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## 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.

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## 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

[Jervell-Lange Nielsen syndrome (JLNS)], whereas patients without deafness are referred to as autosomal recessive Romano-Ward syndrome (AR RWS) [7].

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 bidirectionally on ABI 3500 Genetic Analyzer (Applied Biosystems, UK).

#### 2.3.3. Detection of variants

Analysis of the electrophorogram 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.

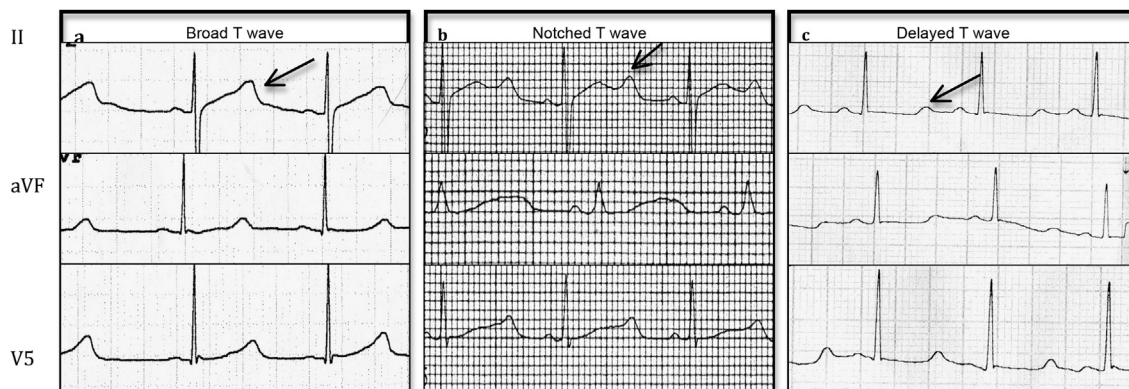


Fig. 1. Classification of LQTS type on the T wave morphology on ECG. LQT1 (a), LQT2 (b) and LQT3 (c).

## 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.genomed.org/lovd2/home.php?action=switch\\_db](http://www.genomed.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](http://sift.jcvi.org/www/SIFT_enst_submit.html)). In addition, FATHMM (<http://fathmm.biocompute.org.uk>), I-Mutant (<http://folding.biofold.org/i-mutant/i-mutant2.0.html>), PROVEAN ([http://provean.jcvi.org/seq\\_submit.php](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](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 at least 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  $530.7 \pm 40.34$  ms in LQT1 group whereas the mean QTc in LQT2 and LQT3 was 650 ms and  $533 \pm 66$  ms respectively. Syncope 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 syncope, seizures and family history. In LQT3 patients, syncope 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

