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

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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.

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.
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\textsuperscript{nd}, 3\textsuperscript{rd}, or 4\textsuperscript{th} 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\muL volume using conventional 10x 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).

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.
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.

Table 1. Clinical characteristics of patients

| Probands | 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 |
|------------|----------------------|------------------|--------------------------------------------|------------------|-------------------------------|-------------------------|
| LQTS<sup>c</sup> |                     |                  |                                            |                  |                               |                         |
| 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                                       | ICD              | 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                     |

BrS<sup>a</sup> |                     |                  |                                            |                  |                               |                         |
| 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                           |                         |

SSS<sup>e</sup> |                     |                  |                                            |                  |                               |                         |
| I-2        | 42                   | Physical activity | -                                         | Pacemaker implantation | Yes                          |                         |
| II-3       | 25                   | Physical activity | -                                         | Pacemaker implantation | Yes                          |                         |
| III-1      | 29                   | Physical activity | -                                         | Pacemaker implantation | Yes                          |                         |
| III-2      | 22                   | Physical activity | -                                         | Pacemaker implantation | 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

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-
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).

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

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

![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)](image)

![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](image)
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 LOTS, 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.

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