Phenotype guided characterization and molecular analysis of Indian patients with long QT syndromes

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ABSTRACT

Background: Long QT syndromes (LQTS) are characterized by prolonged QTc interval on electrocardiogram (ECG) and manifest with syncope, seizures or sudden cardiac death. Long QT 1–3 constitute about 75% of all inherited LQTS. We classified a cohort of Indian patients for the common LQTS based on T wave morphology and triggering factors to prioritize the gene to be tested. We sought to identify the causative mutations and mutation spectrum, perform genotype-phenotype correlation and screen family members.

Methods: Thirty patients who fulfilled the criteria were enrolled. The most probable candidate gene among KCNQ1, KCNH2 and SCN5A were sequenced.

Results: Of the 30 patients, 22 were classified at LQT1, two as LQT2 and six as LQT3. Mutations in KCNQ1 were identified in 17 (77%) of 22 LQT1 patients, KCNH2 mutation in one of two LQT2 and SCN5A mutations in two of six LQT3 patients. We correlated the presence of the specific ECG morphology in all mutation positive cases. Eight mutations in KCNQ1 and one in SCN5A were novel and predicted to be pathogenic by in-silico analysis. Of all parents with heterozygous mutations, 24 (92%) of 26 were asymptomatic. Ten available siblings of nine probands were screened and three were homozygous and symptomatic, five heterozygous and asymptomatic.

Conclusions: This study in a cohort of Asian Indian patients highlights the mutation spectrum of common Long QT syndromes. The clinical utility for prevention of unexplained sudden cardiac deaths is an important sequel to identification of the mutation in at-risk family members.

1. Introduction

Long QT syndromes (LQTS) are inherited autosomal dominant channelopathies associated with prolongation of QT interval on the 12-lead electrocardiogram (ECG) (QTc > 440 ms in men and >450 ms in women). They are caused by mutations in fifteen cardiac ion channels pore forming and auxiliary subunit genes [1–3]. Common symptoms include syncope, seizures, and polymorphic ventricular tachyarrhythmias, that may lead to sudden cardiac death [4]. Among all types of LQTS, Long QT types 1–3 (LQT 1–3) constitute about 75% of the cases [5,6]. LQT1 is also observed as an autosomal recessive trait in patients with sensorineural deafness...
Specific triggering factors and T wave morphology on ECG are consistently described in three common types of LQTS [8–10]. Typically, in LQT1 syndrome, physical exertion triggers arrhythmic events with ECG showing broad T waves; in LQT2 syndrome, emotional stress, auditory stimuli, postpartum period trigger cardiac events with ECG demonstrating a biphasic or notched T wave. In LQT3 syndrome, majority of arrhythmic events occur during sleep or rest and they have delayed onset of T wave on ECG [2,11,12].

LQT1 and LQT2 are caused due to mutations in the potassium channel genes, KCNQ1 (OMIM#607542) and KCNH2 (OMIM#152427) respectively, while LQT3 is caused by mutations in a sodium channel gene, SCN5A (OMIM#600163) [2]. Although hundreds of mutations have been identified in the three genes, studies have shown the presence of common as well as founder mutations in different populations suggesting genetic or allelic heterogeneity [6,13–16].

Molecular testing in LQTS helps to confirm the clinical diagnosis in the proband, perform genotype-phenotype guided management as well identify the at-risk family members who may be asymptomatic and can be missed by ECG studies [12,17]. In this study, we classified a cohort of Asian Indian patients with Long QT syndrome based on T wave morphology and triggering factors. The aim was to identify the causative mutations in patients affected with common types of LQTS, determine the mutation spectrum of this sparsely studied population, perform genotype-phenotype correlation and identify the at-risk family members.

