* codes for a protein known as Kv11.1 potassium ion channel and homotetramer of *KCNH2* coassembles with Mink-related protein1 (*MiRP1/KCNE2*) to form  $I_{Kr}$  and conducts potassium ions out of the cardiac myocytes. Mutations of the alpha subunit of sodium channel gene, *SCN5A*, cause LQT3. This gene is located at chromosome 3p21-24.

LQTS is inherited mainly in dominant fashion with rare cases of recessive inheritance seen in Jervell Lange-Nielsen syndrome (5) Much progress in congenital long QT syndrome has been made with availability of the molecular diagnostic tools and better understanding of the relationship between genotype and phenotype. In this study we analyzed the *KCNQ1*, *KCNH2*, and

*KCNE1* gene of a Korean family with LQT syndrome and discovered a genetic mutation co-segregating with the disorder.

## CASE DESCRIPTION

### Clinical characterization

The proband who was admitted at the St. Mary's Hospital on May 29th, 2001 was a 39-yr-old female patient referred for the evaluation of intermittent palpitation and dizziness. Clinical evaluation including QT interval assessment and T-wave morphology through ECG, 24 hr Holter monitoring, treadmill exercise test, and coronary angiography was performed. Pedigree analysis was also carried out. The QT intervals were measured by Ashman method and corrected for heart rate (QTc) (6). All the family members were also evaluated by ECG.

The patient complained severe dizziness and syncope with palpitation when she was under the stress or emotionally upset. Twenty-four hour Holter monitoring showed a few premature atrial or ventricular complexes. During treadmill exercise test, horizontal ST depressions were appeared on leads II, III, aVF, V<sub>4</sub>-V<sub>5</sub> with chest pain. While we are taking coronary angiography, the patient complained palpitation, dizziness, and loss of consciousness for a few seconds. Then the ECG showed typical 'Torsades de Pointes' with T wave alternans (Fig. 1). She was diagnosed as long QT syndrome and prescribed propranolol 40 mg bid. After medication, the symptom improved and she was discharged with good condition.

Fig. 2A shows the pedigree of this LQTS family. Five family

members (II2, II2, II4, II7, and III1) were diagnosed with LQTS using the diagnostic scoring system (7). The rest 4 members showed normal ECG patterns (II1, II6, II8, and III2) with normal QTc intervals (< 450 ms) and the representative ECG is shown (Fig. 2B). With exception of subject II4, all affected members showed prolonged QTc intervals ranged from 454 to 527 ms and the proband's ECG is shown in Fig. 2B. Subject II4 was diagnos-

