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Genotype–phenotype correlation in long QT syndrome families

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ABSTRACT

Heterogeneity in clinical manifestations is a well-known feature in Long QT Syndrome (LQTS). The extent of this phenomenon became evident in families wherein both symptomatic and asymptomatic family members are reported. The study hence warrants genetic testing and/or screening of family members of LQTS probands for risk stratification and prediction.

Of the 46 families screened, 18 probands revealed novel variations/compound heterozygosity in the gene/s screened. Families 1–4 revealed probands carrying novel variations in *KCNQ1* gene along with compound heterozygosity of risk genotypes of the *SCN5A*, *KCNE1* and *NPPA* gene/s polymorphisms screened. It was also observed that families- 5, 6 and 7 were typical cases of “anticipation” in which both mother and child were diagnosed with congenital LQTS (cLQTS). Families- 16 and 17 represented aLQTS probands with variations in *IKs* and *INa* encoding genes. First degree relatives (FDRs) carrying the same haplotype as the proband were also identified which may help in predictive testing and management of LQTS. Most of the probands exhibiting a family history were found to be genetic compounds which clearly points to the role of cardiac genes and their modifiers in a recessive fashion in LQTS manifestation.

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Introduction

Long QT Syndrome (LQTS) is associated with prolongation in ventricular repolarisation and is diagnosed by a prolonged QTc value exceeding 450 ms and documented syncope episodes. However, QTc measurement on electrocardiogram (ECG) is not always a reliable surrogate marker for prolonged repolarisation as not all patients who present the LQTS phenotype exhibit prolonged QTc interval and some asymptomatic individuals may have prolonged QTc. The detection of a genetic defect within a family allows for the identification of all subjects at risk of developing cardiac events. This information has a direct impact on the clinical management of prophylactic therapy for defective gene carriers against fatal arrhythmias. Hence, a pressing need arises to screen the LQTS-associated genes for pathological mutations in high-risk individuals [1]. Molecular screening of family members of probands would unmask many affected relatives, thus allowing effective preventive measures [2].

Genetic disorders have been associated with carrying common haplotypes mostly carrying risk alleles. Identification of such a common haplotype can be of importance in the prognostic evaluation of first degree relatives (FDRs). The Finnish population represents a genetic isolate due to effective geographical and linguistic separation within the historical period. The geographical distribution of the birthplaces for the oldest KCNQ1-Fin carriers closely matches the internal migration wave commenced from the southeast in the sixteenth century. The founder gene phenomenon is further supported by the common haplotype associated with the disease in virtually all affected individuals examined [3].

Previously, a founder KCNQ1 mutation has been reported to occur in five South African families based on identical haplotype around the disease locus [1]. Thus, one can envision not only genotype-specific treatment algorithms but even mutation-specific considerations. The clinical message is that in the future attention should be paid to families with a high percentage of symptomatic individuals and that, once the disease-causing mutations have been identified, collaborative studies should be undertaken to test the possibility of identifying other clinically malignant mutations. This will contribute to the development of a more accurate risk stratification grid for patients affected by LQTS [4].

Considering the previous reports and suggestions, screening of available family members is warranted in the present study. Along with controls and patients, the available family members were also screened for any variations in IKs (slowly activating delayed rectifier potassium current) and INa (sodium current) channel encoding genes (already implicated in LQTS), and single nucleotide polymorphisms of gene/s which may have a modifying effect on LQTS i.e. beta-adrenergic receptor-1 & 2 (ADRB 1 & 2), atrial natriuretic peptide (NPPA) and tumor necrosis factor-alpha (TNF- α) gene/s. The present study on LQTS families is in continuation to our previous report which revealed mutations in a JLN Syndrome proband and his family members [5]. In this study, mutations were identified in three probands and their family members. It was also observed that the probands were carrying more than one risk allele leading to compound heterozygosity/genetic

compounds. Predictive genetic testing was also carried out for family members to establish specific haplotype segregating as in the proband, to identify if the risk of such haplotypes to LQTS can co-exist in the siblings and first degree relatives (FDRs) and to predict prognosis of the condition.

Methodology

Study subjects

Blood samples were collected for molecular and genetic analyses from confirmed 46 LQTS probands and 69 first degree relatives referred to Care Hospitals, Hyderabad, Sri Jayadeva Institute of Cardiovascular Science and Research, Bangalore, Institute of Maternal and Child Health, Calicut Medical College, Calicut and Krishna Institute of Medical Sciences, Hyderabad. Samples were collected from 2009 to 2013 due to the rarity of the disorder. The QTc of the LQTS patients and their FDRs was confirmed by electrocardiogram. LQTS patients with prolonged QTc with/without syncope and family history of sudden cardiac death were included in the study. Available first degree relatives of the LQTS probands (with/without any history of cardiovascular disease) were also included. This study has been approved by the Institutional Ethics Committee, Dept. of Genetics, Osmania University, Hyderabad and informed written consent was obtained from the probands and their available family members. Blood samples from 150 controls (75 M: 75 F), without any history of cardiovascular or systemic conditions, was collected from Osmania General Hospital, Hyderabad for comparative analysis.

Inclusion and exclusion criteria

Patients who satisfied the diagnostic criteria Schwartz et al. [24] for LQTS referred by the cardiologists were included in the study. And cases exhibiting hearing loss other than JLN syndrome were excluded.

Molecular analyses

Genomic DNA was isolated from peripheral blood samples by standard protocols in 150 controls, 46 probands and their family members. PCR was carried out for all the gene/s mentioned in a thermal cycler (Eppendorf, Germany) using specific primers obtained from published reports and mutation database. The primers for KCNQ1, KCNE1 and SCN5A were as described by Syrris et al., [6] ADRB1 were by Maqbool et al. [7] and for ADRB2 by Martinez et al. [8]. NPPA primers used were as given by Kato et al. [9] For amplification of TNF- α gene –308 G/A SNP, primers sequences from Wu et al. [10] further modified by Verjans et al. [11] were taken. For –1031 T/C and –238 G/A SNPs, primers described by Soga et al. [12] and Malivanova et al. [13] respectively were used.

For KCNQ1, KCNE1 and SCN5A PCR-SSCP analyses was carried out on native PAGE gels followed by silver staining. And for ADRB, NPPA and TNF- α polymorphisms, PCR-RFLP and ARMS-PCR products were checked on 10% native PAGE gel

followed by silver staining for genotyping of these polymorphisms.

In-silico analysis

In-silico analysis was carried out to examine the influence of variations on transmembrane protein structure (<http://bioinf.cs.ucl.ac.uk/psipred/>) [14], Tertiary protein structure prediction by RAPTOR-X (<http://raptorx.uchicago.edu/>) [15] were elucidated.