2. Materials and methods

2.1. Patients

Thirty patients of Asian Indian origin from unrelated families were included in the ongoing study on life threatening Long QT syndromes, from January 2011 through July 2015 at Sir Ganga Ram Hospital, New Delhi, India. They were “suspected LQTS” on the basis of prolongation of QTc interval >440 ms in men and >450 ms in women. All probands were <40 years of age, had a structurally normal heart on echocardiography, and were classified in three different groups of LQTS (LQT1, LQT2, LQT3) depending on the ECG morphology (Fig. 1) and triggers. They also had either a) symptoms of syncope/seizures/resuscitated sudden cardiac death (SCD) and/or b) a family history of SCD/LQTS. All those with unclear ECG morphology were excluded. The clinical details such as age at onset of symptoms, age at diagnosis, trigger for arrhythmias, type of cardiac events and the family history, up to three generations was noted for each proband. Patients with structural cardiac abnormality, electrolyte imbalance or on medications known to prolong QT interval were excluded.

An informed consent was obtained from all the probands and their family members included in this study. This study was approved by the Ethics Committee of Sir Ganga Ram Hospital vide no EC/01/12/337.

2.2. Sample collection and DNA extraction

Blood samples (6 ml EDTA) were collected from the patient, their parents and siblings as available. Genomic DNA was extracted using the salt precipitation method. The isolated DNA was quantified by a spectrophotometer at the absorbance ratio of 260/280.

2.3. Mutation screening

2.3.1. PCR amplification

Primers flanking the exon-intron boundaries of all coding exons in three common genes (KCNQ1 [NM_000218], KCNH2 [NM_000238] and SCN5A [NM_198056]); were designed using web-primer software available in Saccharomyces genome database (http://www.yeastgenome.org/cgi-bin/web-primer). Primer sequences are available on request. On the basis of the T wave morphology on ECG and triggering factors causing the episodes, the candidate gene was selected to be PCR amplified and sequenced in these patients.

2.3.2. Sanger sequencing

PCR products were purified using MicroAmp purification plate and subjected to direct sequencing based on dideoxynucleotide termination methodology using the BigDye terminator cycle sequencing Ready reaction kit (Applied Biosystems, Perkin Elmer Corporation, Foster City, CA). Sequencing was performed bi-directionally on ABI 3500 Genetic Analyzer (Applied Biosystems, UK).

2.3.3. Detection of variants

Analysis of the electropherogram was performed by aligning the patient sequences with the reference sequences of KCNQ1, KCNH2 and SCN5A available in the database using Blat tool (https://genome.ucsc.edu/cgi-bin/hgBlat). Forward and reverse primer sequencing results were compared to confirm the presence of the variation. All variations were also verified by sequencing a second amplified amplicon.
2.4. Confirmation of the pathogenicity

If variations identified in this study were described in previous literature or listed in mutation database (http://triad.fsm.it/cardmoc/ and http://www.genomede.org/lovd2/home.php?action=switch_db), then these were considered disease causing.

For novel variations, in-silico analysis was performed. Frameshift variations caused by deletion or duplication as well as nonsense variations responsible for truncation of a protein were considered to be pathogenic. Missense variations were evaluated using bioinformatic softwares such as Mutation Taster (http://www.mutationtaster.org), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), and SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html). In addition, FATHMM (http://fathmm.biocompute.org.uk), L-Mutant (http://folding.biofold.org/i-mutant/i-mutant2.0.html), PROVEAN (http://provean.jcvi.org/seq_submit.php), PSIPred (http://bioinf.cs.ucl.ac.uk/psipred/), RaptorX (http://raptorx.uchicago.edu/StructurePrediction) and ProtParam (http://web.expasy.org/protparam), were used to predict the effect of the missense mutations on the secondary structure of protein and to calculate the instability index of the mutated proteins. Splice-site variations were investigated using BDGP (http://www.fruitfly.org/seq_tools/splice.html), AUGUSTUS (http://bioinf.uni-greifswald.de/augustus/predictions/), GeneID (http://genome.crg.es/software/geneid/), GENSCAN (http://www.genes.mit.edu/GENSCAN.html) and NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/). Conservation of residues was examined across different species using PhyloP and PhastCons (http://www.mutationtaster.org). Hundred control samples (200 alleles) were screened to assess the frequency of the novel variations in the general population. Novel variations were also checked in dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP), 1000 Genomes (http://browser.1000genomes.org/index.html) or Exome Variant Server (EVS) (http://evs.gs.washington.edu/EVS). Frequency information was also obtained from the literature or from public exome/genome databases — 1000 Genomes and EVS and variations with a MAF (minor allele frequency) of <1% in all population were considered as mutations. The novel variations were only considered pathogenic if they were absent in these control populations and atleast five bioinformatic softwares predicted the change to be pathogenic.

