Molecular Analysis of KCNQ1, KCNH2 and SCN5A Genes in Iranian Patients with Long QT Syndrome

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Abstract

Background: Long QT syndrome is a cardiac ion channelopathy characterized by corrected QT interval prolongation on electrocardiograms, leading to syncope and sudden death.

Methods: In this study, the genetic screening of four Iranian LQTS families, including two Romano Ward syndrome families and two families with Jervell and Lange-Nielsen syndrome, was performed by Sanger sequencing and haplotype analysis for three of the most common LQTS genes, KCNQ1, KCNH2 and SCN5A.

Results: A de novo mutation c.1838C>T in the KCNH2 gene associated with LQT2 was identified in a RWS family. A homozygous mutation c.477+5G>A was found in the KCNQ1 gene of the two JLNS families, and a novel recessive KCNQ1 variant c.934A>T (p.T312S) was identified in the KCNQ1 of another RWS family. The structural, functional and pathogenicity evaluation of the novel KCNQ1 missense variant in silico predictive programs along with the segregation and population studies revealed that the variant was a likely pathogenic mutation.

Conclusion: To the best of our knowledge, p.T312S is the first mutation identified for an AR-RWS family in the Iranian families. This assay can be used to screen individuals to provide useful information for the identification of the LQTS in the Iranian population. It is yet to be proven that the detection of different types of LQT will result in a more effective therapy.

Keywords: Long QT syndrome; Cardiac repolarization; Genetics; De novo mutation; Iran

Introduction

Hereditary long QT syndrome (hLQTS) is one of the most prevalent causes of sudden cardiac death usually in the young people characterized by an abnormality in cardiac repolarization [1]. LQTS can exist either as an autosomal dominant (Romano Ward syndrome, RW, MIM# 192500) or autosomal recessive (Jervel and Lange-Nielsen syndrome, JLNS, MIM# 220400) disorder. Both syndromes lead to recurrent syncope and prolongation of the QT interval in the electrocardiogram (ECG) [2]. In addition to these symptoms JLNS patients suffer from sensorineural deafness [3]. Mutations in sixteen genes encoding cardiac ion channels and associated proteins have so far been identified as responsible for all genotype-positive LQTS [4,5]. KCNQ1 (also known as KVLQT1) and KCNH2 (also known as human ether-a-go-go-related gene, HERG) are the two most common LQTS genes that mutations in which account for approximately 95% of cases [6]. In this report, four patients with LQTS were analyzed to identify the mutations present in the three most common LQTS genes: KCNQ1, KCNH2 and SCN5A.

Materials and Methods

Clinical evaluation

For further clinical assessments, four unrelated LQTS families were referred to the emergency unit at the Rajaei Cardiovascular Medical and Research Center, Tehran, Iran.

LQTS patients

Family 1 (RW): The family study was conducted using three members in two generations. The proband was a 3-year-old boy (2:1 in Figure 1A), who was referred to the emergency unit of hospital Rajaei in Iran, due to recurrent syncope, multiple episodes of seizure and convulsion. A 12-lead electrocardiography was done. QTc interval was 560 ms (Figure 2A). The echocardiography showed normal cardiac structure. No auditory phenotype was detected in the proband. A proband’s sibling died suddenly at 16 weeks of age (2:2 in Figure 1A). The patient’s parents did not show any symptom, although they were also subjected to ECG studies.

Family 2 (RW): The proband (3:1 in Figure 1B) was a 15-years-old boy with a history of repeated syncopal events since the age of 2 years and seizure since the age of 9 years. He also had a history of palpitation around the age of 8 years. His QTc interval was 510 ms (Figure 2B). He showed a normal cardiac structure in the echocardiography and no auditory phenotype was detected in the proband.