Results

Family-1

The proband referred as a 6-year old boy was categorized as a patient of JLN syndrome, a recessive form of Long QT syndrome (LQTS) based on the clinical diagnostic criteria with a history of parental consanguinity, neonatal sibling deaths, age at onset of 6 months and deafness associated with mutations in *KCNQ1* gene [16,17]. The electrophorogram (on repeated sequencing) of the parents, sibling and maternal grandmother revealed a carrier status while the proband harbored recessive variations. In-silico analysis also revealed the variations may lead to a change in the secondary structure of mRNA and shift in the position of splice site. The variations also resulted in a change of position of the splicing enhancer/inhibitor in *KCNQ1* and exonic variations leading to truncated S2–S3 fragment of *KCNQ1* transmembrane protein in cardiac cells with aberrant repolarization causing prolonged QTc. Apart from this, the epithelial cells of inner ear are reported to be affected causing deafness. These variations identified in this proband have been described in our earlier study [5]. Additional findings of compound heterozygosity and relatives at risk are being described in this study.

Compound heterozygosity

Apart from these novel mutations in *KCNQ1*, the proband also expressed the risk genotypes – ‘AA’ of *KCNE1* S38G, ‘AA’ of *SCN5A* H558R and ‘GG’ of *SCN5A* 98297G > A polymorphisms identified by SSCP analysis. This compound heterozygosity may lead to the formation of aberrant INa and IKs channels leading to prolonged QTc in the proband. The combination of above reported mutations along with these risk genotypes may also contribute to severity and disease progression which

is well correlated with a QTc duration of 520 msec and recurrent syncope.

Relatives at risk

Parents, sibling and maternal grandmother of the proband were asymptomatic, but their electrophorograms revealed heterozygosity for the variation and the carrier status further confirms to the recessive inheritance pattern.

Family-2

The patient is an 11 day old female neonate. Bradycardia was detected in the fetus, hence caesarian section was performed but the baby continued to have bradycardia. The electrocardiogram (ECG) showed a prolonged QTc with a family history of 2 neonatal deaths and sudden death of maternal grandfather due to myocardial infarction (Fig. 1). ECG of the family members was found to be normal. Blood samples were collected from the proband and her parents.

Molecular results

The PCR-SSCP analysis revealed band pattern variations in the samples of proband and her mother in exon 15 of *KCNQ1* gene (Fig. 2). Repeated commercial sequencing showed a novel polymorphism at C338033T in a heterozygous state (Fig. 3) causing R594X which was confirmed by NCBI BLAST (Fig. 4) and registered with dbSNP (ss947849412).

In-silico analysis

Since the variation is located in an exon, the protein 3D structures from RaptorX (Fig. 5) and transmembrane structures from PSIPRED were compared to the wild type (Fig. 6). The 3D structures revealed a loss in alpha helix and gain of 2 beta sheets causing changes in conformation of the protein.

Transmembrane structure predictions revealed conformational changes in 3D structure as exon 15 encodes the C-terminal region of *KCNQ1* (Fig. 5).

Compound heterozygosity

In addition to this novel mutation in *KCNQ1*, the proband also expressed the risk genotypes – ‘AA’ of *KCNQ1* S546S and IVS13 + 36A > G, ‘AA’ of *SCN5A* H558R and ‘GG’ of *SCN5A* 98297G > A polymorphisms identified by SSCP analysis. It can be hypothesized that the compound heterozygosity observed may lead to aberrant INa and IKs channels leading to prolonged QTc in the proband in a recessive manner.

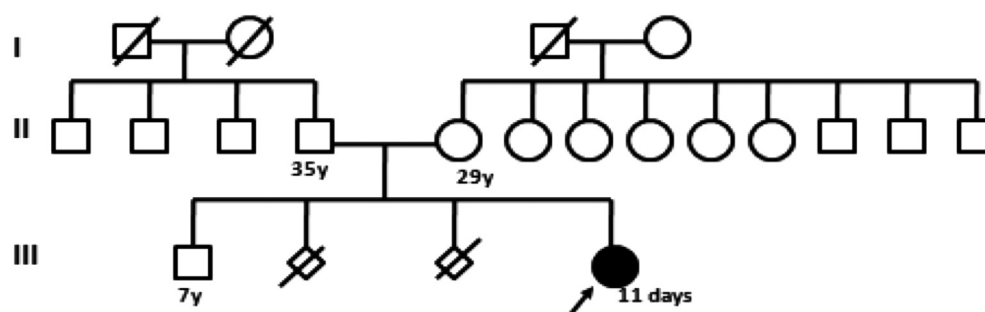


Fig. 1 – Pedigree of family-2.

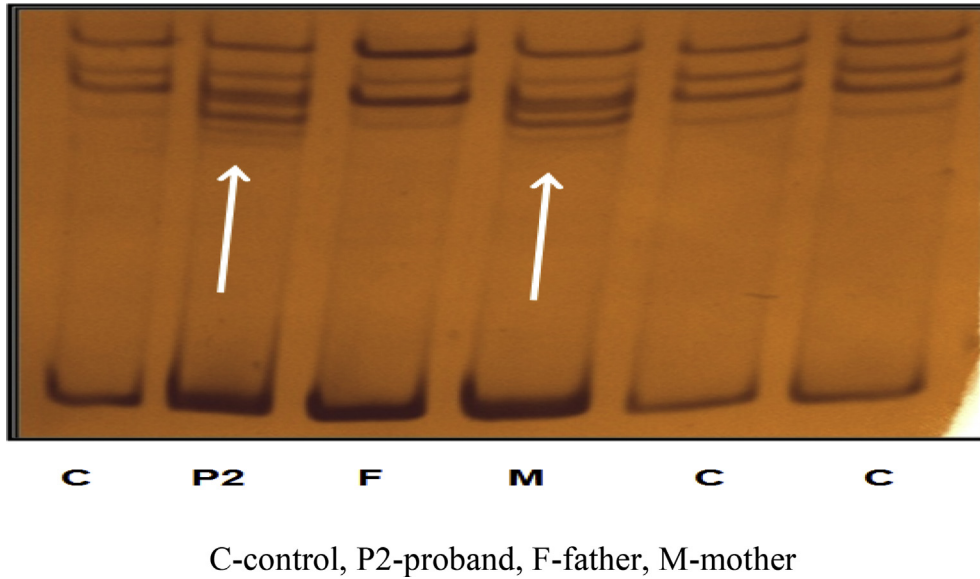


Fig. 2 – SSCP band pattern variations of exon 15 of KCNQ1 gene.