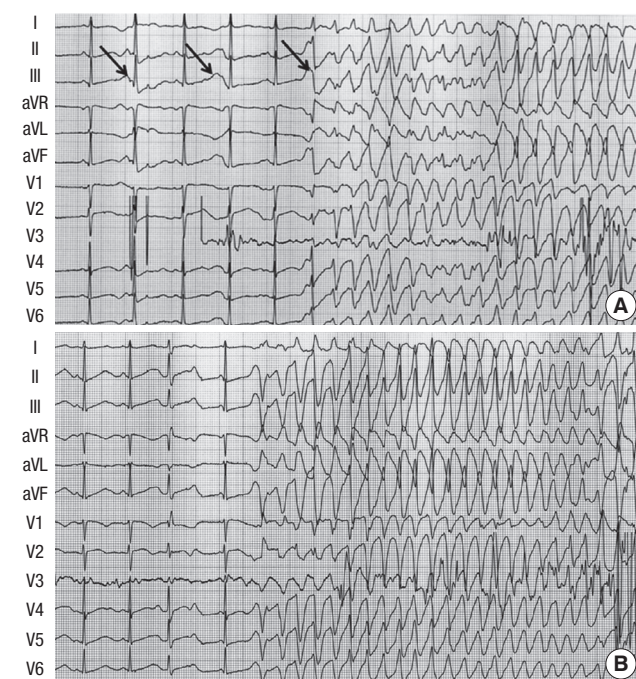
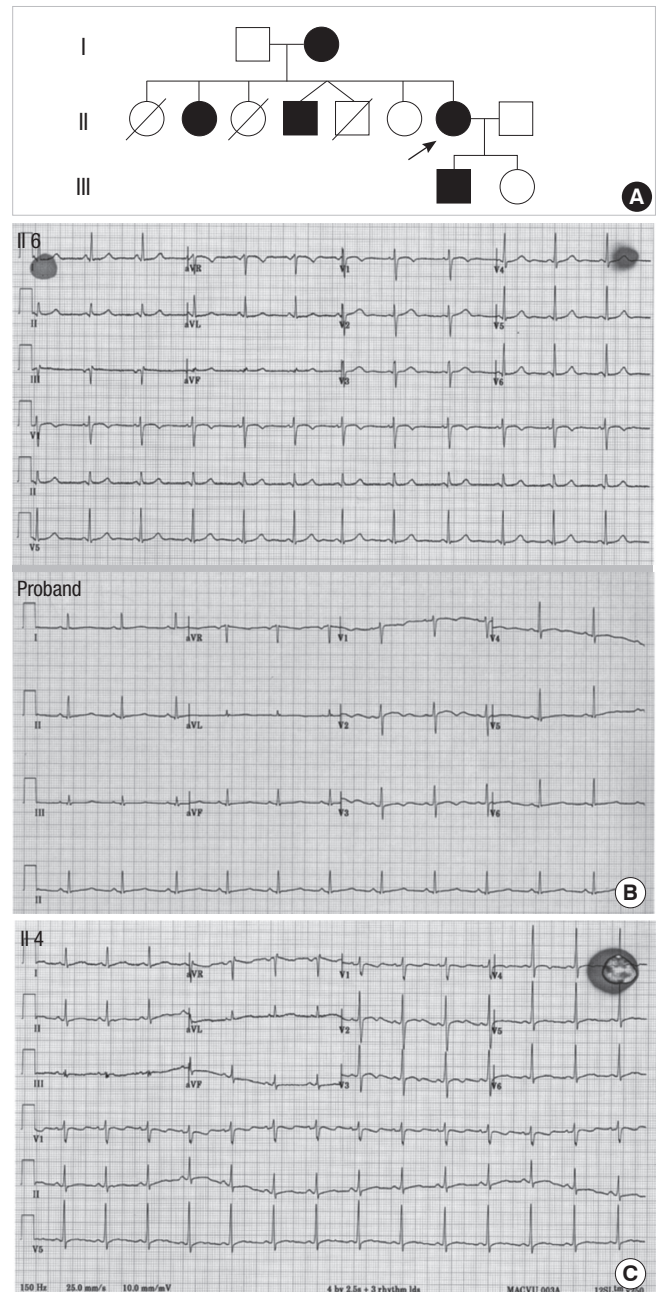


Fig. 1. It showed typical figures of 'Torsades de Pointes'. In (A), the black arrows indicate long QT interval and T wave alternans.

Fig. 2. Pedigree and Electrocardiogram. (A) Pedigree of the LQTS family; filled symbols represent affected members whereas empty symbols normal members. Arrow indicates the proband. (B) Electrocardiogram of normal II6 and the proband II7. (C) Electrocardiogram of II4 displays atypically short QTc interval than those of the other family members. This patient also contains del453 C mutation (151Pfs+15X) in the *KCNH2* gene.

