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Novel frameshift mutation in the *KCNQ1* gene responsible for Jervell and Lange-Nielsen syndrome

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ARTICLE INFO	ABSTRACT
	Objective(s): Jervell and Lange–Nielsen syndrome is an autosomal recessive disorder caused by mutations in *KCNQ1* or *KCNE1* genes. The disease is characterized by sensorineural hearing loss and long QT syndrome. *Materials and Methods* Here we present a 3.5-year-old female patient, an offspring of consanguineous marriage, who had a history of recurrent syncope and congenital sensorineural deafness. The patient and the family members were screened for mutations in *KCNQ1* gene by linkage analysis and DNA sequencing. *Results:* DNA sequencing showed a c.1532_1534delG (p. A512Pfs*81) mutation in the *KCNQ1* gene in homozygous form. The results of short tandem repeat (STR) markers showed that the disease in the family is linked to the *KCNQ1* gene. The mutation was confirmed in the parents in heterozygous form. *Conclusion:* This is the first report of this variant in *KCNQ1* gene in an Iranian family. The data of this study could be used for early diagnosis of the condition in the family and genetic counseling.

Introduction

Jervell and Lange–Nielsen syndrome (JLNS) is a rare autosomal recessive disease (OMIM# 220400) characterized by congenital sensorineural deafness and significant QT interval prolongation often more than 500 msec in the electrocardiogram. The patients with long QT syndrome (LQTS) are prone to polymorphic ventricular arrhythmias, syncope, and sudden death (1). At least, 16 genes have been reported causing autosomal-dominant Romano-Ward syndrome (RWS) and two genes as the cause of JLNS (2). Mutations in *KCNQ1, KCNH2,* and *SCN5A* genes constitute more than 75% of pathogenic alleles in LQTS (3, 4).

Homozygous or compound heterozygous mutations in the *KCNQ1* gene and less frequently in the *KCNE1* gene lead to JLNS (5, 6). The α- and β-subunits of the potassium ion channel, which transport potassium actively out of the cardiac and inner ear cells, are encoded by *KCNQ1* and *KCNE1* genes, respectively (7). QT intervals in patients with *KCNQ1* mutations (90% of the cases) are longer than those with *KCNE1* mutations,

and the risk of arrhythmic events is also higher in these patients (8). In homozygous patients with JLNS, potassium transport is also affected in the inner ear cells, which causes sensorineural hearing loss. Usually, JLNS heterozygous carriers are asymptomatic; however, they might have mild QT prolongation without deafness.

In this report, a female patient clinically diagnosed JLNS is described, and molecular assays were then performed for confirming the diagnosis.

Methods

Case Report

A 3.5-year-old girl was referred to our emergency unit at the Rajaei's Cardiovascular Medical and Research Center (Tehran-Iran) due to syncope.

The medical history showed recurrent syncope and deafness and the history of bradycardia during the fetal period. The patient's first syncopal episode occurred at the age of 18 months. Cochlear implantation had been performed at age 2. There was no family history of convulsions or sudden cardiac death in three generations.

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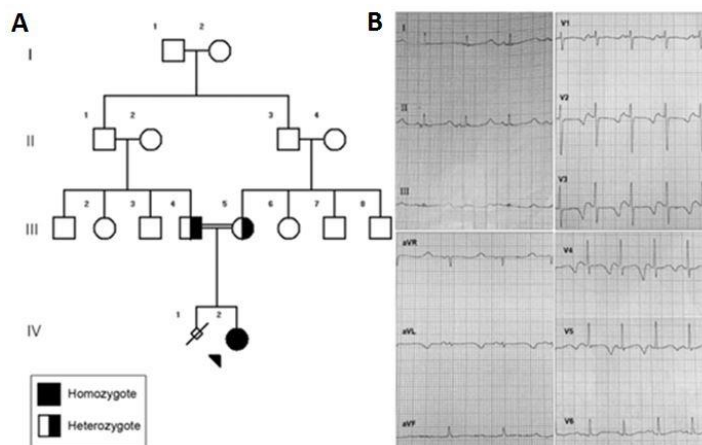
Figure 1. The Pedigree of the family (A): proband is shown with a black filled circle and an arrow head. Half filled squares or circles denote heterozygotes for *KCNQ1* gene mutation, transmitted from parents (other family members have not been studuied), and ECG of the patient (B): demonstrating prolonged QTc interval (581 msec)

In addition, the patient was the offspring of a first-degree consanguineous marriage (Figure 1-A). Her mother reported one stillbirth in the previous pregnancy.