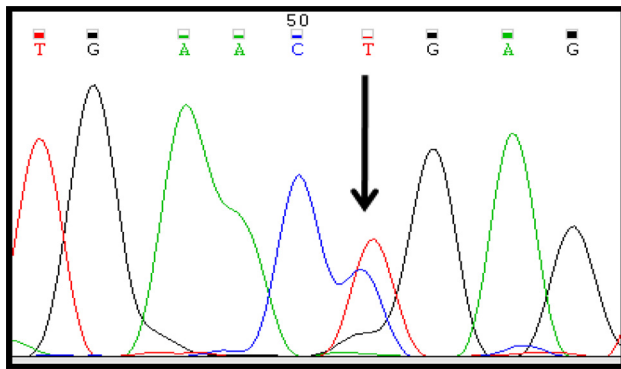


Fig. 3 – Electropherogram showing C338033T.

Relatives at risk

The mother of proband was asymptomatic, but the electro-pherograms revealed the same variation of R594X observed in the proband in a heterozygous state indicating the potential risk to mother at a later stage.

Both the parents of the proband were found to express the same haplotype as the proband, increasing the risk to develop LQTS at later stage (Table 1). This highlights the age related penetrance of the gene.

Though the proband did not express the trademark symptoms of syncope and deafness, a prolonged QTc with a maternal history of two miscarriages and myocardial infarction in maternal grandfather was reported. The proband's 7-yr old sibling and parents were asymptomatic. Molecular screening by PCR-SSCP analysis revealed band pattern variation in 15th exon of KCNQ1 gene in proband and her mother which on commercial sequencing was confirmed as R594X mutation in a heterozygous state (338033 C/T). This mutation was found to be present in the C-terminal domain resulting in modification of 3D structure of the protein. The dominant-negative effect of this mutation may result in the interaction of this protein with novel/unknown proteins in the cell leading to altered interactions of the K⁺ channel ending in bradycardia. Tester et al. [18] reported a similar case on the prenatal molecular analysis of the amniotic fluid of a fetus at 16 weeks gestation which revealed a mutation in KCNQ1 gene (LQT1). When bradycardia was detected in the fetus during

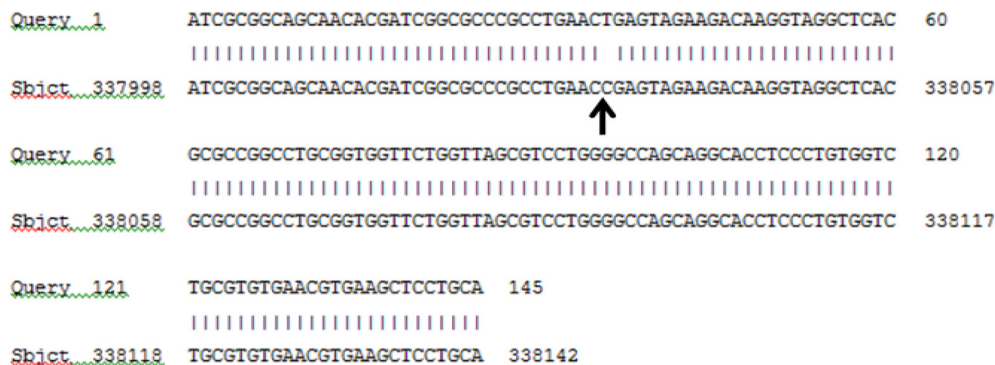


Fig. 4 – NCBI BLAST Novel Polymorphism at C338033T causing R594X (ss947849412).

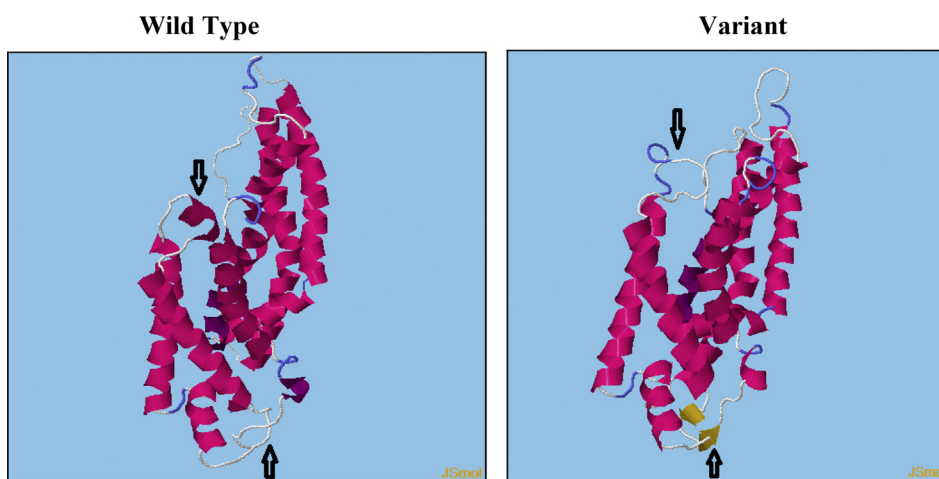
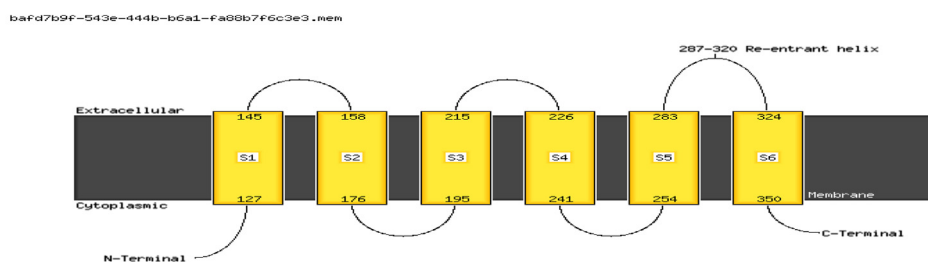


Fig. 5 – 3D Structure of Wild type and Variant Protein.

Wild-Type



Variant

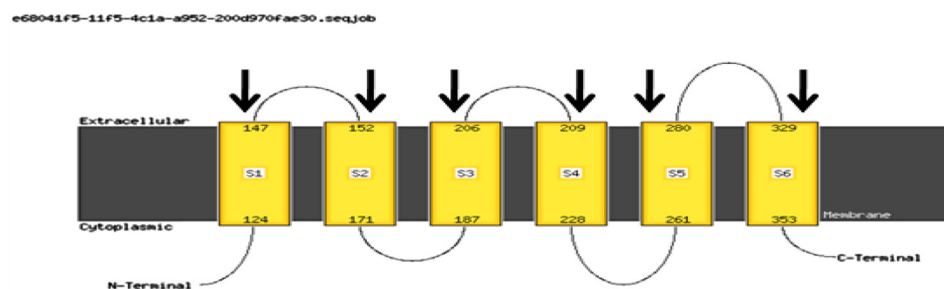


Fig. 6 – Transmembrane Structure of Wild-Type and Variant (Exon 15 variations) Protein.

labor, therapy with β -blockers was initiated immediately. Genetic testing for congenital long QT syndrome (cLQTS) would thus become routine due to the inherent risks involved in amniocentesis [18].