**Table 1.** Clinical characteristics of the pedigree

Family	Age	Mutation	ECG baseline					Clinical characteristics
			Heart rate (bpm)	PR Interval (ms)	QRS duration (ms)	QT/QTc	P-R-T axes	
I 1	87	-	61	142	72	432/435	58 46 66	- Normal sinus rhythm - Normal ECG
I 2	78	+	63	172	138	442/473	21 1 22	- Right bundle branch block - Abnormal ECG
II 2	54	+	74	158	82	474/526	47 -33 85	- Normal sinus rhythm - Left axis deviation - ST&T wave abnormality, Consider anterolateral ischemia - Prolonged QT - Abnormal ECG
II 4	46	+	85	130	86	334/397	48 30 11	- Nonspecific T wave abnormality - Abnormal ECG
II 6	43	-	71	102	74	396/430	-1 -2 24	- Normal sinus rhythm with short PR - Otherwise normal ECG
II 8	44	-	117	122	90	306/427	76 58 79	- Sinus tachycardia - Otherwise normal ECG
III 1	17	+	84	136	88	384/454	54 62 36	- Normal sinus rhythm - Normal ECG
III 2	14	-	78	94	70	362/412	65 81 42	- Normal sinus rhythm - Normal ECG

**Table 2.** PCR primers used for mutational analysis

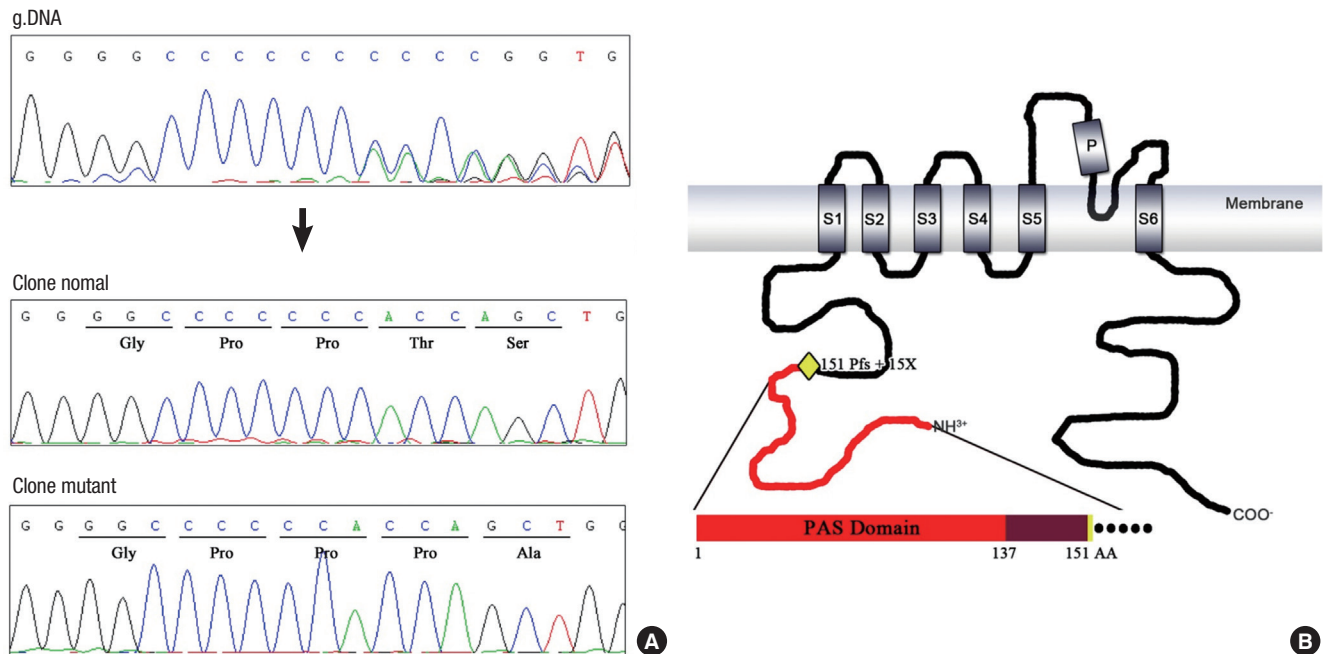
No.	Gene	Exon	Primer SEQ (5'-3')	bp	Annealing (°C)	No.	Gene	Exon	Primer SEQ (5'-3')	bp	Annealing (°C)
1	<i>KCNQ1</i>	Ex1	CCCCGGCAGGCCCTCCTCGTT GGGGCAGGTGGCGCTGGGACA	493	65	17	<i>KCNH2</i>	Ex1	GCCACCCGAAGCCTAGTGCT GAGTGTGCAGGGAAGGGATG	437	54
2		Ex2	GCTCTGTCCGGGAAGGGAAC CCGCCACCCATAACTGACCA	446	65	18		Ex2	CCAGCCATGCTTCTCT GTG CGTCACACCCCCACAGAAC	585	56
3		Ex3	ATCCCTCGTGCAGACTACC AGGCAGCCATGACATCCAGC	312	65	19		Ex3	CCATACGGGGAGGCAGAAGT AAGCCACATCCTCAGGGTACAG	448	58