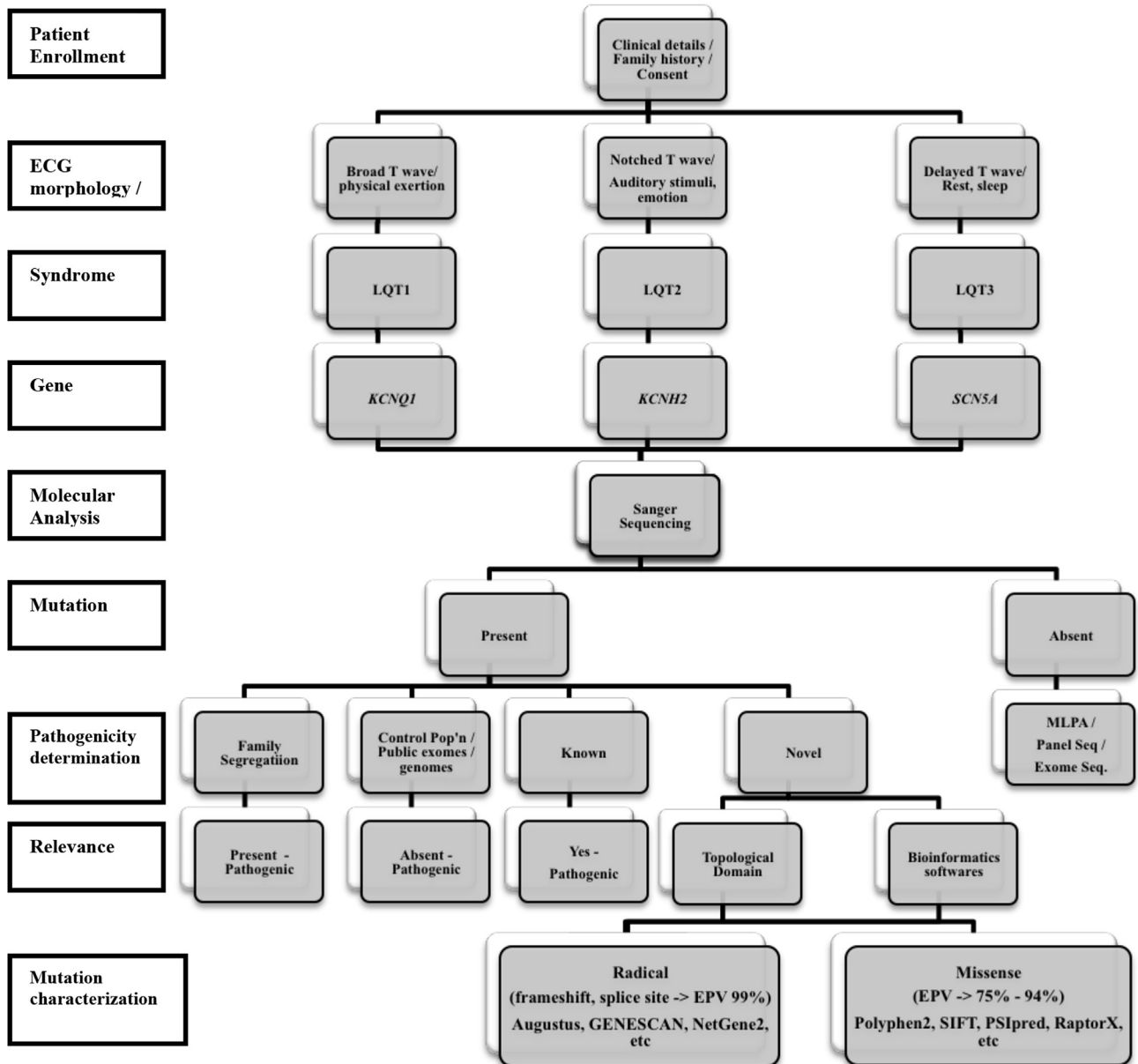


Fig. 2. Work flow for clinical and molecular characterization of patients with LQTS. EPV: estimated predicted value.

patient, events occurred with emotion and sleep. Sudden noises also acted as a trigger for cardiac events in this proband. As compared to LQT3 cohort, where one of them was asymptomatic and the second proband had syncopal events during sleep. These results are summarized in Table S2. Statistical analysis reported no significant difference between age of onset, diagnosis, QTc interval or clinical symptoms in LQT1, LQT2 and LQT3.

#### 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 ( $551 \pm 61$ ) as compared to AD RWS ( $532 \pm 30$ ) and AR RWS ( $506 \pm 8$ ). 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, BDPG, 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