The proband's mother also expressed the same R594X mutation. Predictive testing revealed that both the parents were carrying the same haplotype as the proband highlighting them as potential 'at risk' individuals for developing LQTS correlated to anticipation and age related penetrance of gene.

Family-3

A 32 year old female presented with syncope and prolonged QTc and no significant family history. Blood samples were collected from parents, children, siblings and nephews for molecular analysis (Fig. 7). Molecular analysis revealed band pattern variations in exon-14 of KCNQ1 gene in proband and all the family members (parents, children, siblings and nephews) were subjected to screening (Fig. 8).

Table 1 – Common haplotype group – risk identification in proband's parents of family-2.

KCNE1 S38G	KCNQ1 S546S	KCNQ1 IVS13 + 36A > G	SCN5A H558R	SCN5A 98297G > A	Identified in
G	A	A	A	G	Proband, Mother, Father

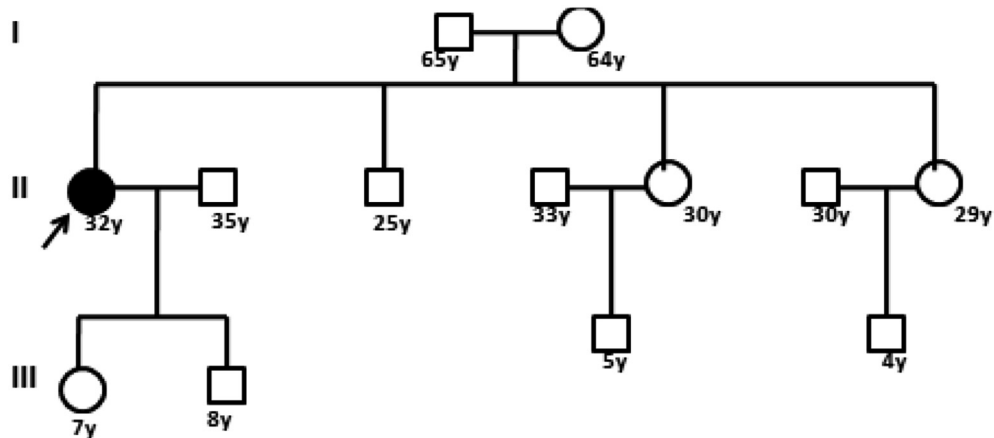
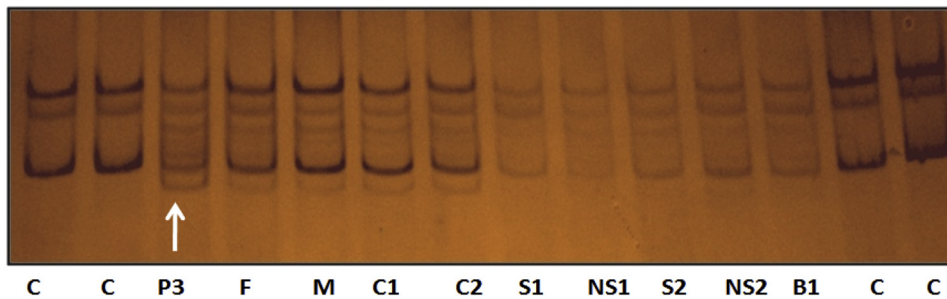


Fig. 7 – Pedigree of Family-3.



C-control, P3-proband, F-father, M-mother, C1-son, C2-daughter, S1-sister-1, NS1- nephew (son of sister-1), S2-sister-2, NS2- nephew (son of sister-2), B1-brother

Fig. 8 – SSCP band pattern variation in proband and family members compared to controls.

DNA samples of the proband and family members were subjected to repeated commercial sequencing. Electropherograms of the amplified samples revealed a heterozygous state in all the family members and proband (Fig. 9). The heterozygosity in the electropherograms was observed even on repeated sequencing. This indicates the possibility of an occurrence of insertions or deletions in the DNA preceding

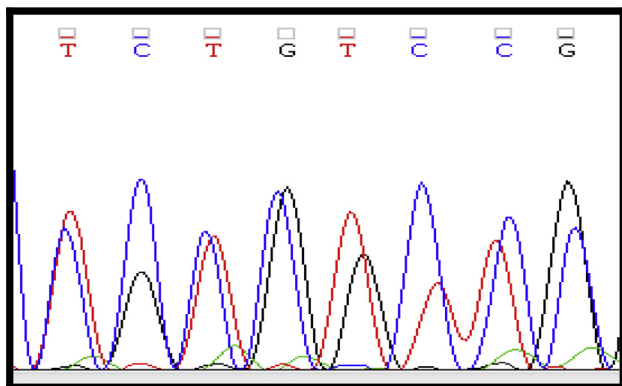


Fig. 9 – Electropherogram of proband-3 indicating heterozygosity in exon-14 of KCNQ1 gene.

exon-14. Such heterozygosity may create a dominant-negative effect leading to truncated KCNQ1 transmembrane structure.

Compound heterozygosity

Apart from the variation in exon-14 of KCNQ1, the proband also expressed the risk genotype ‘GG’ of SCN5A 98297G > A polymorphism identified on SSCP analysis. The compound heterozygosity observed in the proband may lead to aberrant electrophysiological properties of the heart causing prolonged QTc in the proband.

Relatives at risk

Both the parents, children, siblings and nephew-1 of the proband were also found to harbor the same haplotype as the proband (Table 2). The electropherograms of exon-14 of KCNQ1 gene revealed the same variation as observed in the proband in a heterozygous state indicating the potential risk to these family members and follow up studies are therefore warranted in such cases.

KCNQ1 gene screening of proband-3 revealed band pattern variations in exon-14 which on sequencing revealed a heterozygous state. The parents, siblings and children of proband-3 also exhibited the similar heterozygosity. The

Table 2 – Common haplotype group – risk identification in proband’s family members of family-3.

KCNQ1 S546S	KCNQ1 IVS13 + 36A > G	SCN5A H558R	SCN5A 98297G > A	Identified in
A	A	A	G	Proband, Mother, Father, children, 3 siblings and nephew NS1 (son of sister-1)

heterozygosity was observed in exon-14 and since exon-13 and 15 did not reveal any variation, it can be presumed that this heterozygous state is the result of non-homologous recombination involving exon-14 due to transposon/retrotransposons.