3. Family screening

Once a variation was identified in a proband, the parents were sequenced to determine the inheritance pattern. Screening of the siblings and first-degree relatives was performed if their samples were available. The results from segregation studies were used for performing genotype-phenotype correlation.

The entire work plan is demonstrated in Fig. 2.

4. Results

4.1. Patient characterization

In this cohort, on the basis of the T wave morphology (Fig. 1), 22 (73%) of 30 were identified to have broad or tall T waves and were classified as LQT1 patients. Exercise typically acted as a trigger in 12 (54%) of 22 patients. Of these, five (17%) patients had associated sensorineural hearing loss. Similarly, 2 (7%) had notched T waves and 6 (20%) of 30 had delayed onset of T waves classifying them as LQT2, and LQT3 syndromes, respectively. In the LQT2 group, emotion and sound acted as a trigger in one patient and the second patient experienced syncopal events in the postpartum period. Three of six patients in the LQT3 group experienced cardiac events during sleep. The detailed phenotypes of probands classified as LQT1, LQT2 and LQT3 are summarized in Table S1.

4.2. Molecular characterization

Of 30 patients with LQTS, mutations were identified in 20 (67%). In the LQT1 patient cohort, mutations were present in 17 (77%) of 22 in KCNQ1; one proband had mutation in KCNH2 of 2 patients with LQT2 phenotype; and 2 of 6 LQT3 patients had mutations in SCN5A. The genotypic information of these patients is summarized in Table 1.

4.2.1. LQT1 syndrome (KCNQ1)

In this group of 22 patients, 17 were identified with mutations. Of these, seven patients had a heterozygous mutation and ten had biallelic mutations of which three were compound heterozygous and seven were homozygous for mutations in KCNQ1. Five of the latter ten probands with biallelic mutations had associated sensorineural hearing loss (JLNS) and the rest without deafness were classified as AR RWS.

In the cohort of 17 mutation positive patients, 20 mutations in KCNQ1 were identified. Two common novel missense mutations (G186D, F351L) were present in two and three unrelated probands, respectively. Of the remaining 17 different mutations, nine were missense and eight were radical mutations including three splice site, three deletions, and two duplications. Majority of mutations (5/17; 29%) were located in exon 3; two each in exon 6, 13 and 15; and one each in exon 2, 5, 7, 8, 11 and 14. Eleven mutations (65%) were in or between transmembrane domains (S1–S6) and six (35%) were in C-terminal region of the protein (Fig. 3). Eight mutations identified were novel.

4.2.2. LQT2 syndrome (KCNH2)

One KCNH2 heterozygous missense mutation was identified in a proband with LQT2 syndrome. This is a reported mutation in exon 7 of the genomic region and transmembrane (S6) domain of the protein.

4.2.3. LQT3 syndrome (SCN5A)

Two patients were identified with heterozygous missense mutations in SCN5A. These mutations were located in exon 10 and 27 of the genomic region and transmembrane (D1–S6) and C-terminal domain of the protein, respectively. R1897Q was a novel variant.

4.3. Genotype—phenotype correlation

4.3.1. LQT1, LQT2 and LQT3 mutation positive patient groups

In the cohort of LQT1 patients, the male to female ratio was 15:2 while patients with LQT2 and LQT3 were all female. Though the age of onset was from birth upto 11 years of age in LQT1 patients, there was a delay in establishing the diagnosis with the wide range of 2 years–16 years. In LQT2, the age of onset and diagnosis was 29 and 31 years respectively, while in LQT3 cohort, age of onset and diagnosis for one patient was 6 yrs and 16 yrs while for the other, it was 0.5 yrs and 4 yrs. The mean age of onset and diagnosis in LQT1 cohort was 2.43 yrs and 7.94 yrs. QTc interval was >500 ms in 82% and the mean QTc was 530.7 ± 40.34 ms in LQT1 group whereas the mean QTc in LQT2 and LQT3 was 650 ms and 533 ± 66 ms respectively. Syncpe occurred in 82% and seizures in 65% and previous family history of prolonged QTc, LQTS and/or SCD was noted in 88% of LQT1 patients as compared to LQT2 patient that presented with syncpe, seizures and family history. In LQT3 patients, syncpe and family history was noted in one proband. All of them had broad T wave on ECG and 65% experienced cardiac events during or after some form of exercise in LQT1 group. In the LQT2
4.3.2. AD RWS, AR RWS and JLNS mutation positive patient groups