No abnormalities except for hearing impairment were detected during physical and neurological examinations. In the para-clinical studies, routine tests, the level of electrolytes and hormones was normal. Resting 12-lead electrocardiogram (ECG) exhibited a markedly prolonged QTc interval of more than 580 msec (corrected with Bazzet's formula) and T wave alternans (Figure 1-B). Also, a structurally normal heart was detected by echocardiography.

Propranolol with the dose of 3 mg/kg/day, divided into three daily doses, and started for the patient. Because of the high-risk situation, an endocardial single chamber implantable cardioverter defibrillator (ICD) was implanted. Thereafter, the patient was event-free and followed up for 16 months. The ECGs recorded from the father (QTc = 427 msec) and the mother (QTc = 461 msec) (data not shown) were normal (normal range <440-460 msec).

Genetic study

The blood samples were collected in tubes containing EDTA from the patient, parents and available family members. After obtaining written informed consent, genomic DNA was extracted from peripheral blood leukocytes using the salting-out method (9). For detecting the related genes in such heterogeneous cardiac diseases, twelve short tandem repeat (STR) markers (D11SD8.3, D11SU10.9, D11SU2.2, D11SU0.6, D11SD13.6, D11SI, D21SU10, D21SU1.1, D21SD13, D21SD22, D21SD23, D21SD28) within or surrounding the *KCNQ1* and *KCNE1* genes were selected and amplified for the family members. Size determination of the repeats was performed on ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA). Primers were designed for coding exons and flanking exon-intron boundaries encompassing splice sites of *KCNQ1*

[NM_000218] gene (primers are available upon request). For exon 12, the following primers were used: (forward primer: 5'- GGACATGGCCTAAGTATCTCC-3', reverse primer: 5'- CCTATCTGAGACCTGACAGTGC-3'). PCR amplification was carried out in a 25-µl reaction volume containing 3.2 mM dNTP, 2.5 µl 10X Buffer, 2 mM MgCl2, 0.5 unit/µl SmarTaq DNA Polymerase (Cinaclon, Iran) and 200 ng of genomic DNA. PCR amplification was performed in PCR Master Cycler (Eppendorf, Germany) with the following thermal conditions: 95 ℃ for 5 min, thirty-five cycles in 95 ℃ for 90 sec, 62 ℃ for 90 sec, 72 ℃ for 40 sec and final extension at 72 ℃ for 10 min. The amplicons were directly sequenced bidirectionaly on ABI 3130XL Genetic Analyzer by Kawsar Biotech Co. (KBC, Tehran, Iran) (10). The sequencing data were aligned against the RefSeq genomic accession number (NG_008935.1). The variants were compared with the database of mutations in *KCNQ1* gene:
(http://www.genomed.org/lovd2/home.php)

Results

STR markers results showed that the disease in the family is linked to the *KCNQ1* gene (data not shown). Bidirectional Sanger Sequencing of the *KCNQ1* gene for the patient revealed a novel frameshift mutation c.1532_1534delG (p. A512Pfs*81) (ClinVar accession number: SCV000584010) in homozygous form (Figure. 2). This 1-bp deletion in exon 12 results in a premature stop codon at residue 592. True homozygosity was confirmed by parental testing; parents were both heterozygous for the mutation (Figure 2). The mutation has not been reported in the literature and *KCNQ1* gene mutation database previously.

Discussion

JLNS, an autosomal recessive form of LQTS, is characterized by congenital bilateral hearing loss and prolonged QT interval (1, 11, 12). JLNS patient in this
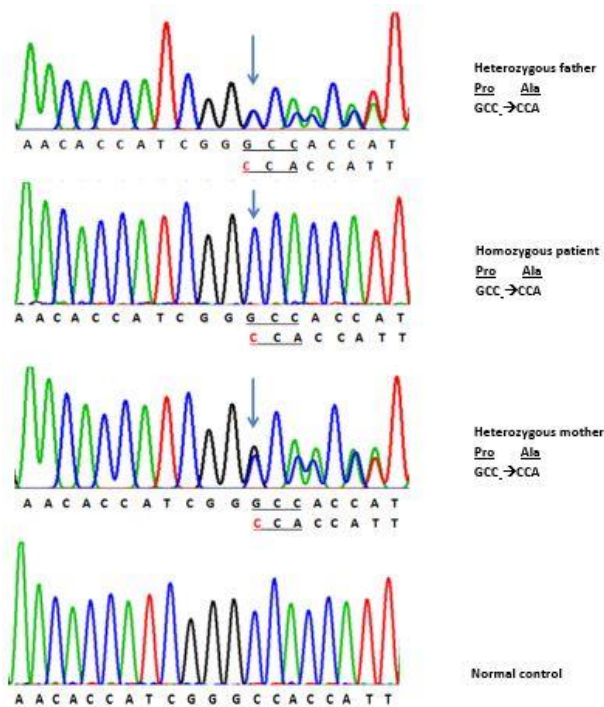
Figure 2. Sanger sequencing results of *KCNQ1* gene: Arrows show the location of c.1532_1534delG (p. A512Pfs*81) mutation in the heterozygous parent and the homozygous patient compared with the normal control