**Table 1**  
List of identified mutations.

Patients	cDNA change	Protein change	Mutation type	Zygoty	Exon	Affected domain	Syndrome	References
<b>KCNQ1</b>								
Patient 1	c.557G > A <sup>#</sup> SCV000240217	p.G186D <sup>#</sup>	Missense	Homozygous	3	S2–S3	JLN1	Vyas et al. (Unpublished results)
Patient 2	c.569G > A SCV000240225; rs120074178	p.R190Q	Missense	Homozygous	3	S2–S3	JLN1	Kaplinger et al., 2009; Vyas et al. (Unpublished results)
Patient 3	c.502G > A SCV000240226; rs179489	p.G168R	Missense	Homozygous	3	S2–S3	JLN1	Marquez et al., 2006; Vyas et al. (Unpublished results)
Patient 4	c.1051T > C <sup>#</sup> SCV000240218 c.443delA <sup>#</sup> SCV000240219	p.F351L <sup>#</sup> p.Y148Lfs*89 <sup>#</sup>	Missense Deletion	Compound Heterozygous	8 2	S6 S1	JLN1	Vyas et al. (Unpublished results) Vyas et al. (Unpublished results)
Patient 5	c.1733-1G > C <sup>#</sup> SCV000240220		Splice site	Homozygous	15	C-ter	JLN1	Vyas et al. (Unpublished results)
Patient 6	c.535G > A SCV000240227; rs199473394	p.G179S	Missense	Homozygous	3	S2–S3	AR LQT1	Splawski et al., 2000; Vyas et al. (Unpublished results)
Patient 7	c.758C > G <sup>#</sup> SCV000240221	p.S253C <sup>#</sup>	Missense	Homozygous	5	S4–S5	AR LQT1	Vyas et al. (Unpublished results)
Patient 8	c.1480dupG <sup>#</sup> SCV000240222	p.E494Gfs*21 <sup>#</sup>	Insertion	Homozygous	11	C-ter	AR LQT1	Vyas et al. (Unpublished results)
Patient 9	c.1686-2A > G <sup>#</sup> SCV000240223 c.1597C > T SCV000240228; rs199472793		Splice site	Compound Heterozygous	13	C-ter	AR LQT1	Vyas et al. (Unpublished results)
Patient 10	c.1762A > T <sup>#</sup> SCV000240224 c.1051T > C <sup>#</sup> SCV000240218	p.I588F <sup>#</sup> p.F351L <sup>#</sup>	Missense Missense	Compound Heterozygous	15 8	C-ter S6	AR LQT1	Vyas et al. (Unpublished results) Vyas et al. (Unpublished results)
Patient 11	c.828_830delCTC SCV000255629	p.S277del	Deletion	Heterozygous	6	S5	LQT1	Napolitano et al., 2005; Present study
Patient 12	c.1703G > C rs199472806; SCV000255628	p.G568A	Missense	Heterozygous	14	C-ter	LQT1	Chen et al., 2005; Present study
Patient 13	c.824_826delTCT rs397508126; SCV000255626	p.F275del	Deletion	Heterozygous	6	S5	LQT1	Aizawa et al., 2007; Present study
Patient 14	c.1032G > A rs1800171; SCV000255625	p.A344A	Splice site	Heterozygous	7	S6	LQT1	Struijk et al., 2006; Present study
Patient 15	c.557G > A <sup>#</sup> SCV000240217	p.G186D <sup>#</sup>	Missense	Heterozygous	3	S2–S3	LQT1	Present study
Patient 16	c.1051T > C <sup>#</sup> SCV000240218	p.F351L <sup>#</sup>	Missense	Heterozygous	8	S6	LQT1	Present study
Patient 17	c.524_535dupTCTGGTCCGCC SCV000255627	p.G179Sfs*267	Duplication	Heterozygous	3	S2–S3	LQT1	Lupoglazoff et al., 2004; Present study
<b>KCNH2</b>								
Patient 1	c.1920C > A SCV000240231	p.F640L	Missense	Heterozygous	7	S6	LQT2	Van Langen et al., 2003; Present study
<b>SCN5A</b>								
Patient 1	c.1231G > A SCV000240230; rs72549410	p.V411M	Missense	Heterozygous	10	D1–S6	LQT3	Tester et al., 2005; Present study
Patient 2	c.5690G > A <sup>#</sup> SCV000240229; rs199472970	p.R1897Q <sup>#</sup>	Missense	Heterozygous	27	C-ter	LQT3	Present study