Majority of patients in all three groups were males (88%). The mean age of onset was comparatively clinically earlier in AR RWS cohort (1.29 yrs) as compared to AD RWS (3.35 yrs) and JLNS (2.3 yrs) whereas the mean age of diagnosis was earlier in AD RWS (5.36 yrs) as compared to AR RWS (10.2 yrs) (p 0.034, significant by Kruskal Wallis) and JLNS (9.3 yrs). The mean QTc was higher in JLNS (551 ± 61) as compared to AD RWS (532 ± 30) and AR RWS (506 ± 8). Syncope was observed in AR RWS and JLNS patients whereas there was an equal distribution of patients with syncope and seizures in AD RWS cohort. All patients with AD RWS had family history whereas only 80% of patients in other two cohorts were noted with it. These results are summarized in Table S2. There was no significant difference between age of onset, QTc interval or any clinical symptoms in this group.

4.4. Pathogenicity

Among the 20 different mutations identified in patients with LQT1, LQT2 and LQT3, 11 mutations are known pathogenic as suggested by literature (Table 1). The eight novel mutations in KCNQ1 in this cohort were studied for their pathogenicity by PSIPred, BDGP, AUGUSTUS, GENSCAN and GeneID (Unpublished results). Here, we evaluate the same using additional softwares (Table 2). Mutation (p.R1897Q) in SCN5A was novel and is first described in this study. These novel mutations were absent in 200 control alleles.
and public exomes/genomes. Two novel splice site variations in KCNQ1 were evaluated by NetGene2 and a change in the number of acceptor sites was predicted as elaborated below. Wild type allele showed the presence of three acceptor sites at position (87, 151 and 364) as compared to wild type with the mutant type (c.1682-2A > G) had only two acceptor sites (87, 151). Similarly, the other splice site mutation (c.1733-1G > C) also showed the absence of the most probable acceptor sites (168, 170, 185) as compared to wild type with five acceptor sites (163, 168, 170, 174, 185).

Secondary and 3D structure modeling by RaptorX and PSIPred predicted a change in the number of helixes, beta sheets and loops in all secondary structures of mutant proteins as compared to the wild type KCNQ1 and SCN5A causing a change in the conformation of the protein (Fig. 4a and b). For the splice site changes in KCNQ1, the sequence predicted by GENSCAN was used as a template for RaptorX. PSIPred software also predicted the R1897Q mutation in the sequence predicted by GENSCAN.

Table 1
List of identified mutations.

