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Original Article

Lack of association between coding region of *KCNE2* gene and the congenital long QT syndrome in an Iranian population

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Abstract

Introduction: Congenital long QT syndrome (LQTS) is a cardiac disorder characterized by QT interval prolongation at basal ECG. Different LQTS genes encode ion channel subunits or proteins involved in regulating cardiac ionic currents. *Long QT syndrome type 6* (*LQT6*) is caused by mutation in the *KCNE2* gene. Our research aimed to analyze genetic variants of *KCNE2* gene causing the disease in Iranian population.

Methods: Twenty nine patients consented for participation in the study. They were diagnosed based on Schwartz's criteria. After DNA extraction from peripheral blood cells, two exons of the *KCNE2* gene were amplified. Afterwards, PCR-SSCP was carried out for screening the possible mutated gene variants. As the last verification step, direct sequencing was done to determine the sequence.

Results: All samples were detected by PCR-SSCP and sequenced. None of the patients had the mutation in the *KCNE2* gene.

Conclusion: Investigating a genetic variant associated with LQTS, in Iranian patients clinically diagnosed with LQT6, no association was found between the disease and *KCNE2* gene. Other previously identified genes, especially the major genes, should be considered for further investigation.

Keywords:
KCNE2 gene;
Long QT syndrome;
Polymorphism;
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Introduction

The congenital long QT syndrome (LQTS) is an autosomal dominant genetic disorder of cardiac electrical repolarization. It is characterized by delayed repolarization of the ventricular myocardium, QT prolongation, and increased risk for torsades des

pointes (TdP)-mediated syncope, seizures, and sudden cardiac death (SCD), in otherwise healthy young individuals with normal heart (Vincent, 1998; Mohler et al., 2003). Its incidence has been reported to be around 1 in 10,000 births. To date, three major LQTS and ten minor LQTS susceptibility genes that account for nearly 80% of the disorder have been identified (Abriel et al., 2001). This disorder is caused by mutations mostly in genes encoding for cardiac ion channels. The defective gene causes an altered ion channel function with prolonged action potential and propensity to torsade de pointes ventricular tachycardia (Goldenberg et al., 2008; Aizawa et al., 2015). Indeed, ion channels do not operate in isolation, rather function as macromolecular complexes consisting of the ion channel pore-containing α subunits as well as auxiliary β subunits and other regulatory proteins that interact with and influence ion channel activation and deactivation/inactivation (Summers et al., 2010). Nearly 75% of patients who have been clinically diagnosed with LQTS, have mutations in one of three major LQTS susceptibility genes that encode ion channel α subunits and are critical for the coordination of the cardiac action potential: KCNQ1-encoded IKs potassium channel, KCNH2-encoded IKr potassium channel, or SCN5A-encoded INa sodium channel. The ten minor LQTS susceptibility genes encode other ion channel α subunits (CACNA1C, KCNJ5), key cardiac potassium (AKAP9, KCNE1, KCNE2) and sodium-channel (CAV3, SCN4B, SNTA1) interacting proteins, or calcium-binding messenger proteins (CALM1, CALM2) (Splawski et al., 2000; Hedley et al., 2009; Kloth et al., 2015). Because these genes play a minor role in the genetic basis of LQTS, only limited genotype-phenotype correlations have been established. The *KCNE-1* and *KCNE-2* genes encoding β auxiliary subunits minK and MiRP 1, were the first found to be implicated in the pathogenesis of LQTS through their