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

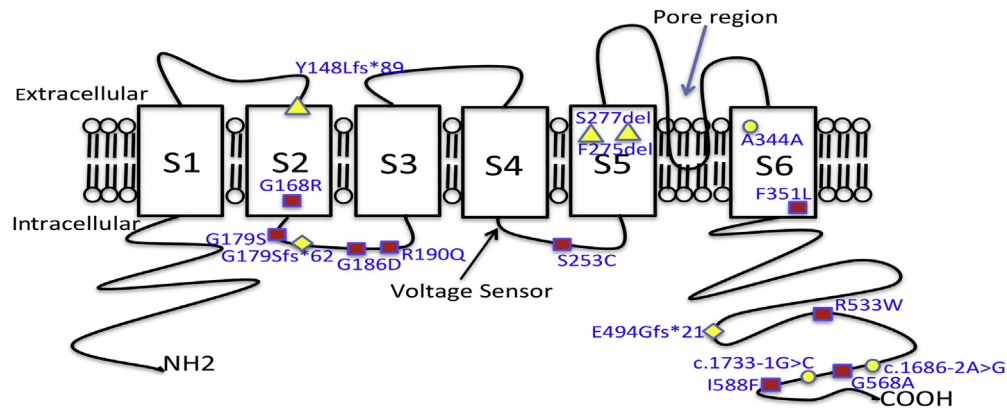
and public exomes/genomes. Two novel splice site variations in *KCNQ1* were evaluated by NetGene2 and a change in the number of acceptor sites was predicted as elaborated below. Wild type allele showed the presence of three acceptor sites at position (87, 151 and 198), while the mutant type (c.1682-2A > G) had only two acceptor sites (87, 198). Similarly, the other splice site mutation (c.1733-1G > C) also showed the absence of the most probable acceptor sites (168, 170, 174, 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 helices, 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 *SCN5A* to cause changes in the secondary structure of the protein (Fig. 4c), which in turn is likely to affect the quaternary structure and/or function.

#### 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*



**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.

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 LQTS (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  $\alpha$ -subunit of  $I_{Ks}$  and  $I_{Kr}$ , respectively, of the delayed rectifier potassium currents and *SCN5A* encodes  $\alpha$ -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].

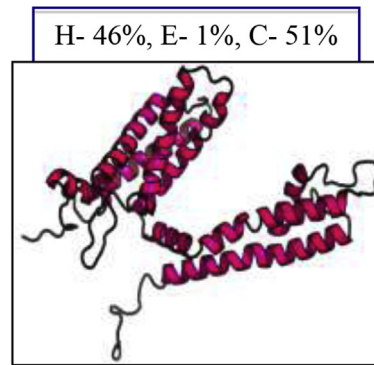
In the cohort of 20 patients positive for mutations, 20 different mutations were identified. Heterozygous mutations were identified in 10 (50%) of 20 patients; 7 (35%) in *KCNQ1*, one in *KCNH2* and two in *SCN5A*. In a study of 903 LQTS Caucasian patients with mutations identified, 91% had heterozygous 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 pattern 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.

**Table 2**  
*In-silico* analysis of novel mutations.

Gene/ Protein	Coding substitution	Protein consequence	Family segregation	Mutation taster	Polyphen2	SIFT	FATHMM	I- Mutant3	PROVEAN	ProtParam (II)	Conservation PhyloP/ PhastCons	MAF (dbSNP, 1000G, EVS)	Other
<i>KCNQ1</i>	557G > A	G186D	Yes	0.999, Disease Causing	1.000, Probably Damaging	0, Damaging	−4.58, Damaging	Decrease, 7	Deleterious, −6.188	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, Damaging	−4.40, Damaging	Decrease, 6	Deleterious, −5.704	41.24	4.003/1	Absent in all	PSIpred – structure alt., RaptorX
	443delA	Y148Lfs*89	Yes	1.000, Disease Causing	–	–	–	–	–	–	–	Absent in all	
	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, Damaging	−5.16, Damaging	Decrease, 5	Deleterious, −4.860	41.13	5.221/1	Absent in all	PSIpred – structure alt., RaptorX
	1480dupG	E494Gfs*21	Yes	1.000, Disease Causing	–	–	–	–	–	–	–	Absent in all	
	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, Damaging	−6.46, Damaging	Decrease, 8	Deleterious, −2.939	41.42	1.682/0.978	Absent in all	PSIpred – structure alt., RaptorX
<i>SCN5A</i>	5690G > A	R1897Q	Yes	0.999, Disease Causing	0.982, Probably Damaging	0.08, Tolerated	−3.82, Damaging	Decrease, 7	Deleterious, −2.576	45.99	4.159/1	Absent in all	PSIpred

