Identification of a Novel KCNQ1 Frameshift Mutation and Review of the Literature among Iranian Long QT Families

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ABSTRACT

Background: Long QT syndrome (LQTS) is characterized by the prolongation of QT interval, which results in syncope and sudden cardiac death in young people. KCNQ1 is the most common gene responsible for this syndrome. Methods: Molecular investigation was performed by DNA Sanger sequencing in Iranian families with a history of syncope. In silico examinations were performed for predicting the pathogenicity of the novel variant. Results: A novel homozygous KCNQ1 frameshift mutation, c.1426_1429delATGC (M476Pfs*4), was identified, and then the current literatures of five patients were reviewed regarding the LQTS. Conclusion: The novel frameshift mutation has been reported for the first time among the Iranian population. Our finding along with the case study series of LQTS patients illustrates the importance of genetic and case series in precise detection of the frequency of LQTS carriers. DOI: 10.29252/ibj.23.3.228

Keywords: Jervell-Lange-Nielsen syndrome, KCNQ1, Long QT syndrome, Romano-Ward syndrome

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INTRODUCTION

Long-QT syndrome (LQTS) is characterized by prolonged QT interval on the electrocardiograms (ECG), syncope, and cardiac arrest[1,2]. It has been reported that mutations in 16 genes can result in inherited LQTS[3]. There are two main types of inherited LQTS: the autosomal dominant termed Romano-Ward syndrome (RWS) and the autosomal recessive type with less recurrence defined as Jervell-Lange Nielsen Syndrome (JLNS)[3]. Mutations in KCNQ1 gene may create both the RWS and JLNS disorders.

JLNS is a condition in which QTc prolongation accompanying with congenital two-sided deafness occurs due to compound heterozygous or homozygous mutations in KCNQ1 and/or KCNE1 genes[4]. RWS is recognized as the aggregation of symptoms such as syncopal attacks and QT interval alteration in ECG without congenital deafness[5]. Although RWS is mostly inherited in an autosomal dominant pattern[6], autosomal recessive inheritance is associated with homozygosity[7]. In addition, the association of JLNS with compound heterozygosity[8] has been observed in some cases; the fact indicates that the pattern of inheritance for this disease could be more intricate.

In this study, we present a novel homozygous frameshift mutation of KCNQ1 gene and review recent literatures on novel frameshift and recessive KCNQ1 mutations of Iranian families.
MATERIALS AND METHODS

Clinical evaluation
Six unrelated LQTS patients (patients A-F) were referred to Emergency Department of Rajaie Cardiovascular, Medical and Research Center (Tehran, Iran) for further clinical evaluations and molecular testing.

Genetic study
Informed consent and the study approval by the Ethics Committee of Pasteur Institute of Iran and Rajaie Cardiovascular, Medical and Research Center (adopted from the 1975 Helsinki Declaration) were obtained. Blood samples along with the ECG were collected from the patient and family members. Using the standard salting-out protocol, genomic DNA was extracted from the peripheral blood samples. Primer design was performed by Gene Runner and Primer3 online software for coding exons and exon-intron boundaries and also untranslated regions of KCNQ1 (NM_000218). Primers for KCNH2 (NM_000238) and SCN5A (NM_198056.2) were designed as well (Table 1). By PCR, exons of the genes were amplified, and by Sanger sequencing technique, the amplicons were sequenced. Sanger sequencing results were compared with the RefSeq genomic accession numbers, and the variants were evaluated by Mutation Database. For predicting the functional impact of the variant on the protein, MutationTaster, HSF, Mutation Assessor, SIFT, PolyPhen-2, and FATHMM were used.

RESULTS
Patient A was a five-year-old girl with a history of syncope and seizure disorders. She was offspring of a consanguineous marriage and suffered from congenital sensorineural deafness. She had experienced fainting around the age of 2.5. There was no family history of convulsions, but a sudden cardiac death happened in proband's sibling at six months of age (4:2 in Fig. 1). One stillbirth was reported by her mother in the previous pregnancy. Her first ECG demonstrated normal sinus rhythm with the prolonged QT interval of over 500 milliseconds (ms), as depicted in Figure 2, while her parents showed normal ECG.