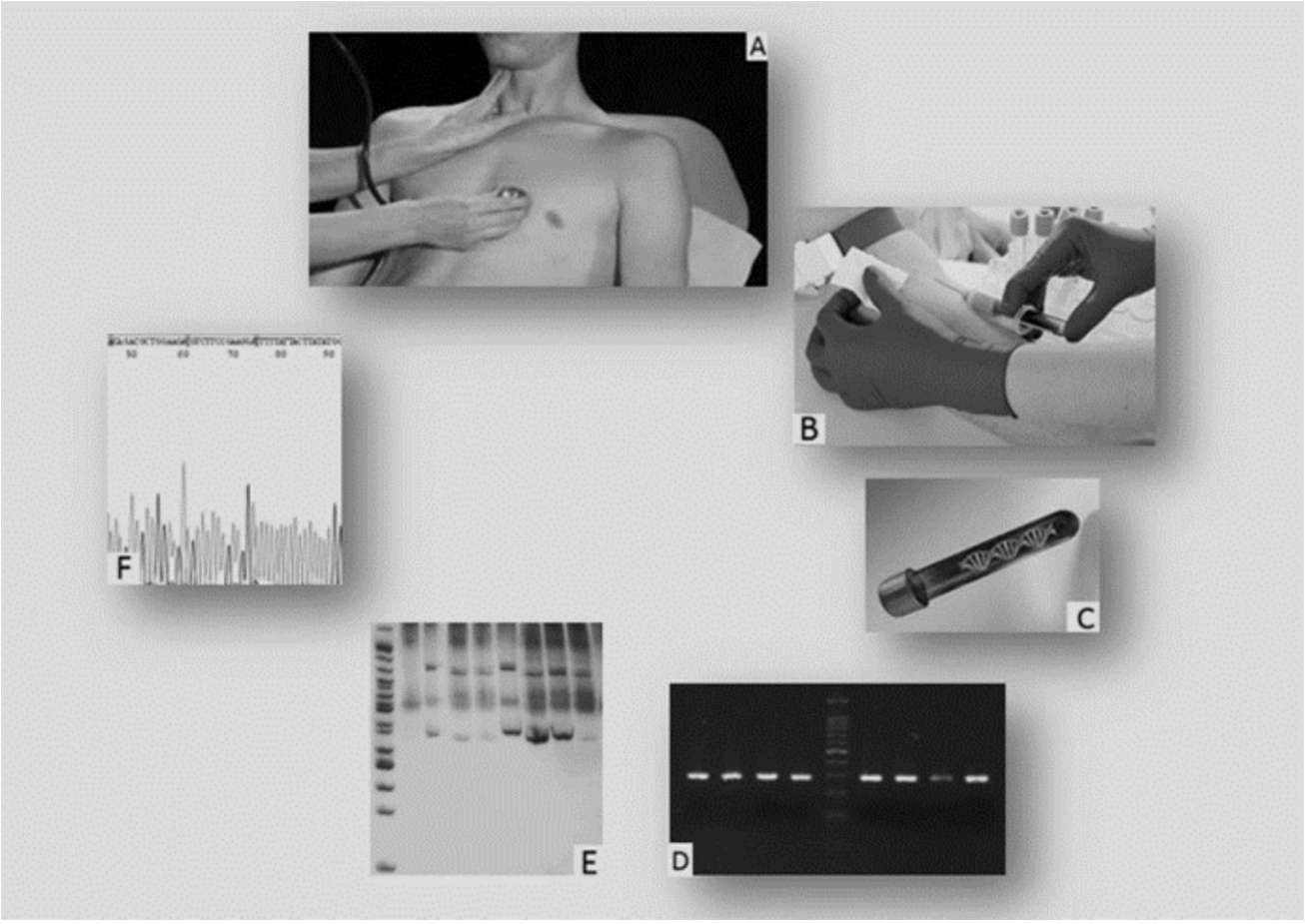

Fig.1. An overview of the research process: (A) The patients were diagnosed by cardiologist and (B) peripheral blood was taken. Then, (C) genomic DNA was purified and polymerase chain reaction was performed using KCNE2 specific primers. agarose gel electrophoresis was carried out to confirm amplification (D). SSCP was performed to determine the possible nucleotide changes. (E). Finally, the suspected cases were selected for the direct sequencing**.**

Table 1: PCR primers for coding regions of *KCNE2* gene

Primer name	**Oligonucleotide sequence, from 5′ to 3′**	**Amplicon size (bp)**	**Reference**
EX2.1	TCCGTTTTCCTAACCTTGTTC	255	(Larsen et al., 2001)
	GCCACGATGATGAAAGAGAAC		
EX2.2	GATGCTGAGAACTTCTACTATG	289	(Larsen et al., 2001)
	GTCTGGACGTCAGATGTTAG		

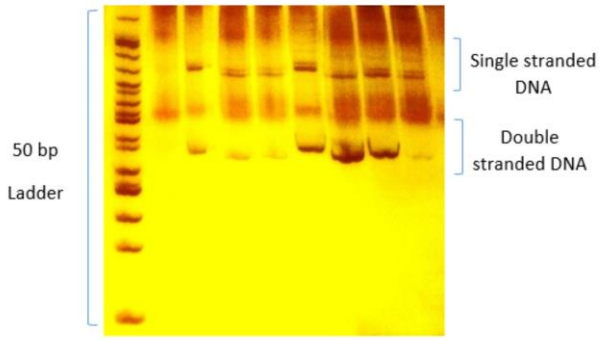

Fig.2. SSCP pattern of PCR samples in native PAGE. The direction of electrophoresis was from top to bottom.

modulatory effect on potassium channels (Nakajo and Kubo, 2015). Despite the large number of variants have been identified so far, the search for more gene variants associated with LQTS is an ongoing process. In our study, we intended to analyze the *KCNE2* gene in terms of mutations in an Iranian population.

