

# Genetic analysis of cardiac SCN5A Gene in Iranian patients with hereditary cardiac arrhythmias

Marzi Asadi, Roger Foo<sup>1</sup>, Zahurul Alam Bhuiyan<sup>2</sup>, Mohammad Reza Samienasab\*, Ahmad Reza Salehi, Shahab Shahrzad<sup>3</sup>, Rasoul Salehi

Departments of Genetics and Molecular Biology and \*Cardiovascular Diseases, School of Medicine, Isfahan University of Medical Sciences; Isfahan-Iran  
<sup>1</sup>Cardiovascular Research Institute, and Genome Institute of Singapore, Yong Loo Lin School of Medicine, National University of Singapore, Singapore-Singapore

<sup>2</sup>Service of Medical Genetics, Department of Laboratories, the University Hospital of Lausanne; Lausanne-Switzerland

<sup>3</sup>Electrophysiology Department, Shaheed Rajaei Cardiovascular Medical and Research Center, Tehran University of Medical Sciences; Tehran-Iran

## ABSTRACT

**Objective:** *SCN5A* encodes alpha subunit of the major sodium channel (Nav1.5) in human cardiac tissue. Malfunction of this cardiac sodium channel is associated with a variety of cardiac arrhythmias and myocardial inherited diseases.

**Methods:** Fifty-three members from three families each diagnosed with long-QT syndrome type 3 (LQTS3), Brugada syndrome (BrS), or sick sinus syndrome (SSS) were included in this observational, cross-sectional study. In this study, we analyzed the sequences of coding region of the *SCN5A* gene.

**Results:** Eleven members of the LQTS family (39%) showed *p.Gln1507-Lys1508-Pro1509del* mutation, 8 of BrS family (50%) showed *p.Arg222Ter* nonsense mutation, and 5 of 9 SSS family members (55%) showed a novel *p.Met1498Arg* mutation in the *SCN5A* gene.

**Conclusion:** *p.Gln1507-Lys1508-Pro1509del* mutation, *p.Arg222Ter* nonsense mutation, and *p.Met1498Arg* in LQTS, BrS, and SSS, respectively, are reported for the first time in the Iranian population. Information regarding underlying genetic defects would be necessary for verifying certain clinically diagnosed arrhythmia types, carrier screening in affected families, and more precise therapy of the patients are required. (*Anatol J Cardiol* 2016; 16: 170-4)

**Keywords:** *SCN5A*, familial arrhythmias, LQTS, BrS, SSS, genetic analysis

## Introduction

*SCN5A* encodes alpha subunit of the major sodium channel (Nav1.5) in human cardiac tissue. The channel is expressed in multiple tissues with similar structure. The gene is located on chromosome 3p21 with 28 exons and encodes a 227KDa alpha subunit (1). Malfunctioning of this cardiac sodium channel is associated with a variety of cardiac arrhythmic and myocardial inherited diseases, such as long-QT syndrome (LQTS) (type 3), syndrome of right precordial ST elevation [Brugada syndrome, (BrS)], cardiac conduction disease (CCD), sinus node dysfunction (SSS), atrial fibrillation (AF), atrial standstill, and dilated cardiomyopathy (2). The role of *SCN5A* mutations in each of these diseases is being investigated and new aspects are being discovered every day. Previous studies showed that 10%–30% of subjects with BrS carry a mutation in *SCN5A*, including missense mutations, nonsense mutations, and nucleotide deletions or insertions (3, 4). More than 100 *SCN5A* mutations are linked to BrS. Virtually all mutations that are heterologously expressed (<50%) lead to sodium channel loss of function (3). On the other

hand, LQT-3 is linked to mutations in *SCN5A*, and covers approximately 13% of all genotyped individuals with LQTS. Until now, more than 80 *SCN5A* mutations have been identified in patients with LQTS-3 and nearly 50% of them have been heterologously studied. Most of these mutations are missense mutations, and are found to cause sodium channel gain of function (3). Recently, a number of studies have linked genetic defects in ion channels, including human Nav1.5 (hNav1.5), to familial SSS. Until now, 14 *SCN5A* mutations have been associated with this disease (5).

Variations in the *SCN5A* gene are associated with several other heart conditions; this includes familial heart block, an abnormality of the heart's electrical system that increases the risk of syncope and sudden death (6, 7). The role of *SCN5A* mutations in diagnosis and risk stratification of the attributed syndromes is still a topic of debate. Furthermore, some mutations in *SCN5A* are not associated with any sodium channel dysfunction (8).