family had homozygous frameshift mutation (c.1532_1534delG) in the *KCNQ1* gene with a QTc≥580 msec experience of cardiac arrhythmias and repeated syncopal event. Although the electrophysiological study was not performed in this case, the reported frameshift mutation was predicted to have a pathogenic effect with an estimated predicted value (EPV) of 99% (6). Neyroud *et al.* (1999) (13) described an insertion of a cytosine at position 1893 (1893insC) in exon 15 in a LQT family that led to disruption in the C-terminus coding region of the gene. It is expected that the truncated protein caused by the frameshift mutation in the exon 12 causes a severe form of the disease. Moreover, it might cause substantial structural changes in the encoded potassium channel subunits, which is likely to severely reduce or abolish the channel function. Also in a report in 2003 (14), a single nucleotide C insertion, c.1338insC, leading to a premature stop codon and truncation of the *KCNQ1* C-terminus was characterized in a LQT patient who died suddenly while swimming at the age 11. The frameshift mutations in *KCNQ1* have also been reported as protein truncating (15) in which the loss of the C- terminus part of the protein in truncating form prevents the formation of tetramer from monomers and likewise, it was suggested that truncating mutations resulted in the loss of function (16). Furthermore, Qureshi *et al.* (2013) described prediction of the protein secondary structure in comparison with the wild-type protein and revealed that 1- nucleotide deletion of a 'G' (2592597delG)

(G216Afs*21) in exon 4 led to altered secondary structure and subsequently diminution of the protein strands production (17). There are challenges about phenotype correlation of RWS or JLNS to the type of mutation (missense, nonsense, frameshift, etc.) in *KCNQ1* gene. RWS with a dominant-negative effect on the tetrameric *KCNQ1* channel is usually induced by missense variants, whereas nonsense and frameshift mutations tend to cause autosomal recessive JLNS (15). However, exceptions exist in which JLNS could also be caused by missense mutations (5). In JLNS patients, QTc prolongation is much more than in any other LQTS patients, which is probably related to the presence of mutations in two alleles (8). Our patient with a homozygous frameshift mutation displayed the prolonged QT interval, the clinical manifestations of T-wave alterations and multiple episodes of syncope.

Unexpectedly, the R518X mutation in exon 12 of *KCNQ1* gene was found in association with LQTS without deafness (18). The absence of hearing loss symptom in the presence of mutations in *KCNQ1* gene may cause misdiagnosis of JLN syndrome.

As many of these patients are referred to the audiologist, ENT specialist, speech therapist or general practitioner, the education of healthcare providers about the possibility of the underlying cardiac disease may lead to earlier diagnosis of JLNS. Among children with JLNS, who are not treated, more than half die prior to age 15 (9, 19). Given the poor prognosis of long QT syndrome when it presents in combination with sensorineural deafness, an ICD was implanted in our patient shortly after diagnosis. In addition, she was in the high-risk group of JLNS patients due to a history of early childhood syncope, a longer QT interval (>580 msec) and a detected mutation in *KCNQ1*. Correct diagnosis allows preventive actions, including β-blockade, reduction of physical and emotional stress, avoidance of QT-prolonging drugs, and implantation of an ICD.

Conclusion

This study is the first report of a novel mutation in the *KCNQ1* gene causal to JLNS in an Iranian family. Its implication as a diagnostic tool in the clinical setting and in genetic counseling has been discussed. Our results suggest that evaluation of all sensory neural deafness children should be completed by ECG and very careful medical history. In cases with JLNS, genetic analysis could help us to predict prognosis of arrhythmia.

Acknowledgment

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