Materials and methods

Study subjects

Peripheral blood samples were collected from 29 patients referred to arrhythmia clinics and specialized heart hospitals between 2011 to 2013. The control group consisted of 34 healthy individuals matched for age and sex. LQTS patients were diagnosed by expert cardiologists according to Schwartz criteria and all inclusion and exclusion criteria were also defined (Schwartz, 1985). The study was approved by the Ethics Committee of Golestan University of Medical Sciences and written informed consent was obtained from all subjects.

DNA extraction and genotyping

Genomic DNA was extracted and purified from whole blood using the boiling method. PCR was performed in a total reaction volume of 25µl including, 100 ng of total DNA,5 mM KCl, 200 mM Tris-HCl (pH 9), 20 mM MgCl2, 10 mM of dNTP mix (2.5mM of each dNTP), 10 pm of each forward and reverse primer and 1 U of Taq. PCR conditions were as follows: 95ºC for 5 minutes for initial denaturation, 95ºC for 30 seconds, 60ºC for 30 Sec., 72ºC for 40 Sec., 30 times for the last 3 steps and 72ºC for 5 min for final extension and 8ºC for 3 min for holding temperature. The PCR primers are noted in table 1. To achieve conformational complexes, we applied 2X SSCP gel loading dye (95% formamide and 20 mM EDTA) and heated the mixture in 90ºC for 5 minutes to denature the DNA strands, and then immediately cooled the mixture on ice to get the proper conformation. A positive control sample for KCNE2 gene was also used to ensure the accuracy of the experiment. The 12% PAGE was prepared with acrylamide/ bisacrylamide, TBE 5X, 10% APS (ammonium persulfate), TEMED (Tetramethylethylenediamine) and distilled water. We adjusted the PAGE temperature to 4ºC to gain a proper resolution of single-stranded DNAs. The samples were detected by silver nitrate staining, shown in figure 2.

Data analysis

This pilot study was carried out to identify the variants

Table 2: *KCNE2* gene polymorphisms of different populations

Population	Sample size	Mutation detectionmethod	SNP/ mutation	New SNP /mutation	References
Ashkenazi Jewish	579	Pyrosequencing	8	No	(Gordon et al., 2008)
Singaporean	13	Sanger sequencing	0	No	(Koo et al., 2007)
Chinese	NA[1]	Sanger sequencing	0	No	(Du et al., 2007)
Danish	254	SSCP	2	No	(Christiansen et al., 2014)
Italian	430	dHPLC[2]	3	2	(Napolitano et al., 2005)
European	40	SSCP-HD[3]	2	1	(Larsen et al., 2001)
Japanese	NA	SSCP	0	No	(Iwasa et al., 2000)
Iranian	63	SSCP-Sanger sequencing	0	No	Present study

[1]Not Available
[2]Denaturing High Performance Liquid Chromatography
[3]Heteroduple

Table 3: Known mutation types in *KCNE2*

Accession Number	Mutation type	Codon number	Amino acid change	Disease/phenotype	Ethnicity	Reference
CM003449	Missense	8	Thr-Ala	Long QT interval, drug induced, association with	Caucasian	(Sesti et al., 2000)
CM993507	Missense	9	Gln-glu	Cardiac arrhythmia	African American	(Abbott et al., 1999)
CM055291	Missense	10	Thr-Met	Long QT syndrome	-	(Tester et al., 2005)
CM097710	Missense	14	Val-Ile	Long QT syndrome	-	(Kapplinger et al., 2009)
CM097711	Missense	20	Ile-Asn	Long QT syndrome	-	(Kapplinger et al., 2009)
CM097712	Missense	27	Arg-His	Long QT syndrome	-	(Kapplinger et al., 2009)
CM042723	Missense	27	Arg-Cys	Cardiac arrhythmia	Chinese	(Yang et al., 2004)
CM993508	Missense	54	Met-Thr	Cardiac arrhythmia	Caucasian	(Abbott et al., 1999)
CM993509	Missense	57	Ile-Thr	Cardiac arrhythmia	Hispanic	(Abbott et al., 1999)
CM064064	Missense	60	Phe-Leu	Long QT syndrome	French	(Millat et al., 2006)
CM021618	Missense	65	Val-Met	Long QT syndrome	Germany	(Isbrandt et al., 2002)
CM097713	Missense	65	Val-Leu	Long QT syndrome	-	(Kapplinger et al., 2009)
CM097714	Missense	77	Arg-Gln	Long QT syndrome	-	(Kapplinger et al., 2009)
CM064063	Missense	77	Arg-Trp	Long QT syndrome	French	(Millat et al., 2006)
CM097715	Missense	94	Glu-Gly	Long QT syndrome	-	(Kapplinger et al., 2009)
CM003450	Missense	116	Ala-Val	Cardiac arrhythmia	Caucasian	(Sesti et al., 2000)
CD057241	Small deletions	51	TCATC(51)CTGTACCTCAT	Long QT syndrome	-	(Napolitano et al., 2005)
CD097716	Small deletions	122	AAATG(122)TCCC	Long QT syndrome	-	(Kapplinger et al., 2009)
CI057269	Small insertions	55	CCTCATG(55)GTG	Long QT syndrome	Italian	(Napolitano et al., 2005)
CM055291	Missense	10	Thr-Met	Cardiac arrhythmia	Ashkenazi Jewish	(Gordon et al., 2008)