Data of Sanger sequencing demonstrated a novel homozygous frameshift mutation, c.1426_1429 delATGC (M476Pfs*4; ClinVar accession number: SCV000678249.1), in the KCNQ1 gene (Fig. 1A). The mutation was confirmed in the father of proband in heterozygous form, but the DNA sample of her mother was not available. The variant was considered as a frameshift mutation according to MutationTaster software. All the aforementioned predictive tools predicted it as a damaging and disease-causing variant.

Table 1. Primers of KCNQ1, KCNH2, and SCN5A genes for coding regions

| Gene/exon | Primer sequence | Gene/exon | Primer sequence |
|-----------|----------------|-----------|-----------------|
| KCNQ1 /1  | F: AGCGGGATAGATGACACGAG R: CTTCTCTGAGACCTGTTGTTG | SCN5A /2 | F: CCTCTCTGCAAATGTGCTGC R: GGAATGATGCACCTGTTGCTTC |
| KCNQ1 /2  | F: TACACAGTATGGAGTACCTG R: GGTGACCTCGTCTCTGGGTATA | SCN5A /3 | F: CTGACCTGCAAATGTGCTGC R: CCTAAGCACAATGCTTGGCAT |
| KCNQ1 /3  | F: TGGACATGAGCTGTAAGTCG R: ACCCATGATCGGCTGCTGC | SCN5A /4 | F: TGCTATTAGGTTGTCATGGAG R: CTTCCTGCAATTAATGGTGG |
| KCNQ1 /4  | F: GTCTCTCCGTTTAGATGCTGC R: GGAATCTGGGAGATGTCTGC | SCN5A /5 | F: CGTCTAGCCGACAGTGGATGC R: CATGCCCATGAGCTTGC |
| KCNQ1 /5  | F: CTGCTTGGGATGGCAGCATATCC R: CCACACATGACGCTTGGAGT | SCN5A /6 | F: TCATTATCCACCGGTAAAGTCG R: GGCTATTGGGCACTGAGCAT |
| KCNQ1 /6  | F: ACCGGAGTGGAGGATGTTGG R: CCAAGGAGGTCTCAACACTGAGC | SCN5A /7 | F: GAATATCGAGACATACCTGAGC R: AGGACAGCGGGAGATCACAGC |
| KCNQ1 /7  | F: TTACATGTCTGTTGGGACA R: CTGGAGATAGATCGGCTGAGAAG | SCN5A /8 | F: TCCCTGCTCTCCAGGAGCA R: GCACAGGAGACAGCAGAGC |
| KCNQ1 /8  | F: CTTCACGACTGACCATACT R: GCTGTTGACAACCTGTTGAGA | SCN5A /9 | F: ACAGCAGCGACAAAGTGACG R: AGGATGTCATGTCCTGGTGA |
| KCNQ1 /9  | F: CCACCTTGTGCAAGTCTCCTC R: CGATGCTAGGTTCCTGCAATC | SCN5A /10 | F: CTTCGGGTTGCAGTACCTGCC R: GTGAATGTTGGATCGGAGTA |
| Gene/exon | Primer sequence | Gene/exon | Primer sequence |
|-----------|----------------|-----------|----------------|
| KCNQ1/10 | F: CTGTGTGAAGACACTGGAGCTG  
R: GTGCTCTGAGACAAGCGTGAAG | SCN5A/11 | F: CTGTCTGAGTTTATCTCCATGATG  
R: CCATAAGATGAGGTTCCATT |
| KCNQ1/11 | F: TGATTGTTCAGTGTGAGGCT  
R: GTGCTATATCTCAGTTGAGC | SCN5A/12 | F: CAAGGCCCAGTTAGTTGTTGAG  
R: CTCTAGGTCAGTTGAGC |
| KCNQ1/12 | F: GGACATGCGCTAATGAGGAGCTG  
R: GTGCTATCTGACGACTGAG | SCN5A/13 | F: TTGCTCGATAATCTCCATGAG  
R: CGTGAAGAAATGAGATGTGAG |
| KCNQ1/13 | F: CGGTTGATAGACAGCAGCTGG  