The dominant-negative effect of the variation may lead to the formation of an abnormal K⁺ channel which plays a role in etiology of the disorder by interacting with novel and unknown protein/s. Since this variation is also detected in the family members of proband-3, the individuals harboring the variation/mutation are hence at a potential risk of manifesting the disorder.

Family-4

The proband-4 is a 45 year old female who presented with syncope and prolonged QTc and a family history of sudden death of a sibling. Family members were not available for sample collection (Fig. 10). PCR-SSCP analysis revealed variation in exon-14 of KCNQ1 gene (Fig. 11). A heteroduplex was observed in the ds DNA of the SSCP gel.

On repeated commercial sequencing a heterozygous electropherogram was observed all throughout the amplified exon-14 of KCNQ1 gene (Fig. 12). It can be hypothesized that this observed heterozygosity may be the result of non-homologous recombination involving exon-14 caused by transposon/retrotransposons or as a result of an indel which occurred upstream to exon-14.

Compound heterozygosity

The proband also expressed the risk genotype of ‘GG’ of SCN5A 98297G > A polymorphism along with the variation in exon-14 of KCNQ1. The dominant-negative effect due to heterozygosity in KCNQ1 and the compound heterozygosity leads to prolongation of QTc due to impaired IKs and INa channels. Predictive

testing in family members is warranted as these variations may be the reason for family history of sudden death and late age at onset in the proband.

Family-5

A 12 year old deaf boy presented with a history of syncope and prolonged QTc on electrocardiogram. He had a history of sudden neonatal death of his twin brother and SCD of another sibling at the age of 12 years (Fig. 13). The proband was diagnosed with cLQTS (JLNS) and administered beta-blockers. The parents were also subjected to routine check-up which revealed prolonged QTc in the mother (diagnosed as cLQTS). The blood samples of the proband and his parents were collected for molecular analyses.

Compound heterozygosity

Molecular analyses showed compound heterozygosity with the haplotype comprising of ‘G’ alleles of SCN5A H558R and G98297A and ‘C’ allele of NPPA C1364A polymorphisms in both the cLQTS patients (proband and his mother) (Table 3).

This family is an interesting case of “anticipation” wherein both the proband and his mother were diagnosed with cLQTS and the age at onset was 2.5 years in proband and the mother was diagnosed at a later age of 38 years, with the intensity of the disorder increasing in generation III with QTc >500 ms in young proband and sudden cardiac deaths in siblings.

Family-6

Fig. 14 shows a family with both proband and her mother affected with cLQTS. They presented with prolonged QTc but no significant family history of sudden deaths. The proband was a 7 year old female and her 28 year old cLQTS mother. Blood samples were collected from the proband, her mother,

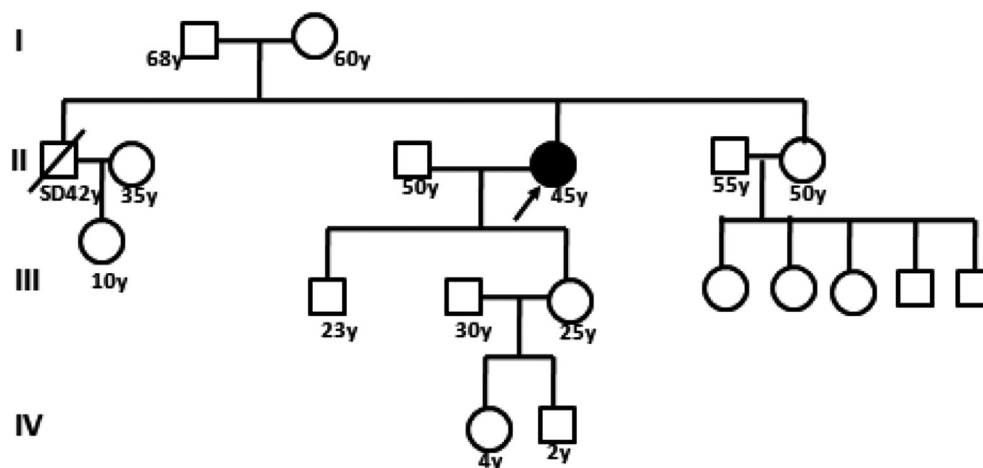


Fig. 10 – Pedigree of family-4.

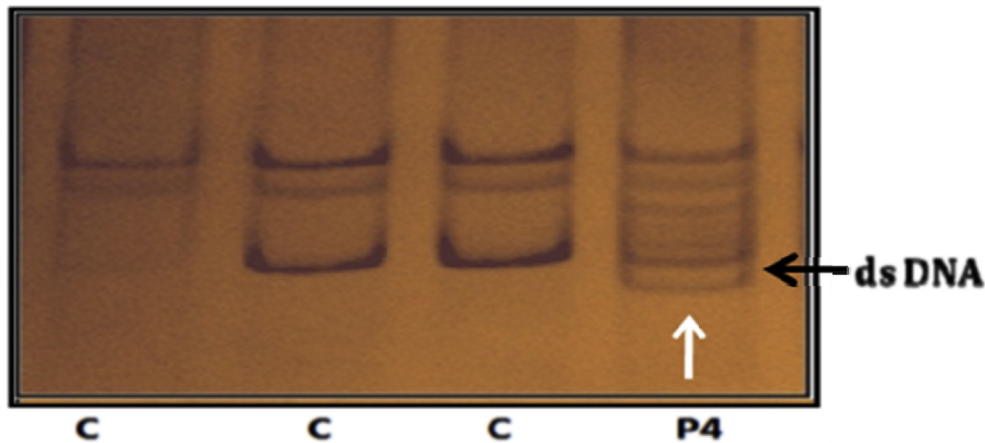


Fig. 11 – SSCP band pattern variation in exon-14 of KCNQ1 gene in proband-4.

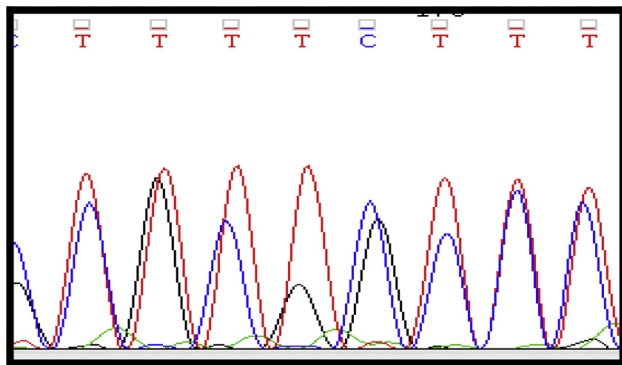


Fig. 12 – Electropherogram showing heterozygosity in exon-14 of KCNQ1 gene.

sibling (a 12 year old normal male) and maternal uncle (a 38 year old normal male).