| Patients | cDNA change | Protein change | Mutation type | Zygosity | Exon Affected domain | Syndrome | References |
|----------|-------------|----------------|---------------|----------|----------------------|----------|------------|
| KCNQ1    |             |                |               |          |                      |          |            |
| Patient 1| c.557G > A* | p.G186D*       | Missense      | Homozygous| 3 S2−S3              | JLN1     | Vyas et al. (Unpublished results) |
| Patient 2| c.650G > A  | p.R190Q        | Missense      | Homozygous| 3 S2−S3              | JLN1     | Kapplinger et al., 2009; Vyas et al. (Unpublished results) |
| Patient 3| c.502G > A  | p.G168R        | Missense      | Homozygous| 3 S2−S3              | JLN1     | Marquez et al., 2006; Vyas et al. (Unpublished results) |
| Patient 4| c.1051T > C | p.F351L*       | Missense      | Compound  | 8 S6                 | JLN1     | Vyas et al. (Unpublished results) |
| Patient 5| c.1733-1G > C| p.Y148Lfs*89*  | Deletion      | Homozygous| 2 S1                 | JLN1     | Vyas et al. (Unpublished results) |
| Patient 6| c.557G > A  | p.G179S        | Missense      | Homozygous| 3 S2−S3              | AR LQT1  | Splawski et al., 2000; Vyas et al. (Unpublished results) |
| Patient 7| c.758C > G  | p.S253C*       | Missense      | Homozygous| 5 S4−S5              | AR LQT1  | Vyas et al. (Unpublished results) |
| Patient 8| c.1480dupG | p.E494Gfs*21*  | Insertion     | Homozygous| 11 C-ter             | AR LQT1  | Vyas et al. (Unpublished results) |
| Patient 9| c.1686-2A > G| p.R533W        | Missense      | Compound  | 13 C-ter             | AR LQT1  | Vyas et al. (Unpublished results) |
| Patient 10| c.1762A > T | p.J588F*       | Missense      | Compound  | 15 C-ter             | AR LQT1  | Vyas et al. (Unpublished results) |
| Patient 11| c.828_830delCTC | p.S277del  | Deletion      | Homozygous| 6 S5                 | LQT1     | Napolitano et al., 2005; Present study |
| Patient 12| c.1703G > C | p.G568A        | Missense      | Homozygous| 14 C-ter             | LQT1     | Chen et al., 2005; Present study |
| Patient 13| c.824_826delCTC | p.F275del  | Deletion      | Homozygous| 6 S5                 | LQT1     | Aizawa et al., 2007; Present study |
| Patient 14| c.1051T > G | p.A344A        | Splice site   | Heterozygous| 7 S6                 | LQT1     | Struijk et al., 2006; Present study |
| Patient 15| c.557G > A  | p.G186D*       | Missense      | Homozygous| 3 S2−S3              | LQT1     | Present study |
| Patient 16| c.1051T > G | p.R1897Q*      | Missense      | Homozygous| 27 C-ter             | LQT3     | Present study |

N-ter: N-terminal; C-ter: C-terminal; *: Novel.

4.5. Family screening and genotype–phenotype correlation

Screening of parents identified mutations in 17/20 families (85%) as described in Table S3. One proband had a de-novo...
mutation; one proband was adopted and for the third proband, family members were not available for screening. In these 17 families, 26 parents were identified to be heterozygous carriers of which 24 (92%) parents were asymptomatic. Of the ten siblings screened, eight were identified with mutations (80%) of which five were asymptomatic (62%). Three had biallelic mutations similar to the probands and they were symptomatic with syncope and/or seizures. Two other siblings of the recessive cohort were heterozygous for the familial mutations and had isolated prolonged QTc. Three siblings of probands with dominant inheritance who were asymptomatic with prolonged QTc were identified with heterozygous mutations.

5. Discussion

Till date, majority of reports published on Indian patients with LQTS are of clinical nature, with only few single case reports on molecular studies [18–22]. The present study is the first to assess the spectrum of mutations in KCNQ1, KCNH2 and SCN5A responsible for causing LQT1, LQT2 and LQT3, respectively in an Asian Indian cohort. In this study, 1) Mutations were identified in 20 of 30 patients with LQT1 (17 in KCNQ1, 1 in KCNH2 and 2 in SCN5A), 2) Among the 20 mutations, nine were novel mutations (8 in KCNQ1 and 1 in SCN5A) and predicted to be pathogenic by bioinformatics softwares, 3) Molecular modeling was performed for these mutations that predicted a change in the conformation of the protein, 4) Family screening identified mutations in 92% of asymptomatic parents and 62% of asymptomatic siblings.