R: GAGTCTCTGCTCCTCAACAC | SCN5A/14 | F: TGCGCTGATGAGGTTTACAGG  
R: TGCATGACATGAAATGAAAGC |
| KCNQ1/14 | F: AACTAGCTCCGTTCTGATCAGG  
R: TGCGCTGATGAGGTTTACAGG | SCN5A/15 | F: GCCACCCGAAGCCTAGTGC  
R: GGAAACTCAGCTCAGGCTTTTGG |
| KCNH2/1 | F: GCCACCCGAAGCCTAGTGC  
R: GGAAACTCAGCTCAGGCTTTTGG | SCN5A/16 | F: CCATCTCTCTCAAGAAGTGAG  
R: CCAGACCTCAGCAACCAACCAATCAT |
| KCNH2/2 | F: CTGTGTGAGTGGAGAATGTGG  
R: GGAGTTGCTAGGCTGTGGGT | SCN5A/17 | F: AGCTTCTAGACTCAGCACCAG  
R: TCCATCTGCTCAGTCGAG |
| KCNH2/3 | F: GCAGAAGAAAGGATCATAGCC  
R: CCAGATCAGACTGCTTGG | SCN5A/18 | F: GAGGAGTCTTCAGTGAGATGGAG  
R: CTCTAGGTCAGTTGAGC |
| KCNH2/4 | F: TGAAGTGTCACCTCAGATAGG  
R: GCTTCTGCTCAGTCGAG | SCN5A/19 | F: CTCTACTGCTGTTGCTTGG |  |
| KCNH2/5 | F: TGCTGTCTCTTCTTAGAGTGG  
R: GCCACCCGAAGCCTAGTGC | SCN5A/20 | F: CCATCTCTCTCAAGAAGTGAG  
R: CCAGACCTCAGCAACCAACCAATCAT |
| KCNH2/6 | F: GGGGGACCTCTGAGTGAAGCT  
R: GTGCTTCTGCTCAGTCGAG | SCN5A/21 | F: GAGGAGTCTTCAGTGAGATGGAG  
R: CTCTAGGTCAGTTGAGC |
| KCNH2/7 | F: AGGAGGGGGTCTAGGAAGTC  
R: TCGAGTCTGAGTGAAGAC | SCN5A/22 | F: CCAGAAGCGAGATCTGCTTGG |  |
| KCNH2/8 | F: TGGAGGCGAGATGTAAGACGG  
R: CACAGTCTAGTGAAGGACCTG | SCN5A/23 | F: GGAACACGAAGCTGCTGAGTGGAA  
R: CAGTTCTGAAACCAGATACATG |
| KCNH2/9 | F: CTGATGCTTCCGAGATCTCC  
R: GAGGAGGAGGGTCTAGGAAGTC | SCN5A/24 | F: CTGACCACCCAGGCGATTAG  
R: GTCATCTGCTGAGTGAAGAC |
| KCNH2/10 | F: TGGAGGCGAGATGTAAGACGG  
R: CACAGTCTAGTGAAGGACCTG | SCN5A/25 | F: TAGTGACCTTCTCAGATACACCC  
R: CCTGTTAGAAGCAGGGAG |
| KCNH2/11 | F: CTGATGCTTCCGAGATCTCC  
R: GAGGAGGAGGGTCTAGGAAGTC | SCN5A/26 | F: GGGTGATACCTGAGTATTCGAG  
R: AGGTGATGAAAGATGATGATAC |
| KCNH2/12,13 | F: AAGACATGCTGCTGAGTGAAGACGG  
R: CACAGTCTAGTGAAGGACCTG | SCN5A/27 | F: GGAACACGAAGCTGCTGAGTGGAA  
R: CAGTTCTGAAACCAGATACATG |
| KCNH2/13 | F: CTGATGCTTCCGAGATCTCC  
R: GAGGAGGAGGGTCTAGGAAGTC | SCN5A/28.1 | F: GCTCCTGAGTATATGACAGACC  
R: CATGATGAAAACTGATACCAT |
| KCNH2/14 | F: CTGATGCTTCCGAGATCTCC  
R: GAGGAGGAGGGTCTAGGAAGTC | SCN5A/28.2 | F: GACATTGTCCAATCTCCAGACCT  
R: GGTTATCCAGAGGCTCCTCC |
| KCNH2/15 | F: GCTCCTGCTTCCGAGATCTCC  
R: GGAACACGAAGCTGCTGAGTGGAA  
R: CACAGTCTAGTGAAGGACCTG | SCN5A/28.3 | F: GGAACACGAAGCTGCTGAGTGGAA  
R: CACAGTCTAGTGAAGGACCTG |

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This mutation introduces three novel amino acids after codon 475 (methionine 476, as the first affected amino acid, was changed to proline) and premature stop codon at 480 that resulted in a truncated protein.