of *KCNE2* gene possibly involved in congenital LQT6 in an Iranian population since there is no data available about the genetic polymorphisms of this gene in our country. To investigate the variants, all sequence data were aligned with the reference sequence using CodonCode Aligner software.

Results

Twenty-nine patients and 34 controls were analyzed for *KCNE2* gene variants using conventional PCR, and the specific amplicons were confirmed by gel electrophoresis. SSCP method was used for the final detection of the single nucleotide polymorphisms.

DNA sequencing was randomly carried out in both directions by Sanger sequencing method using the primer pairs (shown in table 1) by ABI 3730 XL Sanger sequencing instrument. The concentration of PCR amplicons and sequencing primers were 120ng/µl and 10pmol/µl respectively. Based on the obtained results, none of the fragments showed novel or previously known mutations (table 3).

Discussion

Long QT syndrome is a rare and serious disease because of its high rate of mortality (Schwartz et al., 2012; Khori et al., 2013; Changizi et al., 2015). Detection of mutations involved in this disease would support better diagnosis and make better prognosis (Allegue et al., 2010; mazandarani et al., 2011). Nonetheless, far little attention has been paid to LQTS in Iran. Among the LQT causative genes, *KCNE2* is known to have a role in long QT syndrome type 6. Its molecular characterization may assist clinicians and researchers to screen exposed people more effectively (Watanabe et al., 2009). SSCP analysis is widely used to screen or detect disease-causing mutations in hereditary disorders (Hoshino et al., 1992; Gasser et al., 2006; Medlock et al., 2012). No SNP and mutation detected in this study proved low allele frequency of *KCNE2* gene. Other researches has also verified the low allele frequency of *KCNE2* gene, as displayed in Table 2. Due to the very low prevalence of disease, and as expected, no mutation was found in the population studied herein. Thus, our results were consistent with previous studies (table 2). Although, no alteration was observed in KCNE2 gene using SSCP-PAGE method, thirty-five *KCNE2* amplicons were sequenced randomly to confirm the SSCP results. The lack of genetic alteration may be partly due to small sample sizes and the diverse populations and ethnicities. Therefore, we recommend the use of a greater sample sizes and more sensitive methods such as next generation sequencing (NGS) as indicated in Gordon et al study (Gordon et al., 2008). It should also be noted that long QT is a multifactorial disease and *KCNE2* gene mutations are responsible for only less than 2% of the cases; that's why, other genes should be also considered (Splawski et al., 2000; Digby et al., 2010). The potential lethality of LQTS necessitates improvements in biological and technological approaches in the management and treatment of the disease to alleviate some public health concerns.

Given that the genetic testing options often reach the market with less cost-effectiveness than do pharmaceuticals or other medical technologies, this could be a cause for elevated public health concerns (Bennett and Guthrie, 2003). Besides, the LQTS genotype is reported to have diagnostic and prognostic value, also have an increased potential to impact the clinical decision-making (Wilde and Pinto, 2009). However, in our study the patients did not show any mutations in the *KCNE2* gene, perhaps harboring mutations in other LQT genes.

Conclusion

Taken together, the patients form Iranian population did not exhibit any mutation in the *KCNE2* gene though, they may carry mutations in other LQT genes. As a result, *KCNE2* gene might not represent the disease-causing mutation in our population. Further research is suggested to examine other genes involved in congenital long QT syndrome. Also, the use of larger sample size and High-throughput screening (HTS) methods are suggested.

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Conflict of interest

None of the authors have any conflict of interest.

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