In this study, 53 patients from three families clinically diagnosed with LQTS, BrS, and SSS were screened for *SCN5A* mutations and three different mutations, including a novel one were found in 23 of them.

**Address for Correspondence:** Dr. Rasoul Salehi, Department of Genetics and Molecular Biology School of Medicine Isfahan University of Medical Science, Isfahan-Iran Phone: 00 98 313 792 24 87 Fax: 00 98 311 3668 85 97 E-mail: r\_salehi@med.mui.ac.ir

**Accepted Date:** 31.03.2015 **Available Online Date:** 05.05.2015

© Copyright 2016 by Turkish Society of Cardiology - Available online at [www.anatoljcardiol.com](http://www.anatoljcardiol.com)  
 DOI:10.5152/akd.2015.6060



## Methods

### Patient selection

#### LQTS patients

The number of cases studied in the LQTS family was 28. The clinical criteria for LQTS diagnosis was the absence of intra- or inter-ventricular or atrio-ventricular conduction abnormalities (by demonstrating narrow QRS in standard 12-lead ECG); both members with normal QTc interval and prolonged interval with interpretable ECG, prolonged QTc was defined as more than 460 msec in females and 450 msec in males calculated with Bazett's Formula [ $QTc = QT \text{ Interval} / \sqrt{(RR \text{ interval})}$ ] (Fig. 1); and exclusion of electrolyte abnormalities and coronary artery disease, which can mimic LQTS ECG pattern in patients with prolonged QTc interval.

#### BrS patients

Sixteen patients, belonging to a family diagnosed with BrS were included in this study.

BrS-ECG is defined as ST segment elevation with type-1 morphology  $>2$  mm in at least 1 lead among the right precordial leads V1, V2, positioned in the 2<sup>nd</sup>, 3<sup>rd</sup>, or 4<sup>th</sup> intercostal space, occurring either spontaneously or following provocative drug test with intravenous administration of class I antiarrhythmic drugs (4) (Fig. 2). Other abnormalities mimicking BrS ECG pattern, including electrolyte abnormalities, coronary artery disease (by exercise stimulation test), and cardiac structural abnormalities (by normal echocardiography), were ruled out.

#### SSS patients

The inclusion criteria for the 9 selected patients from a family diagnosed with SSS were: consumption of no drug that can mimic SSS such as beta blockers, exclusion of other abnormalities mimicking SSS ECG pattern including electrolyte abnormalities and coronary artery disease (by myocardial perfusion scan) (Fig. 3), and we finally defined SSS as either symptomatic sinus bradycardia of less than 40 beats per min or symptomatic sinus pause of more than 3 s in 48 h Holter monitoring, or symptomatic patients with corrected sinus node recovery time of more than 525 msec in electrophysiological studies.

#### Genetic analysis

Genomic DNA was isolated from peripheral blood lymphocytes using QIAamp DNA blood mini Kits (Qiagen, USA). The entire coding region (exons, 2–28) and exon-intron boundaries of *SCN5A* were amplified using specific oligonucleotide primers (9). PCR amplification performed in 25  $\mu$ L volume using conventional 10 $\times$  buffer, annealing temperature varied from 52 to 66 for different exon amplification. All amplicons were subjected to direct sequencing and then compared with the *SCN5A* sequence in the Ensemble database (reference *SCN5A* ENSG00000183873; www.ensembl.org).

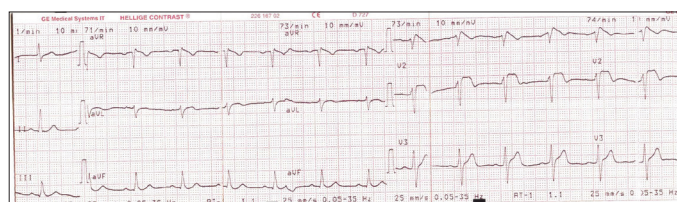


Figure 1. LQTS ECG of 1 of our patients (individual II-7 of LQTS pedigree)



Figure 2. Type 1 ECG pattern of BrS (individual II-7 of BrS pedigree)