Patients B and C were referred to the clinic due to syncope and a prolonged QTs interval of >600 and 560 ms, respectively. Except for hearing defect, physical and neurological examinations were totally normal, and also there was no electrolyte imbalance. Sanger sequencing of the KCNQ1 gene for both cases showed a homozygous frameshift mutation, c.733_734delGG (p.G245Rfs*39), in the exon 5. This frameshift results in a premature stop codon and develops a truncated protein. Examination of the parents of two patients showed heterozygosity for this mutation. In our previous study \[15\], using STR markers, we demonstrated this frameshift mutation in two obviously unrelated families with the same origin, which may represent a founding effect.

Patients D and E were presented by the recurrent episodes of syncope and a prolonged QTs interval of >550 and 600 ms, respectively. Analysis of the DNA sequence in patient D revealed a homozygous missense mutation.
Novel KCNQ1 Mutation and Review of the Literature

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In the present research, the mutation analysis of the KCNQ1, KCNH2, and SCN5A genes were performed in a patient among a cohort of 30 unrelated Iranian LQTS families. The sequence analysis of the index patient demonstrated the absence of mutation in the SCN5A and KCNH2 genes but showed a novel homozygous mutation, c.1426_1429delATGC (M476Pfs*4), in KCNQ1 gene. Five patients screened previously were found to have a missense mutation in two RWS families and two frameshift mutations in three JLNS families.

The novel mutation was detected neither in ExAC nor in 1000 Genome; additionally, it has not been reported in any disease database such as HGMD and LOVD. In the index case, the 4-bp deletion was located at positions 1426 to 1429 in exon 11 of KCNQ1 gene, which led to amino acid sequence alteration and was accordingly considered as a frameshift variant.

It has been shown that frameshift mutations leading to truncated proteins are responsible for the majority of JLNS cases. Wei et al. have reported a one-nucleotide deletion in the position 1188 of KCNQ1 gene, which causes a frameshift and leads to a premature stop codon and consequently, results in a 259-amino-acid deletion in the C-terminal domain. A single nucleotide insertion onto the position 1149 of this gene. In LQTS patients, frameshift mutation has been predicted to be a pathogenic mutation with an estimated predicted value of 99%.

In concordance with the genetic study result, our patient showed the clinical features of seizure, recurrent episodes of syncope, and the family history of serious heart events such as sudden cardiac death in the sibling of the proband who died at six months of age. Likewise, a congenital sensorineural deafness was observed in the proband in agreement with most previous reports in JLNS patients.

The probands B, C, and F were categorized as patients of JLNS, the recessive form of LQTS. A frameshift mutation (p. A512Pfs*81) was found in patient F. In B and C non-consanguineous families, the frameshift mutation, c.733_734delGG, happened in the C-loop between the transmembrane domains S4-S5 of the protein. The C-loop domains have a significant role in changing the potassium channel’s function; therefore, mutations in the residues of this region may impair the voltage-dependent activation of the channel, hence leading to increased risk of lethal cardiac events.

Functional assay showed that mutations in the C-loop of KCNQ1 gene impress the adrenergic regulation of channel, either in the absence or in the presence of wild-type subunits.

To confirm whether the novel missense variant of c.1691A>G (p.D564G) in cases D and E is pathogenic, further analysis was conducted. In silico investigations by the predictive software indicated that the substitution of aspartic acid with glycine at codon 564 may be a disease-causing mutation. SIFT, Polyphen-2, MutationTaster, and FATHMM classified the p.A564G as a damaging variant. The conservation scores predicted by Phylip, PhastCons, and GERPS++ were 4.32, 1, and 4.02, respectively. These analyses also revealed that aspartic acid 564 is located in the C-terminal domain of KCNQ1 channel, in a highly conserved α-helix region. In addition, Mutation Mapper showed the aspartic acid at position 564 was conserved among 100 different species. Consequently, according to the American College of Medical Genetics and Genomics guidelines (ACMG), the recessive variant was categorized as a possible pathogenic mutation.

This is the first report of a novel homozygous frameshift mutation with JLNS. Based on ACMG, this mutation is likely to abolish channel function, severely. Identification of six JLNS and RWS index cases in a period of 24 months in a cardiogenetic clinic in Tehran suggests the high prevalence of LQTS. This high prevalence necessitates a broader surveillance in the country and reveals the importance of genetic and case series studies in precise detection of LQTS carriers’ frequency.
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CONFLICT OF INTEREST. None declared.

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