4		Ex4-5	CTGAAGCACTCAAGGCCGA TCTGAGCAAGTGGATGGGG	930	65	20		Ex4	TCCCCTCTTCCCTTACC GCACCCAGGACGTAGTGAAG	656	61
5		Ex6	TATACCCAGCCTCCCCACCC CCGTTCAAGTACCCCTGCC	375	65	21		Ex5	TTCTTGTGACTCCCCTGGTCTC CTCTCCAAGCTCTCCAAGGT	405	61
6		Ex7	CGTAGGACGCCAGTGATCG GTGCAGGTGAGAAGCCCTG	363	65	22		Ex1 c form	CTGCTCTGGTGGCCAGTCG CTACTTCCCAGCAGCCCTCTC	370	61
7		Ex8	CTCTGTTCCTGGTCTTTCGC TGGGTCTGCTCACAGGGAGG	360	65	23		Ex6	TTTTTGGCCTCCTCCTCATTCT TGGCCTCTCCTCTCCCTACAC	582	60
8		Ex9	ACCATACCTGGCCTTCCACA AGCAGCCAAGGCTTCCACAA	354	65	24		Ex7	GTCTTTGGGGATCTGACCTCTG GTTTCTCCAACCTTGGGTTCTC	562	62
9		Ex10	CTTCCATAAGGGCCCCCG GCTAGCAAGAAGGCCCTGCC	397	63	25		Ex8	CGCTGAGACTGAGACACTGACC TACTGACTGTGACCCCTGAGA	409	64
10		Ex11	GTAGGGCCTGGCAGACGATG GAGACCACAGCCCAAGGGAGA	302	65	26		Ex9	GGTGGGATGGTGGAGTAGAGTG GAACCACATGGCCCTTAGTGAA	690	64
11		Ex12	GCTTCCAGGCACAAGCTCCA AAGGCTTCCCAGCACCAAG	318	65	27		Ex10	CAGCTGAGGGGACATGCTCT ATTCAATGTCACACAGCAAGG	381	64
12		Ex13	AGGGGCAGTGAGGGGATGAC GGTTCGGTGGGGTAGGCACT	315	65	28		Ex11	AGGGAAGGGTTTTT CCAGAG AGGCCTGGGTAAGCAGACAC	349	54
13		Ex14	TCGCTACTGTGCACGCTTGG CGCTGATCATGCAGCTGGG	331	65	29		Ex12,13	AAGAAGCAGCTGGGACAAAC CAGACAGGCCCTCTCCCTCTAC	775	61
14		Ex15	GTGTCCCAAGAGTGGGTGGA GCCACTTTGCTAAGGGTGGG	350	65	30		Ex14	GGCTGCCACACCT CTAGC GACTCTCCTGCCCTACCCTGAG	508	58
15		Ex16	CCACCCCTGGTATTTTGTCA AGTAGGCACTGGCGGAAGG	358	65	31		Ex15	TTCTGTCCCACTG ACTCT GAGCTGTGCTTTCGA GTTCC	412	64
16		Ex17	ACCAGAGGCTGGCACTCATCAC GCAGTCTCCACAGTCTCCTGCC	801	60	32	<i>KCNE1</i>	Ex3	TAAGAGGTGTGCTG GGAAG GGCAGGATGTGTCCA GTTTT	478	58