Compound heterozygosity

Molecular analyses revealed that both the probands were carrying the risk alleles of KCNQ1 S546S and IVS13 + 36A > G, SCN5A 98297G > A, and NPPA C1364A. The presence of such genetic compounds may have lead to impairment of potassium and sodium ion channels leading to prolonged QTc. The

presence of risk allele of the developmental gene NPPA may have lead to malfunction during cardiogenesis leading to congenital LQTS in both the patients.

Relatives at risk

The sibling and maternal uncle were found to carry the same haplotype as the proband and thus are at a risk of developing LQTS at a later stage in life depicting age related gene penetrance (Table 4). In this family also, the concept of “anticipation” is clear with the daughter being affected at an earlier age than the mother.

Family-7

This is a unique case wherein a 10 month female proband and her 38 year old mother were both diagnosed with cLQTS. The proband’s mother had been suffering from episodes of syncope from 6 years of age and was deaf as a result of which she was diagnosed as a JLN syndrome patient (Fig. 15). Her daughter also showed a prolongation of QTc without deafness and was identified as a cLQTS patient, and their blood samples were obtained for genetic analyses.

Compound heterozygosity

The proband and her mother were carrying the same haplotype represented in Table 5 which included risk alleles for – KCNQ1

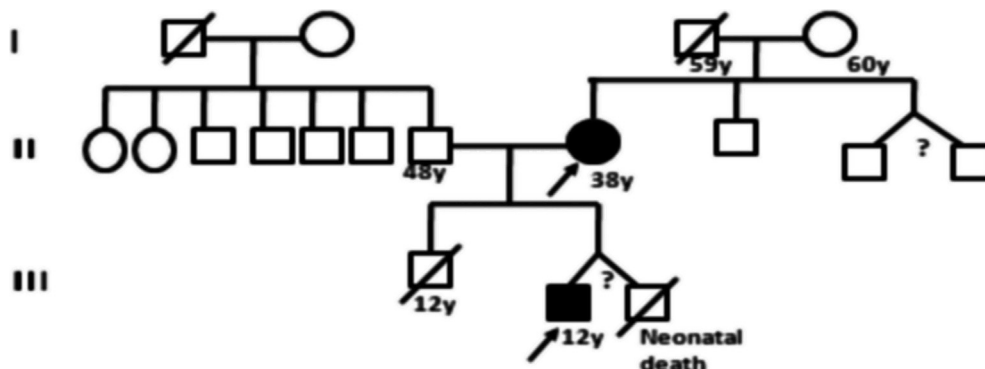


Fig. 13 – Pedigree of Family-5.

Table 3 – Common haplotype group – risk identification in proband's mother of family-5.

NPPA C1364A	SCN5A H558R	SCN5A 98297G > A	Identified in
C	G	G	Proband, Mother (also LQTS)

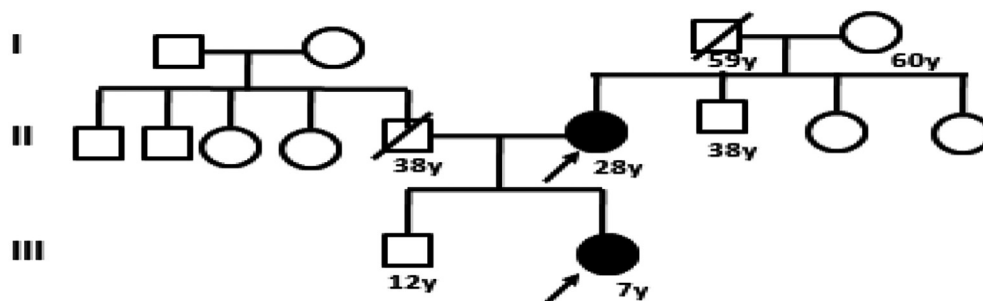


Fig. 14 – Pedigree of Family-6.

Table 4 – Common haplotype group – risk identification in proband's family members of family-6.

KCNQ1 S546S	KCNQ1 IVS13 + 36A > G	NPPA C1364A	SCN5A 98297G > A	Identified in
A	A	C	G	Proband, Mother (also cLQTS), sibling and maternal uncle

S546S and IVS13 + 36A > G, SCN5A 98297G > A polymorphisms. Another interesting finding was the presence of NPPA C1364A risk allele only in the proband (absent in her mother) which may have altered cardiogenesis and lead to presentation of symptoms at an early age. This family represents anticipation with the mother and daughter being affected. This is also a classic case of age related penetrance effects.

Family-8

Proband-8 is a 10 year old female who presented with the symptoms of syncope and prolonged QTc of 530 ms. She had a family history of sudden death of a sibling but no consanguinity (Fig. 16). The patient's mother's sample was collected for analyses.

Compound heterozygosity

The proband was found to be carrying the risk alleles of SCN5A H558R and G98297A polymorphisms indicating the role of this haplotype in the etiology of the disorder.

Relatives at risk

The patient's mother was also found to carry the same haplotype (Table 6) as the proband which poses the mother at risk of developing LQTS at a later stage in life.

Family-9

Proband-16 is a 50 year old female diagnosed with cLQTS, presenting with symptoms of syncope and prolonged QTc at

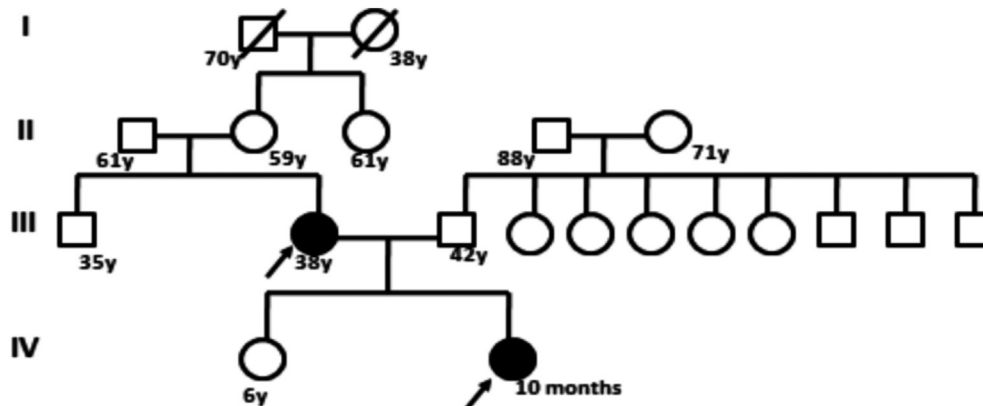


Fig. 15 – Pedigree of Family-7.

Table 5 – Common haplotype group – risk identification in proband's Mother of family-7.