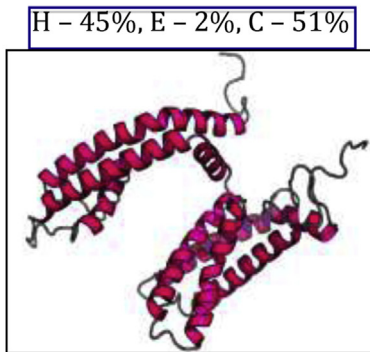
SIFT: Intolerant or deleterious: <\_0.05; Mutation Taster & Polyphen2: probability; FATHMM: scores <0, unfavorable situation, damaging; I-Mutant3: stability, decrease (−0.5 < Kcal/mol), increase (0.5 > Kcal/mol), neutral (−0.5 <\_ Kcal/mol), RI; PROVEAN: Deleterious, <−2.5; ProtParam: Instability index (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.

a. KCNQ1 (Wild Type protein)

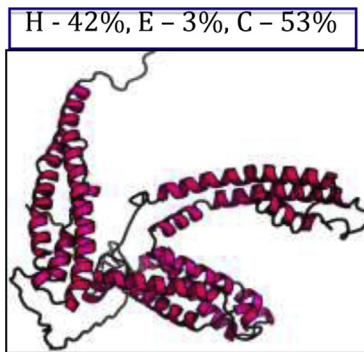


KCNQ1 (Mutant proteins)

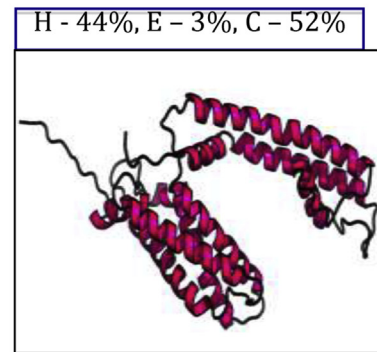
F351L



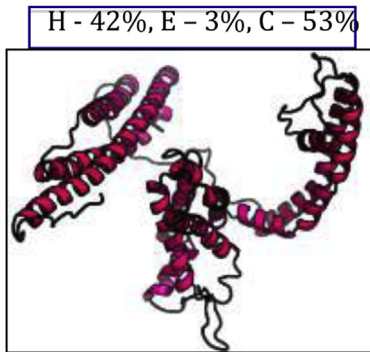
I588F



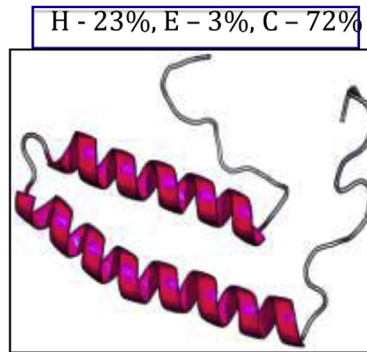
G186D



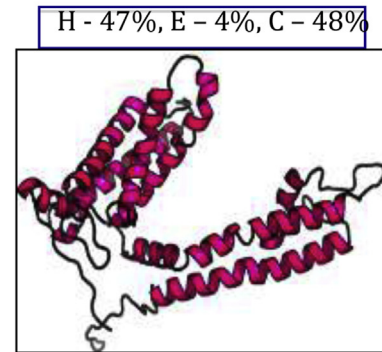
S253C



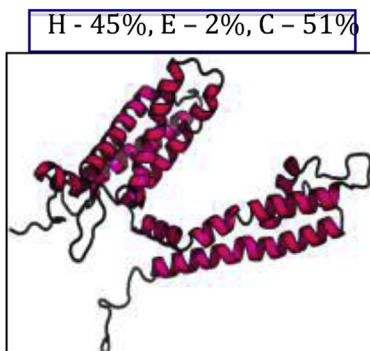
443delA



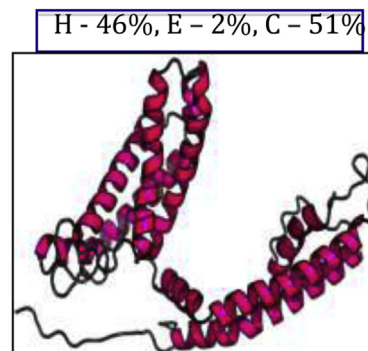
1480\_1481insG



1686-2A>G



1732-1G>C



**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.





### 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  $536 \pm 44.23$  and C-terminal mutations was  $510 \pm 12.36$ . In *KCNH2*, 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 *SCN5A*. 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].