5.1. Patient characterization

The specific T wave pattern observed in LQT1, LQT2 and LQT3 provides the possibility of effectively anticipating the probable gene involved in the pathogenesis of the syndrome [3]. KCNQ1 and KCNH2 encode the α-subunit of IKs and IKr, respectively, of the delayed rectifier potassium currents and SCN5A encodes β-subunit of the voltage gated sodium currents. A decrease in the outward potassium currents or an increase in the inward sodium currents causes a delay of repolarization of cardiac action potential leading to a specific T wave morphology observed in patients with different types of LQTS [23]. This specific ECG morphology was observed in all mutation positive patients with the respective type of LQTS (broad T wave: LQT1, notched T wave: LQT2 and delayed T wave: LQT3). For the patients negative for mutations in the respective common genes, there is a possibility of other genetic and acquired factors that are known to influence ventricular repolarization and manifest with a similar ECG pattern [8]. Gene specific triggers were observed in 11 (65%) of 17 patients with LQT1 (physical exertion), in one patient with LQT2 (sound, emotion) and one of two in patients with LQT3 (sleep). There was an overlap of sleep as a trigger in one LQT1 and one LQT2 patient, but majority of their events occurred with their specific triggers. Though triggers are important for classification, studies have shown their overlap [12]. In this study of 670 LQTS patients, majority of LQT1 patients (62%) experienced events during exercise, emotion (26%) and rest/sleep (3%). Whereas in LQT2 cohort, majority had events during emotion (43%), sleep (29%) and exercise (13%). Similarly, in LQT3 group, majority had events during sleep (39%), emotion (19%) and exercise (13%). In the remaining patients described in this study, triggers were unknown or were other than the three described above.

5.2. Molecular characterization

In the current study, mutations were identified in 20 (67%) of 30 probands suspected to have LQTS. LQT1 formed the largest cohort with mutations identified in 17 (57%) of 30 patients. LQT1 is reported to have the highest frequency of approximately 30–35% among the common subtypes, while LQT2 accounts for 25–30% and LQT3 for 5–10% [24].

In the cohort of 20 patients positive for mutations, 20 different mutations were identified. Heterozygous mutations were identified in 10 (50%) of 20 patients; 7 (35%) in KCNQ1, one in KCNH2 and two in SCN5A. In a study of 903 LQTS Caucasian patients with mutations identified, 91% had heterozygous mutations of which 42% were in KCNQ1, 32% in KCNH2 and 13% in SCN5A [6]. Across worldwide studies, autosomal dominant form of LQT syndrome with heterozygous mutation is described to be more common with a prevalence of 1:2000 in all ethnicities while JLNS is extremely rare and identified in autosomal recessive form of LQT syndrome with homozygous or compound heterozygous mutations in less than 1 per 4 million [2,3]. In the present study, biallelic mutations were present in 10 (50%) of 20 patients; seven patients (7/20; 35%) were homozygous and three patients (3/20; 15%) were compound heterozygous for mutations in KCNQ1. The high rate of homozygous mutations in the absence of high consanguinity (2/9; 22%) in the biallelic group, is possibly due to the endogamous marriages within the same caste or community as previously reported in this population [25,26]. However the cohort size is small and the findings need to be substantiated in a larger sample size.

Fig. 3. Localization of LQT1 mutations in KCNQ1 channel protein subunit domains. Mutated amino acids are shown as follows: missense mutations: square; splice site: circle; deletion: triangle and insertion/duplication: diamond. Missense mutations are represented in red and radical mutations in yellow.
| Gene/Protein | Coding substitution | Protein consequence | Family segregation | Mutation target | Polyphen2 | SIFT | FATHMM | I-Mutant3 | PROVEAN (II) | ProtParam | Conservation PhyloP/PhastCons | MAF (dbSNP, 1000G, EVS) | Other |
|---------------------------------|----------------------|---------------------|-------------------|----------------|----------|------|--------|----------|-------------|-----------|-----------------------------|------------------------|-------|
| KCNQ1                          | 557G > A             | G186D               | Yes               | 0.999, Disease Causing | 1,000, Probably Damaging | 0 | -4.58 | Decrease, Damaging 7 | 41.44 | 5.346/1 | Absent in all | PSIpred – structure alt., RaptorX |
| 1051T > C                      | F351L                | Yes                 | 0.999, Disease Causing | 0.978, Probably Damaging | 0.03, Decrease, Damaging 6 | 42.14 | 4.003/1 | Absent in all | PSIpred – structure alt., RaptorX |
| 443delA                        | Y148Lfs*89           | Yes                 | 1,000, Disease Causing | 0.000, Probably Damaging | - | - | - | - | - | Absent in all | BDGP, AUGUSTUS, GeneID, GENSCAN, NetGene2, RaptorX |
| 1732-1G > C                    | –                    | Yes                 | 1,000, Disease Causing | - | - | - | - | - | Absent in all | BDGP, AUGUSTUS, GeneID, GENSCAN, NetGene2 |
| 758C > G                       | S253C                | N/A                 | 0.999, Disease Causing | 1.000, Probably Damaging | 0, Decrease, Damaging 5 | 41.13 | 5.221/1 | Absent in all | PSIpred – structure alt., RaptorX |
| 1480dupG                       | E494Gfs*21           | Yes                 | 1,000, Disease Causing | - | - | - | - | - | Absent in all | BDGP, AUGUSTUS, GeneID, GENSCAN, NetGene2 |
| 1686-2A > G                    | –                    | Yes                 | 1,000, Disease Causing | - | - | - | - | - | Absent in all | BDGP, AUGUSTUS, GeneID, GENSCAN, NetGene2 |
| 1762A > T                      | I588F                | Yes                 | 0.999, Disease Causing | 0.992, Probably Damaging | 0, Decrease, Damaging 8 | 41.42 | 1.682/0.978 | Absent in all | PSIpred – structure alt., RaptorX |
| SCN5A                          | 5690G > A            | R1897Q              | Yes               | 0.999, Disease Causing | 0.982, Probably Damaging | 0.08, Decrease, Damaging 7 | 45.99 | 4.159/1 | Absent in all | PSIpred |