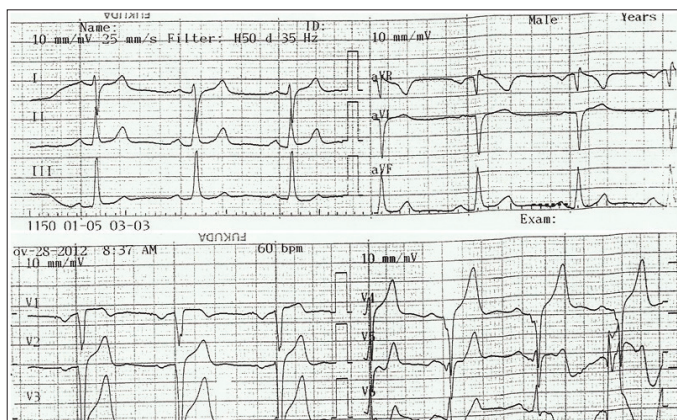


Figure 3. ECG of 1 of SSS patients (individual III-1 of SSS pedigree)

## Results

Fifty-three individuals, belonging to three families of LQTS, BrS, and SSS were studied. Twenty-eight members of the LQTS family, 16 of the BrS family, and 9 of the SSS family comprised our study population. The clinical characteristics of these patients (Table 1), clinical phenotypes of the symptomatic mutation carriers (Table 2), and molecular characteristics of mutations (Table 3) are shown.

#### p.Gln1507-Lys1508-Pro1509del mutation

The pedigree of the LQTS family is shown in Figure 4a. Sequencing results showed that in LQTS family 11 members carried the *p.Gln1507-Lys1508-Pro1509del* mutation. These nine base pairs deletion (CAGAAGCCC) were located in exon 26 of *SCN5A* gene (Fig. 4b). Eleven patients with this mutation had prolonged QTc interval according to our definition; 1 had normal baseline ECG interval, 2 had implanted cardioverter defibrillator (ICD) due to aborted sudden cardiac death, and others were asymptomatic. Sixteen of them had normal ECG with no mutations.

#### p.Arg222Ter nonsense mutation

Pedigree of the BrS family is shown in Figure 5a. Sequencing results in BrS patients revealed *p.Arg222Ter* mutation. This nonsense mutation (*p.Arg222Ter*) is located in exon 6 of the *SCN5A* gene (Fig. 5b). Two of the patients had ICD implant due to aborted sudden cardiac death.

**Table 1. Clinical characteristics of patients**

Proband	Number of patients	Number of positive mutation screened	Age	Male gender (n)	Male with mutation	SCN5A mutation
LQT <sup>a</sup>	28	11	30±2	13	4	Deletion in exon 26
BrS <sup>b</sup>	16	8	32±5	7	4	Nonsense mutation in exon 6
SSS <sup>c</sup>	9	5	25±3	7	4	Missense mutation in exon 26

<sup>a</sup>Long QT syndrome <sup>b</sup>Brugada syndrome <sup>c</sup>Sick sinus syndrome

**Table 2. Clinical characteristics of symptomatic mutation carriers and healthy mutation carriers**

Patient ID	Age of first symptom	Triggering factor	Cardiac arrest (LQT, BrS) (How many times)	Present treatment	Responding or not to treatment	Clinical diagnosis Index
<b>LQTS<sup>c</sup></b>						<b>QTcd (ms)</b>
II-1	35	Emotional stress	-	Beta blocker high dose	No	455
II-2	40	Emotional stress	-	Beta blocker high dose	No	460
II-4	40	Emotional stress	Once	ICDb	Yes	490
II-6	33	Emotional stress	-	Beta blocker high dose	No	480
II-7	36	Emotional stress	-	Beta blocker high dose	No	484
II-9	40	Stress	-	Beta blocker high dose	No	472
II-11	45	Crying	Twice	ICD	Yes	602
III-2	10	Emotional stress	-	Beta blocker high dose	No	463
III-3	15	Emotional stress	-	Beta blocker high dose	No	452
III-8	20	Crying	-	Beta blocker high dose	No	462
III-9	19	-	-	-	-	450
<b>BrS<sup>a</sup></b>						<b>ST segment elevation, mm</b>
II-7	26	Running	Twice	ICD	No	2.5
I-2	60	-	-	-	-	<2
II-1	47	-	-	-	-	<2
II-3	46	-	-	-	-	<2
II-5	45	-	-	-	-	<2
III-2	16	-	-	-	-	<2
III-3	12	-	-	-	-	<2
III-6	14	-	-	-	-	<2
<b>SSS<sup>e</sup></b>						<b>Bradycardia</b>
I-2	42	Physical activity	-	Pacemaker implantation	Yes	Yes
II-3	25	Physical activity	-	Pacemaker implantation	Yes	Yes
III-1	29	Physical activity	-	Pacemaker implantation	Yes	Yes
III-2	22	Physical activity	-	Pacemaker implantation	Yes	Yes
II-2	50	-	-	-	-	No
III-3	20	-	-	-	-	No

<sup>a</sup>Brugada syndrome <sup>b</sup>Implantable cardioverter defibrillator <sup>c</sup>Long QT syndrome <sup>d</sup>Corrected QT interval <sup>e</sup>Sick sinus syndrome

**p.Met1498Arg missense mutation**

The pedigree of the SSS family is shown in Figure 6a. Five members of this family showed *p.Met1498Arg* missense mutation in exon 26 (Fig. 6b). Four of them had pacemaker implantation (DR-PPM Implantation) due to abnormal heart rhythms.