In the current study, nine (45%) of 20 mutations were novel variants (*KCNQ1*: 8 and *SCN5A*: 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 *SCN5A* 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 (G186D, 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, LQT 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
<i>KCNH2</i>	Potassium Channel, Voltage Gated Eag Related Subfamily H, Member 2
<i>KCNQ1</i>	Potassium Channel, Voltage Gated KQT-Like Subfamily Q, Member 1
<i>SCN5A</i>	Sodium Channel, Voltage Gated, Type V Alpha Subunit

### References

- [1] Moss AJ, Kass RS. Long QT syndrome: from channels to cardiac arrhythmias.

- J Clin Investig 2005;115:2018–24. <http://dx.doi.org/10.1172/JCI25537>.
- [2] Mizusawa Y, Horie M, Wilde AA. Genetic and clinical advances in congenital long QT syndrome. *Circ J* 2014;78:2827–33. <http://dx.doi.org/10.1253/circj.CJ-14-0905>.
- [3] Giudicessi J, Ackerman M. Genotype- and phenotype-guided management of congenital long QT syndrome. *Curr Probl Cardiol* 2013;38:417–55. <http://dx.doi.org/10.1016/j.cpcardiol.2013.08.001>.
- [4] Bartos DC, Giudicessi JR, Tester DJ, et al. A KCNQ1 mutation contributes to the concealed type 1 long QT phenotype by limiting the Kv7.1 channel conformational changes associated with protein kinase A phosphorylation. *Heart Rhythm* 2014;11:459–68. <http://dx.doi.org/10.1016/j.hrthm.2013.11.021>.
- [5] Chang YS, Yang YW, Lin YN, Lin KH, Chang KC, Chang JG. Mutation analysis of KCNQ1, KCNH2 and SCN5A genes in Taiwanese long QT syndrome patients. *Int Heart J* 2015;56:450–3. <http://dx.doi.org/10.1536/ihj.14-428>.
- [6] Kapplinger JD, Tester DJ, Salisbury BA, et al. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. *Heart Rhythm* 2009;6:1297–303. <http://dx.doi.org/10.1016/j.hrthm.2009.05.021>.
- [7] Priori SG, Schwartz PJ, Napolitano C, et al. A recessive variant of the Romano-Ward long-QT syndrome? *Circulation* 1998;97:2420–5. <http://dx.doi.org/10.1161/01.CIR.97.24.2420>.
- [8] Moss AJ, Zareba W, Benhorin J, et al. ECG T-wave patterns in genetically distinct forms of the hereditary long QT syndrome. *Circulation* 1995;92:2929–34. <http://dx.doi.org/10.1161/01.CIR.92.10.2929>.
- [9] Chiang C, Roden D. The long QT syndromes: genetic basis and clinical implications. *J Am Coll Cardiol* 2000;36:1–12. [http://dx.doi.org/10.1016/S0735-1097\(00\)00716-6](http://dx.doi.org/10.1016/S0735-1097(00)00716-6).
- [10] Tester DJ, Ackerman MJ. Genetics of long QT syndrome. *Methodist Debakey Cardiovasc J* 2014;10:29–33. PMID: PMC4051331.
- [11] Abrams D, MacRae C. Long QT syndrome. *Circulation* 2014;129:1524–9. <http://dx.doi.org/10.1161/CIRCULATIONAHA.113.003985>.
- [12] Schwartz P, Priori S, Spazzolini C, et al. Genotype-phenotype correlation in the long-QT syndrome. Gene-specific triggers for life-threatening arrhythmias. *Circulation* 2001;103:89–95. <http://dx.doi.org/10.1161/01.CIR.103.1.89>.
- [13] Splawski I, Shen J, Timothy KW, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation* 2000;102:1178–85. <http://dx.doi.org/10.1161/01.CIR.102.10.1178>.
- [14] Brink P, Crotti L, Corfield V, et al. Phenotypic variability and unusual clinical severity of congenital long-QT syndrome in a founder population. *Circulation* 2005;112:2602–10. <http://dx.doi.org/10.1161/CIRCULATIONAHA.105.572453>.