SIFT: Intolerant or deleterious: < -0.05; Mutation Taster & Polyphen2: probability; FATHMM: scores < 0, unfavorable situation, damaging; I-Mutant3: stability, decrease (-0.5 < Kcal/mol), increase (0.5 > Kcal/mol), neutral (0 < Kcal/mol); PROVEAN: Deleterious, < -2.5; ProtParam: Instability index (II) > wild-type protein (KCNQ1: 41.24; SCN5A: 46.21), instable; Conservation (PhyloP/PhastCons): values between 14 and + 6, positive scores for sites predicted to be conserved and negative scores predicted to be fast evolving sites/values from 0 (most probably not conserved) to 1 (most probably conserved); PSIpred: structure alteration (alt.); N/A, not available, IP, incomplete penetrance.
Fig. 4. In-silico analysis for prediction of molecular structures. a. Secondary structure (H: helix, E: Beta sheet, C: loop) and 3D model of wild type and mutant KCNQ1 protein. b. Secondary structure (H: helix, E: Beta sheet, C: loop) and 3D model of wild type and mutant SCN5A protein. c. Secondary structure of SCN5A wild type and mutant protein. The altered residue (p.R1897Q) is shaded in red while the changes in the secondary have been highlighted in green.
b. SCN5A (Wild Type protein)

H - 51%, E - 3%, C - 44%

SCN5A (Mutant protein)

1686-2A>G

H - 51%, E - 4%, C - 44%

c. SCN5A Wild type

R1897Q Mutant

Legend:
- helix
- confidence of prediction
- strand
- predicted secondary structure
- coil
- target sequence

Fig. 4. Continued
5.3. Mutation characterization

In KCNQ1, 11 mutations (65%) were located in or between transmembrane domains (S1−S6) and six (35%) were in C-terminal region of the protein (Fig. 3). The transmembrane mutations are associated with the longer QTc and higher frequency of cardiac events as compared to C-terminal mutations [27,28]. Similar findings were observed in this study with the mean QTc values of patients with transmembrane mutations was 536 ± 44.23 and C-terminal mutations was 510 ± 12.36. In KCN2H2, the one mutation identified was in the transmembrane S6 domain. Missense mutations in this domain have been associated with higher QTc interval and increased risk of SCD [29], as was observed in our patient (QTc 650 ms) with a history of SCD in her son. In patients with LQT3, one mutation was located in the transmembrane region and the second in the C-terminal domain of SCNSA. There is a lack of clear association between the locations of mutation in this gene with risk associated of cardiac events in LQT3 patients. In present study, in patients with LQT3, a transmembrane mutation was associated with a higher QTc and syncope, as compared to the patient with C-terminal mutation and isolated fetal bradycardia. Hence, genotype–phenotype correlation can help in stratifying the clinical course of the disorder in patients with LQT1, LQT2, and LQT3 [2,12,27].