KCNQ1 S546S	KCNQ1 IVS13 + 36A > G	SCN5A H558R	SCN5A E1061E	SCN5A S1074R	SCN5A 98297G > A	NPPA C1364A	Identified in
A	A	G	G	C	G	C	Proband
A	A	G	G	C	G	heterozygous	Mother

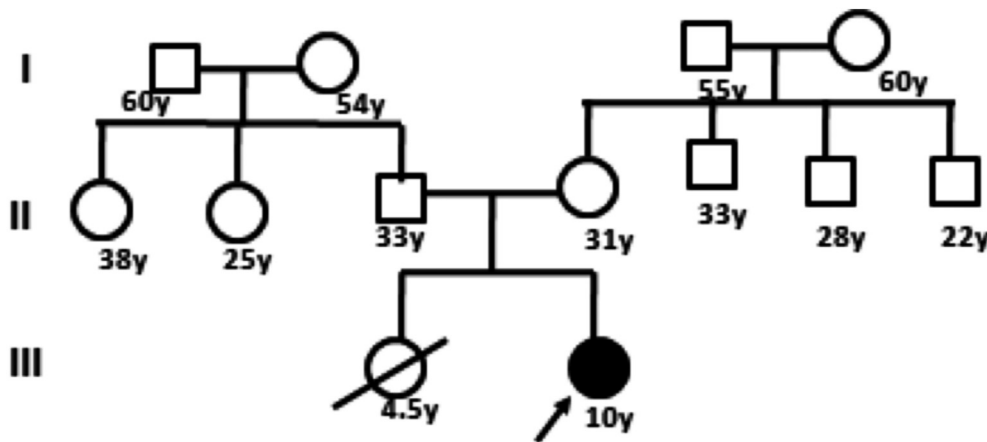


Fig. 16 – Pedigree of Family-8.

34 years of age (Fig. 17). None of the patient's family members were available for genetic analyses.

Compound heterozygosity

The patient was found to carry the risk alleles for – KCNQ1 S546S and IVS13 + 36A > G, SCN5A H558R and 98297G > A polymorphisms. The effect of these risk alleles may alter the functioning of the potassium and sodium ion channels.

Family-10

The proband is a 20 year old male diagnosed with cLQTS and a prolonged QTc of 540 ms presenting with symptoms of syncope from the age of 9 years. He had a significant family history of 4 sudden cardiac deaths and parental consanguinity (Fig. 18). Blood samples were acquired from the proband, his mother, maternal uncle and a cousin for genetic analyses.

Compound heterozygosity

Compound heterozygosity was found in the proband expressing the risk alleles of SCN5A H558R and G98297A polymorphisms. The presence of two risk alleles of SCN5A may lead to variation in the INa channel formation, thus leading to prolonged QTc in the proband. Genetic analysis of the family members neither revealed any genetic variations nor the same haplotype as in the proband.

Family-11

The patient, a 22 year old female was diagnosed with cLQTS after presenting with a prolonged QTc of 475 msec. She had been suffering with syncope from the age of 15 years (Fig. 19). Blood samples were obtained from her parents and a 20 year old brother along with the patient.

Compound heterozygosity

Analyses showed that the patient was carrying risk alleles for – KCNE1 S38G, SCN5A H558R and 98297G > A polymorphisms. The combined effect of these risk alleles may alter the functioning of the IKs and INa channels.

Relatives at risk

The mother of the patient was found to carry the same haplotype as the proband (Table 7) which include the risk allele of SCN5A H558R and 98297G > A polymorphisms posing the mother at risk of developing cardiac symptoms later on in life. Predictive testing with clinical follow up could help in preventing the onset of the disorder or may promote early diagnosis.

Family-12

The proband of this family is an 8 year old female presenting with syncope and prolonged QTc of 490 msec. Her mother had

Table 6 – Common haplotype group – risk identification in proband's mother of family-8.

KCNQ1 S546S	KCNQ1 IVS13 + 36A > G	SCN5A H558R	SCN5A 98297G > A	Identified in
A	A	A	G	Proband, Mother

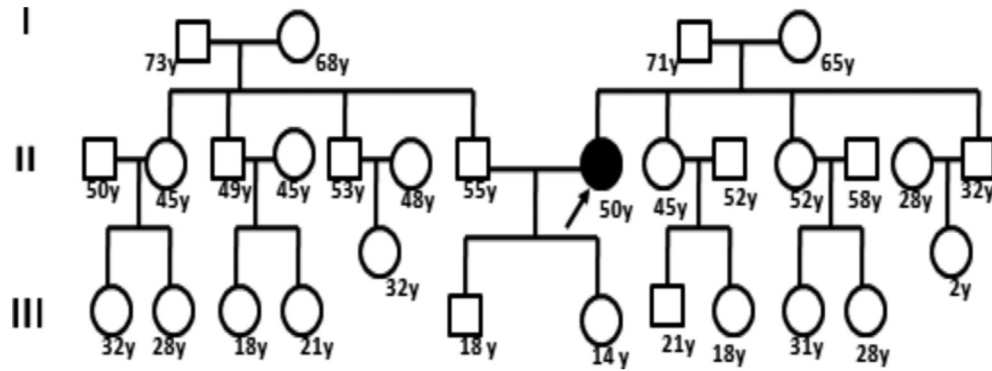


Fig. 17 – Pedigree of Family-9.

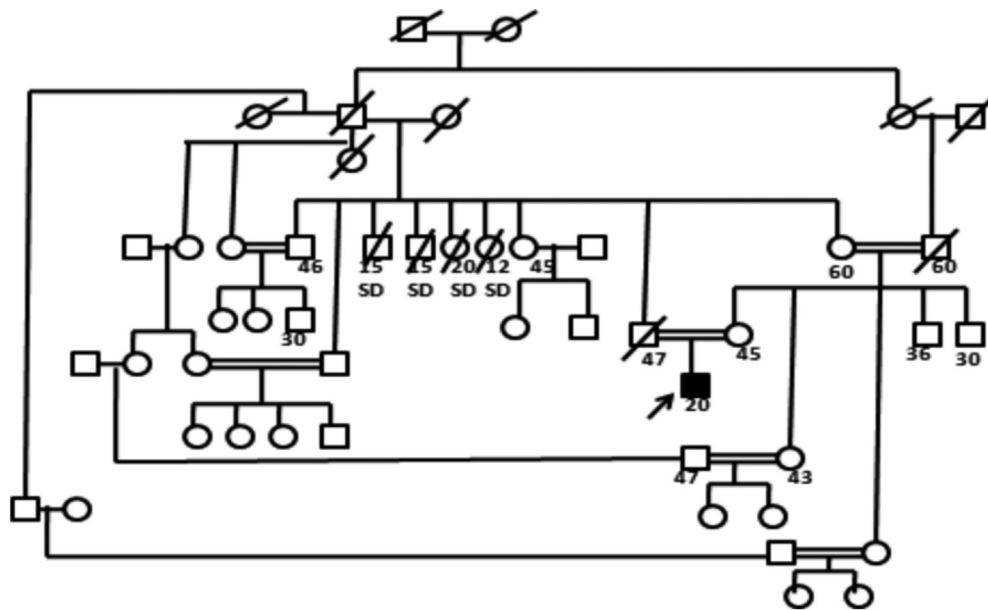


Fig. 18 – Pedigree of Family-10.

a history of miscarriage (Fig. 20). For sample collection, only the proband and her father were available.