Family 3 (JLNS): In this family, there were three siblings (4:5 in Figure 3A) with one deaf who was an 8-year-old boy referred because of recurrent episodes of syncope. He had a history of seizures since the age of 4.5. QTc was markedly prolonged in the index patient, 580

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**Figure 1:** Pedigrees and mutation confirmation. A: Family pedigree for the patient 1. B: DNA Sanger sequencing confirmation for c.1838C>T (p.T613M) mutation in the patient 1 with heterozygote condition (lower) and normal parents (upper and middle). C: Family pedigree for the patient 2. D: DNA Sanger sequencing confirmation for c.934A>T (p.T312S) mutation in the patient 2 with homozygote condition (lower), unaffected heterozygote parents (middle) and normal sequence (upper). SAB: Spontaneously Abortion.

**Figure 2:** ECG of the patients. A: The 12-lead electrocardiogram of the patient A at 3 years of age (QTc 560 ms in lead II). B: The 12-lead electrocardiogram of the patient B at 15 years of age (QTc 510 ms in lead II).
ms (Figure 4A). As a result of the high-risk situation, an endocardial Implantable Cardioverter Defibrillator (ICD) was implanted. Propranolol with a dose of 5 mg/kg/day, divided three times a day, was started for the patient. The Sensorineural deafness was diagnosed at 2.5 years and is managed with hearing aids. Heterozygous carrier parents had no clinical phenotype.

**Family 4 (JLNS):** The proband was a 9-year-old boy (4:6 in Figure 3B) with a history of recurrent syncope and seizures since the age of 2 years. He was deaf and had cochlear implants at 3 years. After referral to our arrhythmia clinic, an endocardial Implantable Cardioverter Defibrillator (ICD) was implanted. Propranolol was started (5 mg/kg/day, divided three times a day), which suppressed his symptoms. The ECG of the proband showed a QTc of 560 ms (Figure 4B). The parents and his brother were without any cardiac events. Further family history was unavailable.

**DNA isolation**

After obtaining informed consents and study approval by the ethics committee of the Pasteur Institute of Iran and Rajaie Cardiovascular Medical and Research Centre (adopted from the 1975 Helsinki Declaration), blood samples were collected from patients and their family members in tubes containing EDTA. Genomic DNA was isolated from the peripheral blood according to the standard salting out protocol [6,7].

**Haplotype analysis**

Haplotype analysis for LQT1–3 (loci) was performed for the detection of related genes in such heterogeneous cardiac diseases, in the families with the utility of a set of 16 polymorphic short tandem repeat (STR) markers which including D11SD8.3, D11SU10.9, D11SU2.2, D11SU0.6, D11SD13.6 (LQT1) as described in detail by Amirian et
al., D7SU7, D7SU4.8, D7SU3, D7SD5, D7SD6, D7SD9 (LQT2); and D3SU11, D3SU10.8, D3SU2.5, D3SI, D3SD5.6 (LQT3) as described in detail by Zafari et al. [8]. All STRs which were unique were selected among penta or tetra-nucleotide repeat markers and were located upstream or downstream of the genes at a distance of less than 1.4 Mb. Size determination of the repeats was performed on ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Mutation analysis

The patients were screened for the pathogenic variant by Sanger sequencing. The primer pairs for all coding exons and exon-intron boundaries and untranslated regions of three common LQTS-causative genes: KCNQ1 [NM_0002218], KCNH2 (NM_0002238) and SCN5A (NM_198056.2) were designed by primer 3 online and gene runner software (the sequence of primers are available upon request). Exons of the three genes were amplified by PCR and the purified PCR products were sequenced with both amplification primers, forward and reversed by sanger sequencing chain termination method on ABI 3130XL Genetic Analyzer by Kawsar Biotech Co. (KBC, Tehran, Iran) [9].

In silico variant analysis

The novel recessive variant found in patient was interpreted by a variety of in silico predictive programs. For predicting the functional impact of the variant on the protein, MutationTaster, HSF, Mutation Assessor, SIFT, PolyPhen-2 and FATHMM were used. The secondary structure of the protein was predicted by I-TASSER server. GERP++, PhyloP and PhastCons methods were also used to predict the conservation score [10-17].

Family and population study

Genealogy and co-segregation analyses were performed for all members of the family with available DNA samples for the novel missense variant. A total of 100 unrelated healthy individuals with the same ethnicity were collected to detect the genotype by amplification refractory mutation system (ARMS) PCR for the identified variant c.934A>T in the population.