- [15] Marjamaa A, Salomaa V, Newton-Cheh C, et al. High prevalence of four long QT syndrome founder mutations in the Finnish population. *Ann Med* 2009;41:234–40. <http://dx.doi.org/10.1080/07853890802668530>.
- [16] Liu W, Yang J, Hu D, et al. KCNQ1 and KCNH2 Mutations associated with long QT syndrome in a Chinese population. *Hum Mutat* 2002;20:475–6. <http://dx.doi.org/10.1002/humu.9085>.
- [17] Riuro H, Campuzano O, Berne P, et al. Genetic analysis, in silico prediction, and family segregation in long QT syndrome. *Eur J Hum Genet* 2015;23:79–85. <http://dx.doi.org/10.1038/ejhg.2014.54>.
- [18] Sathyamurthy I, Jayanthi K, Dash J, Srinivasan KN. Long QT syndrome in children with congenital deafness. *Indian Pediatr* 2009;46:507–8.
- [19] Sundar RJ, Parvathy CS, Thomas JM, Sudarsana G. Congenital long QT syndrome presenting as epilepsy. *Indian Pediatr* 2003;40:1201–3.
- [20] Chinagudi S, Patted SM, Herur A. A study of electrocardiographic changes in congenital deaf school children. *Indian J Otolaryngol Head Neck Surg* 2010;62:44–8. <http://dx.doi.org/10.1007/s12070-010-0008-6>.
- [21] Qureshi SF, Ali A, Ananthapur V, et al. Novel mutations of KCNQ1 in Long QT syndrome. *Indian Heart J* 2013;65:552–60. <http://dx.doi.org/10.1016/j.ihj.2013.08.025>.
- [22] Qureshi SF, Ali A, John P, et al. Mutational analysis of SCN5A gene in long QT syndrome. *Meta Gene* 2015;6:26–35. <http://dx.doi.org/10.1016/j.mgene.2015.07.010>.
- [23] Nakajima T, Kaneko Y, Kurabayashi M. Unveiling specific triggers and precipitating factors for fatal cardiac events in inherited arrhythmia syndromes. *Circ J* 2015;79:1185–92. <http://dx.doi.org/10.1253/circj.CJ-15-0322>.
- [24] Medeiros-Domingo A1, Iturralde-Torres P, Ackerman MJ. Clinical and genetic characteristics of long QT syndrome. *Rev Esp Cardiol* 2007;60:739–52. [http://dx.doi.org/10.1016/S1885-5857\(08\)60010-9](http://dx.doi.org/10.1016/S1885-5857(08)60010-9).
- [25] Bidchol AM, Dalal A, Shah H, et al. GALNS mutations in Indian patients with mucopolysaccharidosis IVA. *Am J Med Genet A* 2014;164:2793–801. <http://dx.doi.org/10.1002/ajmg.a.36735>.
- [26] Dalal A, Bhavani GS, Togarrati PP, et al. Analysis of the WISP3 gene in Indian families with progressive pseudorheumatoid dysplasia. *Am J Med Genet A* 2012;158:2820–8. <http://dx.doi.org/10.1002/ajmg.a.35620>.
- [27] Moss AJ, Shimizu W, Wilde AAM, et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation* 2007;115:2481–9. <http://dx.doi.org/10.1161/CIRCULATIONAHA.106.665406>.
- [28] Shimizu W, Horie M, Ohno S, et al. Mutation site-specific differences in arrhythmic risk and sensitivity to sympathetic stimulation in LQT1 form of congenital long QT syndrome: multicenter study in Japan. *J Am Coll Cardiol* 2004;44:117–25. <http://dx.doi.org/10.1016/j.jacc.2004.03.043>.
- [29] Shimizu W, Moss AJ, Wilde AM, et al. Genotype-phenotype aspects of type 2 long QT syndrome. *J Am Coll Cardiol* 2009;54:2052–62. <http://dx.doi.org/10.1016/j.jacc.2009.08.028>.
- [30] Tester DJ, Will ML, Haglund CM, Ackerman MJ. Effect of clinical phenotype on yield of long QT syndrome genetic testing. *J Am Coll Cardiol* 2006;47:764–8. <http://dx.doi.org/10.1016/j.jacc.2005.09.056>.
- [31] Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. *Circulation* 1999;99:529–33. <http://dx.doi.org/10.1161/01.CIR.99.4.529>.