**Discussion**

In the present study, we analyzed *SCN5A* mutations in cardiac arrhythmia syndromes associated with cardiac sodium channel (Nav1.5) dysfunction. Our study population included 53 members belong to three families. Clinically, they were diag-

**Table 3. Molecular characteristics of gene mutation finding**

Gene name	Nucleotide Change	Aminoacid change	Mutation Type	Location	Omim <sup>a</sup>	Reference (dbSNP)
SCN5A	<i>c.4519_4528del CAGAAGCCC</i>	<i>p. Gln1507-Lys1508-Pro1509del (Hetero)</i>	Deletion (in frame)	Exon 26	LQTS-3	Keller et al. (10)
SCN5A	<i>c.664C&gt;T</i>	<i>p.Arg222Ter (Hetero)</i>	Nonsense	Exon 6	BrS	Kapplinger et al. (11)
SCN5A	<i>c.4493T&gt;G</i>	<i>p.Met1498Arg (Hetero)</i>	Missense	exon 26	SSS	Novel

<sup>a</sup>Online mendelian inheritance in man

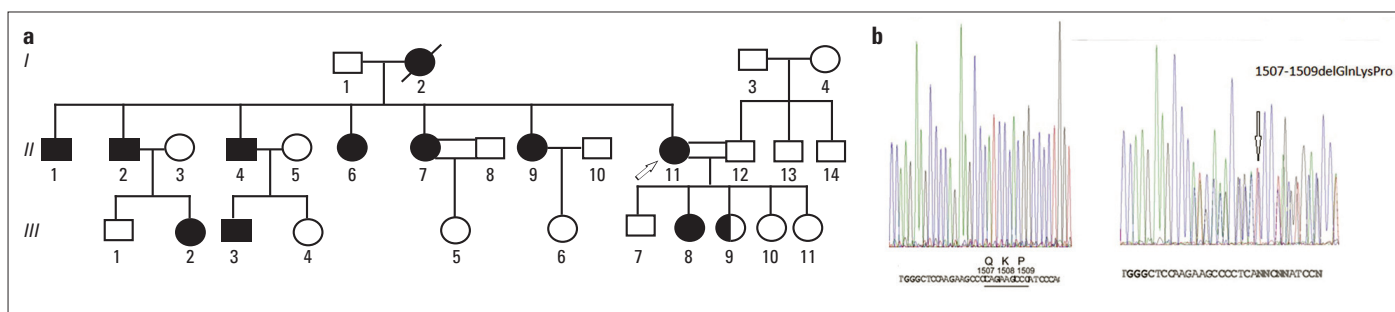
nosed with LQTS, BrS, and SSS; all inherited in an autosomal dominant manner.

The mutation finding in LQTS patients was first reported by Keller et al. (10), is located in the DIII-DIV linker region of *SCN5A* gene, and plays an important role in fast sodium channel inactivation. Regarding BrS patients, *p.Arg222Ter* nonsense mutation was previously reported by Kapplinger et al. (11), is located in the DI-S4 region of Nav1.5, and acts as a loss of function mutation. In the SSS family, we identified a novel *c.4493T>G* or *p.M1498R* missense mutation in DIII-DIV linker region of Nav1.5, which have not been previously reported.