Table 1: In silico analysis results for the variant p.T312S.

| S. no | Predictive Software | Prediction | Score |
|-------|---------------------|------------|-------|
| 1     | SIFT                | Deleterious| 0     |
| 2     | PolyPhen-2          | Probably damaging | 0.98 |
| 3     | Mutation Taster     | Protein features (might be) affected Splice site changes | 0.98 |
| 4     | FATHMM              | Damaging   | -9.35 |
| 5     | SNPs and GO         | Disease    | 7     |
| 6     | PhyloSNP            | Disease    | 4     |
| 7     | HSF                 | Alteration of an Exonic ESE site Potential alteration of Splicing | - |
| 8     | GERP++              | Evolutionary constraint | 3.93 |
| 9     | PhyloP              | Evolutionary conserved | 4     |
| 10    | PhastCons           | Evolutionary conserved | 1     |

LQTS was previously reported for human HERG gene [18]. Paternity test based on microsatellite repeat length (VNTR) and RFLP analysis excluded any non-paternity/non-maternity. Additionally, haplotype analysis showed his parents shared the same haplotype (data not shown). This suggests that the C>T change in codon 613 of the KCNH2 gene in the proband is either a de novo mutation in the proband or a germ line mosaic mutation in the parent.

Family 2 (RWS): Bidirectional Sanger sequencing revealed that a novel homozygous missense KCNQ1 gene variant c.934A>T (ClinVar accession number: SCV000700208) resulted in Threonine to Serine substitution at position 312 (p.T312S) (Figure 1D). The variant was neither reported in 1000 Genome nor Exome Aggregation Consortium (ExAC) and was absent in 200 control alleles in the normal population. The proband shared the same haplotype in the parent (data not shown) and cosegregation analysis confirmed that the variant was associated with LQTS. The p.T312S variant identified in this study has not been reported previously. The predictive software (PhyloP, PhastCons and GERP++) determined that the Threonine 312 position on the protein was identified as a coil structure by I-TASSER server with an almost high confidence value (Figure 5).

Family 3, 4 (JLNS): Haplotype analysis encompassing the three candidate genes showed homozygosity of the STR markers around the KCNQ1 gene in two JLNS index cases (Figure 4). This resulted to evaluation of the KCNQ1 gene in these families. During DNA sequence analysis of the candidate gene, a single, homozygous nucleotide substitution c.477+5G>A was detected in the KCNQ1 gene of the two JLNS probands (Figure 3C). The mutation was found in heterozygous form in the parents (3:1 and 3:2 in Figure 4A and 3:4 and 3:5 in Figure 4B), and also in the other living sister and brother of patient 3 and patient 4, respectively (4:4 in Figure 3A and 4:5 in Figure 3B). This sequence change that resulted to a splice site alteration in intron 2 of the gene has already been reported as pathogenic in the literature [19]. Likewise splice site prediction tools interpreted this splice variant to be pathogenic due to disruption of probable splicing donor site.

Discussion

In the present study KCNQ1, KCNH2, and SCN5A genes were screened in four Iranian families among a cohort of 31 unrelated LQTS patients. A de novo homozygous missense mutation in exon 7 of the KCNH2 gene was detected in a RW patient and a novel homozygous missense KCNQ1 gene variant in another RW patient. JLNS patients in two families had homozygous splice site mutation in the KCNQ1 gene. In LQTS type2 (KCNH2) and type3 (SCN5A) with autosomal dominant
inheritance pattern, and catecholaminergic polymorphic ventricular tachycardia (CPVT) (RyR2), de novo mutations occur frequently [20-22]. The c.1838C>T substitution has previously been reported in Japan, Netherlands, and USA in association with LQTS patients and in a fetus with prenatal diagnosis of LQTS by fast next generation sequencing [23-25]. This is the first time that the same mutation with a de novo pattern was found in an Iranian family. Similar variant (p.T613A) affecting the same residues have been identified in association with Long QT syndrome [18]. Hence, it is possible that the variant detected in this study is more likely pathogenic with a similar effect. Additionally, mutation c.1838 C>T is located in a CpG sequence in the pore helix of the Kv11.1 protein between S5 and S6 potassium channel domains in which common missense mutations such as Y611H, V612L, T613A, and A614V are frequently reported [18,26]. Physiological studies have revealed that p.T613M mutation inhibited normal trafficking of the HERG protein to the surface membrane and caused a loss of function as well as a decrease in cell surface protein [24]. The majority of HERG mutations, more than 200 reported, have loss of function mechanism; which is, a considerable decrease of the protein product caused by the mutated allele [27]. 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 (2:2 in Figure 3A) who died at 16 weeks of age.