ed as LQTS based on the non-specific T-wave abnormality despite of shorter QTc interval than normal one (Fig. 2C). The clinical characteristics are summarized in Table 1.

### Genetic analysis

Genomic DNA was extracted from the peripheral blood of the participants using a Blood extraction kit (Qiagen) following the





**Fig. 3.** Chromatogram of 453delC mutation in the *KCNH2* gene (A) and schematic representation of the *KCNH2* protein (B). Yellow diamond depicts the mutation (151Pfs+15X) described in this study.

manufacturer's protocol. Genomic DNA was used as a template for PCR amplification of the individual exons of the *KCNQ1*, *KCNH2*, and *KCNE1* genes. Gene-specific primer sequences were designed using genomic sequences of each gene (Genbank accession numbers; NG\_008935.1 for *KCNQ1*, NG\_008916.1 for *KCNH2*, and NG\_009091.1 for *KCNE1*). PCR primer sequences and conditions are shown in the Table 2. The purified PCR product was directly sequenced using ABI Prism 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and the sequences analyzed using BLAST program (<http://blast.ncbi.nlm.nih.gov/>). The putative mutation was further investigated in the family members to determine its familial transmission. And the general Korean population was also screened for the presence of this mutation.

The sequencing of the entire coding regions as well as the splicing junctions of the *KCNQ1*, *KCNH2*, and *KCNE1* genes of the proband revealed a typical chromatogram of mixed samples in the sequence read of an exon (Fig. 3A). Subcloning of the PCR products followed by sequencing of individual clones identified a mutant allele with a single nucleotide C deletion in exon 3 of *KCNH2* gene. This C deletion at the nucleotide position of 453 of the *KCNH2* mRNA presumably resulted in frameshift of the coding region thus introducing a premature termination codon after 15 miscoded amino acids (151Pfs+15X) (Fig. 3B). This mutation co-segregated with LQTS in this family, thus I2, II2, II4, II7 (proband), and III1 were all heterozygotes for delC453fs (151Pfs+15X). This mutation was not detected in 100 chromosomes in general Korean population.

## DISCUSSION

The clinical course of patients with LQTS is so variable and is affected by the length of QT interval, sex and the genotype (8). The risk of sudden cardiac death is significantly higher among patients with LQT1 and LQT2 mutations than those with LQT3 mutation (9). Sudden cardiac death usually occurs under specific circumstances in a gene-specific manner. Patients with LQT2 usually experience cardiac events with acute arousal-type emotions, such as the ringing of an alarm clock (10, 11).

A voltage-gated  $K^+$  channel which flows the rapidly activating delayed rectifying  $I_{kr}$  current is formed by tetramer of human *KCNH2* $\alpha$  subunit and *MiRP1* $\beta$  subunit and acts to keep the balance of membrane currents flow during the action potential plateau and repolarization in the heart.

The mutations in the *KCNH2* gene cause LQTS2 which is the second most common cause of the congenital LQTS and responsible for 35%-45% of all genotyped LQTS. The *KCNH2* gene codes for an 1159 amino acid polypeptide which folds into 6 transmembrane  $\alpha$  helices, numbered S1-S6, a pore helix situated between S5 and S6, and cytoplasmic N- and C-termini (12).