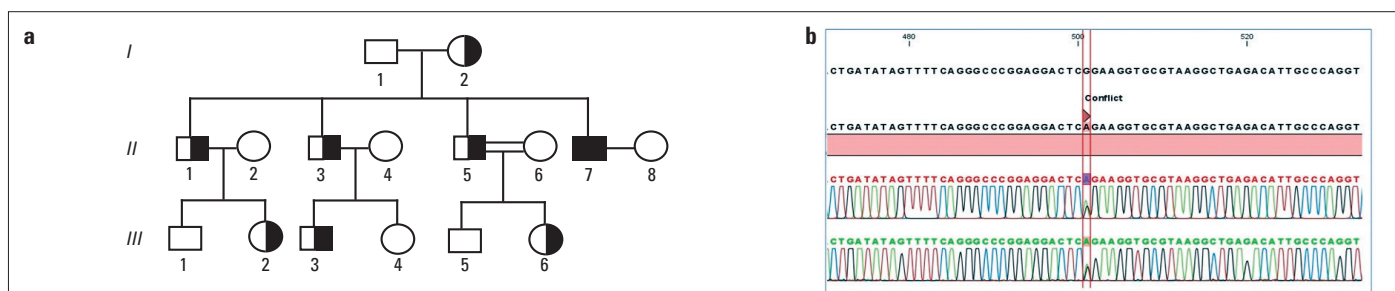
All the three mutations are being reported for the first time in the Iranian population. In the LQTS family, 11 patients (39% cases) had the same mutation; however, one of the mutation carriers remained with no detectable abnormality in his ECG. The remaining 17 members had neither the mutation nor any abnormal clinical findings. In the BrS family, eight patients were detected with *p.Arg222Ter* mutation; nevertheless, six of them had normal ECG in serial monitoring situations. The prevalence of the mutation in our limited study population emerged to be 50%, which is more than previous studies (12-14). The detected mutation in SSS patients was located in the cytoplasmic region

of Nav1.5 with a loss of function effect, i.e., attenuation of cardiac Na current (15). Napolitano et al. (16) reported *p.M1498T* mutation in a patient who was clinically diagnosed with LQTS phenotype; however, our cases showed a phenotype compatible to SSS, on the basis of ECGs findings (Fig. 3). Out of the five patients, two individuals with positive mutations had normal ECG in serial monitoring situations and the rest of them (II-3, III-1,2) had pacemaker implantation.

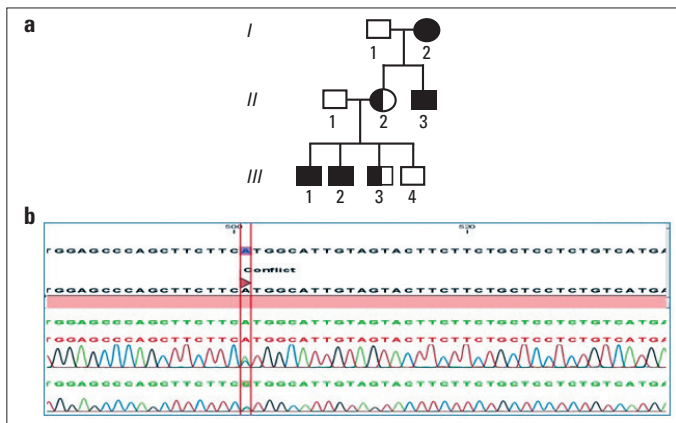
Of the three detected mutations, *c.4519\_4528del CAGAAGCCC*, *c.664C>T*, and *c.4493T>G*, two of them were located in exon 26 (66% of all patients with positive mutations), indicating the hotspot nature of this exon for LQTS-3 and SSS syndromes in the Iranian population, which is in concordance with a previous report (17). The information gained here would be beneficial in planning genetic screening methods for Iranian patients with cardiac arrhythmia. Although we found that in the LQTS family all except 1 (III-9) patient with ECG findings of prolonged QTc having the mutation, the clinical penetrance of this kind of mutation should be further evaluated. Detection of BrS, LQTS-3-associated, or SSS mutations can improve presymptomatic screening, enable better follow-up of asymptomatic patients, and facilitate choosing effective therapies earlier (17).



**Figure 4. a, b. (a) The pedigree of the LQTS family. The filled symbols show symptomatic mutation carrier individuals, half-filled symbols asymptomatic mutation carriers with normal ECG, (b) sequencing results in-frame deletion mutation in LQTS patients (right) in comparison to normal control (left)**



**Figure 5. a, b. (a) The pedigree of the BrS family. The filled symbols show asymptomatic mutation carriers, half-filled symbols asymptomatic mutation carriers with normal ECG, (b) sequencing result *p.Arg222Ter* mutation in a BrS patient**



**Figure 6. a, b. (a) The pedigree of the SSS family. The filled symbols show symptomatic mutation carriers, half-filled symbols asymptomatic mutation carriers with normal ECG, (b) sequencing result *p.Met1498Arg* mutation in SSS patient**

### Study limitations

This study was performed including 3 Iranian families. In future, more families should be included and screened to find out the prevalence of the detected mutations in Iranian population. Furthermore, this study was not aimed at conducting the functional analysis of these mutations.