A new homozygous missense KCNQ1 gene variant (p.T312S) was identified in the pore region of the human cardiac potassium channel of a RW family. The mutation causes a substitution from threonine to serine, which is well known for its ability to disrupt the secondary structure of the protein. Threonine 312 is located in the extracellular loop of the pore forming within a coil structure which is evolutionary conserved in mammals. The pathogenicity of Threonine substitution at codon 312 with Serine as a novel variant was predicted using all the online predictive tools (Table 1). Additionally, the occurrence of a missense variant in the pore forming alpha subunit of the KCNQ1 protein is expected to be likely pathogenic with an estimated predicted value (EPV) of 94% [28]. Based on previous reports disruption in the pore region of KCNQ1 and KCNH2 in RW families because of full loss-of-function of potassium channels Kv7.1 and Kv11.1 could lead to a severe LQTS phenotype [23,29]. Since the recessive mutation did not detect in 200 normal chromosomes, ruled out a typical polymorphism. Overall, according to the American College of Medical Genetics and Genomics guidelines the recessive KCNQ1 variant is classified as a likely pathogenic variant [30]. The clinical manifestations of the prolonged QT interval of about 510 ms, recurrent episodes of syncope, palpitation and seizure were in concordance with the molecular testing result of the patient. The family history of cardiac events such as fainting in the proband’s mother and convulsion episodes in family members has also been presented. This finding indicates that along with clinical evaluations, genetic testing should be done for detection of family members who are at risk.

An intronic homozygous mutation was detected in the splice regulatory site at intron 2 of the KCNQ1 gene in two children from two unrelated families which co-segregate with the LQT1-JLNS phenotype. It is important to emphasize that as a given LQTS-associated variant, the variant must have involved a conserved amino acid or splice site that change the primary structure of the encoded protein [19]. As described in previous studies from Iran [9,31], although JLNS causing mutations in KCNQ1 are mostly truncating and result in complete abolishment of IKs channel function [32], but intronic mutations also besides frameshift mutation lead to full skipping of KCNQ1, thereby causing transcriptional deviance. For preservation of hearing, IKs channel is required for K+ conduction in the inner ear endolymph. Homozygous or compound heterozygous mutations of KvLQT1 result to complete loss of IKs function, insufficient endolymph production, decadence of Corti organ, and consequently sensorineural deafness [33]. Functional analysis with the splice mutant KCNQ1 channel revealed that there was no recognizable IKs current, which is concurrent with the hearing loss [34]. Both homozygous carriers from two unrelated families as a true JLNS phenotype experienced severe cardiac arrhythmias with considerably prolonged QTc and deep congenital neural deafness. Both patients had their first episode of syncope before the age of 2. This splice mutation is associated with a high risk of sudden death if untreated.

**Conclusion**

Altogether, in our cohort, two LQTS missense mutations and one splice site KCNQ1 mutation were identified in four index cases in our cardiogenetic clinic in Iran. This indicates that the prevalence of LQTS is inadmitably much higher in Iran than what is expected as a result, which exigencies a widespread observation in this country. Further, in the current study a novel recessive mutation in an AR-RWS patient was found for the first time in the Iranian population. Till date, only one report of mutation screening in four AR-RWS patients of Iranian origin has been described and the current study adds to this slight literature [35]. This study helps the development of genetic testing for arrhythmia susceptibility. Finally, mutation identification is of importance for pre-symptomatic diagnosis and treatment, in some cases.

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**Conflict of Interests**

None of the authors have any conflict of interests with regard to this publication.
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