Currently, near 300 mutations were reported in the *KCNH2* gene (<http://www.fsm.it/cardmoc/>) for LQT2 and these mutations can lead to LQTS by different mechanisms. These mutations exert influence on  $I_{kr}$  by reduced synthesis of HERG, defective trafficking of *KCNH2* from ER to plasma membrane, defective gating or defect in ion permeation (13, 14). Those mutations include missense mutation (64%), frameshift mutation due to deletion or insertion (~21%), nonsense mutation (8%),

splice error mutation (3.5%), and inframe deletion/insertion mutation (3.5%).

In this study, we described a deletion mutation del453C in N-terminal region of the *KCNH2* gene in a family with long QT syndrome. This mutation has been previously reported in the Finnish LQT families (15, 16) and the affected patients exhibited the mean QTc of 470 + 42 ms. Similarly to this characteristics, all five carriers in this Korean LQTS pedigree manifested LQT phenotype with the prolonged QTc intervals with average  $484.33 \pm 37.31$  ms. and some with abnormal T wave phenotype while non-carriers showed normal ECG. Thus, the penetrance of LQTS in this family was assumed to be 100%. This del453C mutation presumably leads to deficiency in the rapid delayed rectifier potassium channel and resulted in the prolonged the open state of the channel thus reducing in the repolarizing current.

The mutant *KCNH2* (151Pfs+15X) we found in this Korean LQTS family presumably resulted in a frameshift of the coding sequence and thus introduced a stop codon unexpectedly. Therefore, the mutant mRNA may have suffered nonsense-mediated decay of mRNA, which resulted in the decreased production of *KCNH2* protein. In this case, the cells may have experienced haplo-insufficiency of protein causing a disease state. Alternate mechanism could be that the mutant allele mRNA may not be affected in its stability but it was translated and resulted in the truncated *KCNH2* protein due to frame shift. In turn, this truncated protein may have caused abnormal formation of potassium channel leading to dominant negative effect.

N-terminal region of truncated *KCNH2* protein (1-151 AA) contains a Per-Arnt-Sem (PAS) domain (1-137 AA) which is found in many signaling proteins and functions as a signal sensor. Functionally, PAS domain of *KCNH2* was shown to modulate deactivation process of the channel (12, 17) and N-terminal 16 AA have been implicated in slowing the deactivation process and stabilizing the open state (18).

Interestingly, N-terminus (1-135AA) was shown to form a tetramer in the absence of the rest of *KCNH2* protein. In addition, when it was co-expressed with wild type *KCNH2* in which was the case in our patients, truncated mutant inhibited the functional expression of *KCNH2* channel in transfected cells (19). Furthermore, this soluble peptide of N-terminus (1-135 AA) was shown to bind to membrane bound *KCNH2* (12) suggesting that the truncated mutant *KCNH2* protein may affect formation of *KCNH2* tetramer leading to abnormal function of potassium channel. Currently, it is not known whether this mutation 151Pfs+15X caused expression of truncated protein and inhibited formation of functional potassium channel. Molecular approach as well as electrophysiological study with mutant form of this gene can be utilized to elucidate the mechanism underlying LQTS with this mutation.