Based on the current study, nine (45%) of 20 mutations were novel variants (KCNQ1: 8 and SCNSA: 1). Even with the advance in genetic testing, establishing the pathogenicity of the identified rare variation can be difficult. These alterations are known as “Variants of Unknown Significance” (VUS) as their association with disease phenotype is unclear. In such scenarios, one has to rely on family segregation studies, absence of variant in public databases, absence of the variant in population specific control population and bioinformatics analysis [3]. All novel mutations in the present study were absent in public exomes/genomes and in our control population. Segregation studies identified eight of nine novel mutations in probands to be inherited from their parents. One proband was adopted and hence, parental screening was not performed. According to the criteria listed in previous studies for LQTS, frameshift and splice site mutations are known as “radical mutation” and considered probably pathogenic with an estimated predicted value (EPV) of 99% [3]. For the missense variations, the location is of great significance in determining the pathogenicity [27−29]. Mutations in the transmembrane and C-terminal regions of KCNQ1 and SCNSA protein are considered to be probably/possibly pathogenic with an EPV of 94% and 75%, respectively [3]. In addition to this, we have used several prediction tools (Table 2) to confirm the pathogenicity of our novel variants. Molecular modeling using Raptor X and PSIPred further suggested the pathogenic role of these variants as changes were seen in the secondary structure (helix, beta-sheets and coils), which might change the conformation and have an effect on the normal function of the protein (Fig. 3) (Unpublished results).

5.4. Family screening

LQT syndromes are known to present with incomplete penetrance and variable expressivity that suggests that family members with the same LQTS causing mutation can display variable clinical course with some being severely affected to some with no history of symptoms in lifetime [3]. In this study, 26 parents were heterozygous among 17 (94%) of 18 families studied. Of these, 24 (92%) parents were asymptomatic. Similarly, eight of ten siblings screened were identified with mutations (80%) of which five were asymptomatic (62%). These asymptomatic mutation carriers could have been easily missed if they were not genotyped. Although ECG testing is the first line test for LQT diagnosis, studies have shown that about 10−40% of genotype − positive individuals have QTc values with normal range and are known to have “concealed LQTS” [30,31]. Although these individuals have a reduced risk of life threatening cardiac events (4%) compared to those with patients with symptoms (15%), they still have an > 10 fold higher risk than genotype negative relatives (0.4%) [3]. Hence, family screening is very important in Long QT syndromes and can aid in preventing life threatening events in at-risk asymptomatic members.

6. Conclusions

Based on the results of this study, a personalized molecular analysis model for affordable diagnostic approach of patients with Long QT syndromes for Asian Indian population is suggested. The morphology of T wave pattern observed on the ECG can guide in the selection of the candidate gene for molecular testing as observed in the majority of mutation positive patients (67%) in this study. Secondly, presence of hotspots or common mutations in the gene can aid in shorter time for testing. In this study, 29% of the identified mutations in KCNQ1 were in exon 3 and we also identified two common missense mutations (G180D, F351L). These common mutations are novel and may be specific to our population, as they have not yet been reported. If negative, other exons of the specific gene can be sequenced. If a patient is negative for mutations in the most probable candidate gene, screening of other LQTS susceptibility genes (LQT2, LQT3, QT 4−15 (accounting for <5%)) can be done [3]. This step-by-step mutation analysis will help in identification of mutations at a reasonable cost, with limited resources and in a shorter time frame, which will benefit the patient and at-risk family members.

Conflict of interests

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ipej.2016.03.003.

List of abbreviations

AD RWS autosomal dominant Romano-Ward syndrome
AR RWS autosomal recessive Romano-Ward syndrome
JLNS Jervell and Lange-Nielsen syndrome
KCNH2 Potassium Channel, Voltage Gated Eag Related Subfamily H, Member 2
KCNQ1 Potassium Channel, Voltage Gated KQT-Like Subfamily Q, Member 1
SCNSA Sodium Channel, Voltage Gated, Type V Alpha Subunit

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