### Conclusion

In this study, which was conducted for the first time in Iran, we screened 53 cardiac arrhythmia patients for *SCN5A* mutations and found 3 different mutations in 24 individuals diagnosed with different clinical syndromes. *p.Gln1507-Lys1508-Pro1509del* mutation, *p.Arg222Ter* nonsense mutation, and novel mutation *p.Met1498Arg* in LQTS, BrS, and SSS, respectively are reported for the first time in Iranian subjects. Information on the underlying genetic defects of familial arrhythmias in Iranian patients would be extremely helpful in verifying clinical diagnosis, carriers screening, and personalized therapy for the patients.

**Conflict of interest:** None declared.

**Peer-review:** Externally peer-reviewed.

**Authorship contributions:** Concept - R.S., R.F., Z.A.B.; Design - R.S., M.A.; Supervision - R.S.; Materials - M.R.S., R.S.; Research - M. A., R.S.; Data collection &/or processing - M.A., A.R.S., S.S.; Literature search - M.A., R.S.; Writing - R. S., M.A., A.R.S.; Critical review - R.S.

### References

- Gellen ME, George AL, Chen LQ, Chahine M, Horn R, Barchi RL, et al. Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage dependant sodium channel. Proc Natl Acad Sci 1992; 89: 554-8.

- Wilde AM, Brugada R. Phenotypical manifestations of mutations in the genes encoding subunits of the cardiac sodium channel. Circ Res 2011; 108: 884-97.
- Amin AS, Asghari-Roodsari A, Tan HL. Cardiac sodium channelopathies. Pflugers Arch 2010; 460: 223-37.
- Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al., HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPCC in June 2013. Heart Rhythm 2013; 10: 1932-63.
- Gui J, Wang T, Jones RPO, Trump D, Zimmer T, Lei M. Multiple Loss-of-Function Mechanisms Contribute to *SCN5A*-Related Familial Sick Sinus Syndrome. PlosOne 2010; 5: 1-12.
- Butters TD, Aslanidi OV, Inada S, Boyett MR, Hancox JC, Lei M, et al. Mechanistic links between  $Na^+$  channel (*SCN5A*) mutations and impaired cardiac pacemaking in sick sinus syndrome. Circ Res 2010; 107: 126-37.
- Benson DW, Wang DW, Dyment M, Knilans TK, Fish FA, Strieper MJ, et al. Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (*SCN5A*). J Clin Invest 2003; 112: 19-28.
- Baroudi G, Acharfi S, Larouche C, Chahine M. Expression and intracellular localization of an *SCN5A* double mutant R1232W/T1620M implicated in Brugada syndrome. Circ Res 2002; 90: 11-6.
- Wang Q, Li Z, Shen J, Keating MT. Genomic Organization of the Human *SCN5A* Gene Encoding the Cardiac Sodium Channel. Genomics 1996; 34: 9-16.
- Keller DI, Acharfi S, Delacrétaiz E, Benammar N, Rotter M, Pfammatter JP, et al. A novel mutation in *SCN5A*, delQKP 1507–1509, causing long QT syndrome: Role of Q1507 residue in sodium channel inactivation. J Mol Cell Cardiol 2003; 35: 1513-21.
- Kaplinger JD, Tester DJ, Alders M, Benito B, Berthet M, Brugada J, et al. An international compendium of mutations in the *SCN5A* encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. Heart Rhythm 2010; 7: 33-46.
- Hiraoka M. Inherited arrhythmic disorders in Japan. J Cardiovasc Electrophysiol 2003; 14: 431-4.
- Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Brignole M, et al. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: A prospective evaluation of 52 families. Circulation 2000; 102: 2509-15.
- Hashemi A, Shahrzad S, Shahrzad S, Saber S, Taban S, Aslani A, et al. Positive Brugada challenge test in V1R–V3R as a predictor of malignant prognosis in Brugada patients. Ann Noninvasive Electrocardiol 2013; 18: 421-6.
- Kotta CM, Anastasakis A, Stefanadis C. Effects of mutations and genetic overlap in inherited long-QT and Brugada arrhythmia syndromes. Hellenic J Cardiol 2012; 53: 439-46.
- Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J, et al. Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. JAMA 2005; 294: 2975-80.
- Liang P, Liu W, Li C, Tao W, Li L, Hu D. Genetic analysis of Brugada syndrome and congenital long QT syndrome type 3 in the Chinese. J Cardiovasc Dis Res 2013; 1: 69-74.