## REFERENCES

- Moss AJ, Kass RS. *Long QT syndrome: from channels to cardiac arrhythmias. J Clin Invest* 2005; 115: 2018-24.
- Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, Moss AJ, Schwartz PJ, Towbin JA, Vincent GM, et al. *Spectrum of mutations in long-QT syndrome genes: KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation* 2000; 102: 1178-85.
- Hedley PL, Jørgensen P, Schlamowitz S, Wangari R, Moolman-Smook J, Brink PA, Kanters JK, Corfield VA, Christiansen M. *The genetic basis of long QT and short QT syndromes: a mutation update. Hum Mutat* 2009; 30: 1486-511.
- Itoh H, Shimizu W, Hayashi K, Yamagata K, Sakaguchi T, Ohno S, Maikiyama T, Akao M, Ai T, Noda T, et al. *Long QT syndrome with compound mutations is associated with a more severe phenotype: a Japanese multicenter study. Heart Rhythm* 2010; 7: 1411-8.
- Jervell A, Lange-Nielsen F. *Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death. Am Heart J* 1957; 54: 59-68.
- Sagie A, Larson MG, Goldberg RJ, Bengtson JR, Levy D. *An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study). Am J Cardiol* 1992; 70: 797-801.
- Schwartz PJ, Moss AJ, Vincent GM, Crampton RS. *Diagnostic criteria for the long QT syndrome: an update. Circulation* 1993; 88: 782-4.
- Moss AJ, Schwartz PJ, Crampton RS, Tzivoni D, Locati EH, MacCluer J, Hall WJ, Weitkamp L, Vincent GM, Garson A Jr, et al. *The long QT syndrome: prospective longitudinal study of 328 families. Circulation* 1991; 84: 1136-44.
- Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Robinson JL, Priori SG, Benhorin J, Locati EH, Towbin JA, Keating MT, et al. *Influence of genotype on the clinical course of the long-QT syndrome: International Long-QT Syndrome Registry Research Group. N Engl J Med* 1998; 339: 960-5.
- Moss AJ, Robinson JL, Gessman L, Gillespie R, Zareba W, Schwartz PJ, Vincent GM, Benhorin J, Heilbron EL, Towbin JA, et al. *Comparison of clinical and genetic variables of cardiac events associated with loud noise versus swimming among subjects with the long QT syndrome. Am J Cardiol* 1999; 84: 876-9.
- Ali RH, Zareba W, Moss AJ, Schwartz PJ, Benhorin J, Vincent GM, Locati EH, Priori S, Napolitano C, Towbin JA, et al. *Clinical and genetic variables associated with acute arousal and nonarousal-related cardiac events among subjects with long QT syndrome. Am J Cardiol* 2000; 85: 457-61.
- Morais Cabral JH, Lee A, Cohen SL, Chait BT, Li M, Mackinnon R. *Crystal structure and functional analysis of the HERG potassium channel N terminus: a eukaryotic PAS domain. Cell* 1998; 95: 649-55.
- Clancy CE, Rudy Y. *Cellular consequences of HERG mutations in the long QT syndrome: precursors to sudden cardiac death. Cardiovasc Res* 2001; 50: 301-13.
- Thomas D, Kiehn J, Katus HA, Karle CA. *Defective protein trafficking in hERG-associated hereditary long QT syndrome (LQT2): molecular mechanisms and restoration of intracellular protein processing. Cardiovasc Res* 2003; 60: 235-41.
- Laitinen P, Fodstad H, Piippo K, Swan H, Toivonen L, Viitasalo M, Kaprio J, Kontula K. *Survey of the coding region of the HERG gene in long QT syndrome reveals six novel mutations and an amino acid polymorphism*

- with possible phenotypic effects. Hum Mutat 2000; 15: 580-1.*
16. Fodstad H, Swan H, Laitinen P, Piippo K, Paavonen K, Viitasalo M, Toivonen L, Kontula K. *Four potassium channel mutations account for 73% of the genetic spectrum underlying long-QT syndrome (LQTS) and provide evidence for a strong founder effect in Finland. Ann Med 2004; 36: 53-63.*
  17. Chen J, Zou A, Splawski I, Keating MT, Sanguinetti MC. *Long QT syndrome-associated mutations in the Per-Arnt-Sim (PAS) domain of HERG potassium channels accelerate channel deactivation. J Biol Chem 1999; 274: 10113-8.*
  18. Wang J, Myers CD, Robertson GA. *Dynamic control of deactivation gating by a soluble amino-terminal domain in HERG K(+) channels. J Gen Physiol 2000; 115: 749-58.*
  19. Li X, Xu J, Li M. *The human delta1261 mutation of the HERG potassium channel results in a truncated protein that contains a subunit interaction domain and decreases the channel expression. J Biol Chem 1997; 272: 705-8.*