Compound heterozygosity

Molecular analyses revealed that the patient was carrying risk alleles for – SCN5A H558R and 98297G > A

polymorphisms. The effect of these risk alleles may alter the functioning of the INa channels. It was observed that the father of the proband did not reveal any such haplotype or variations. Hence, it can be assumed that mother may be a carrier but needs predictive/genetic testing confirmation.

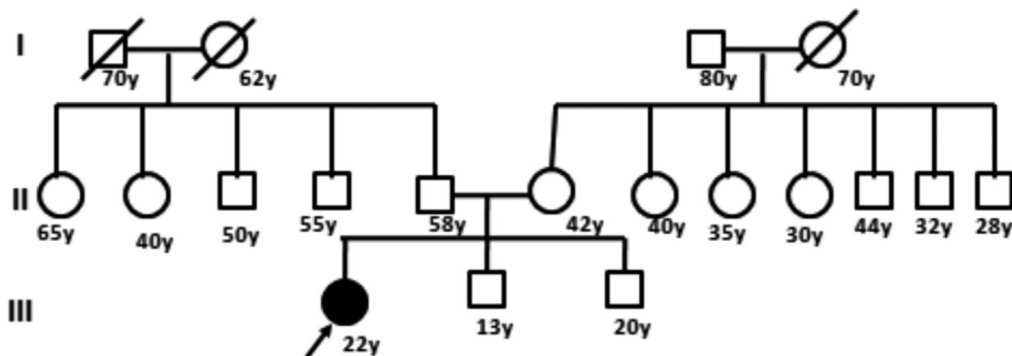


Fig. 19 – Pedigree of Family-11.

Table 7 – Common haplotype group – risk identification in proband's mother of family-11.

KCNQ1 S546S	KCNQ1 IVS13 + 36A > G	SCN5A H558R	SCN5A 98297G > A	Identified in
G	G	A	G	Proband, Mother

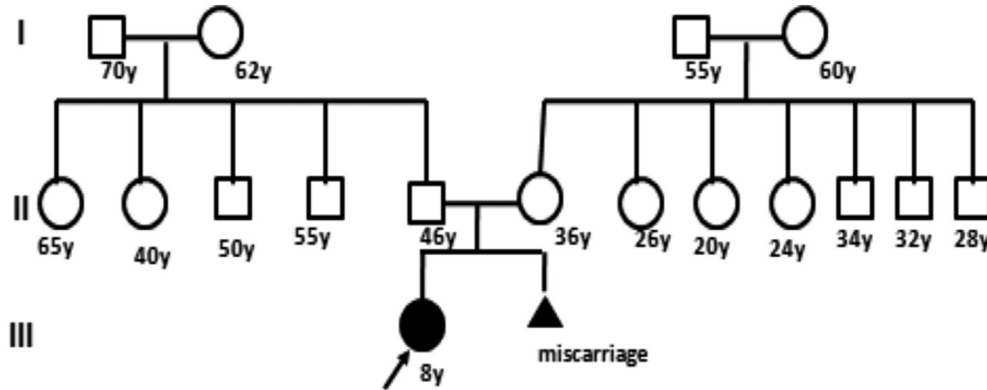


Fig. 20 – Pedigree of Family-12.

Family-13

This family had a 10 year old female proband who presented with symptoms of syncope since 2 years of age. She was finally diagnosed with cLQTS after an electrocardiogram revealed a prolonged QTc. The proband also had a family history of consanguinity and sudden cardiac deaths of two siblings and one cousin at a very young age (Fig. 21). Molecular analyses were carried out on the proband, her parents and her normal sibling.

Compound heterozygosity

The patient was found to be carrying the risk alleles for – SCN5A H558R and 98297G > A polymorphisms. The electrophysiology of sodium ion channel may be affected due to these risk alleles. Parents and sibling of the proband did not reveal any variations leading to the assumption of carrier

status and the two siblings who had SCDs were probably carrying the same haplotype as the proband.

Family-14

The 14 year old male proband of this family was presenting symptoms from 11 years of age. He had episodes of syncope and prolonged QTc of 528 msec. He did not have any significant family history of consanguinity or sudden deaths (Fig. 22). Blood samples were collected from proband and his mother.

Compound heterozygosity

The patient was found to be carrying risk alleles for – KCNQ1 S546S and IVS13 + 36A > G, SCN5A H558R and 98297G > A polymorphisms. This genetic compound may alter the potassium and sodium ion channels leading to prolonged

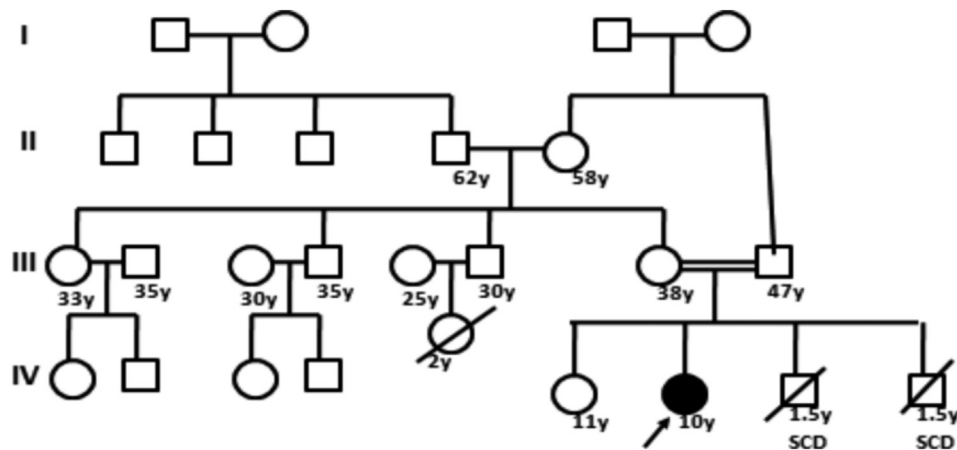


Fig. 21 – Pedigree of Family-13.

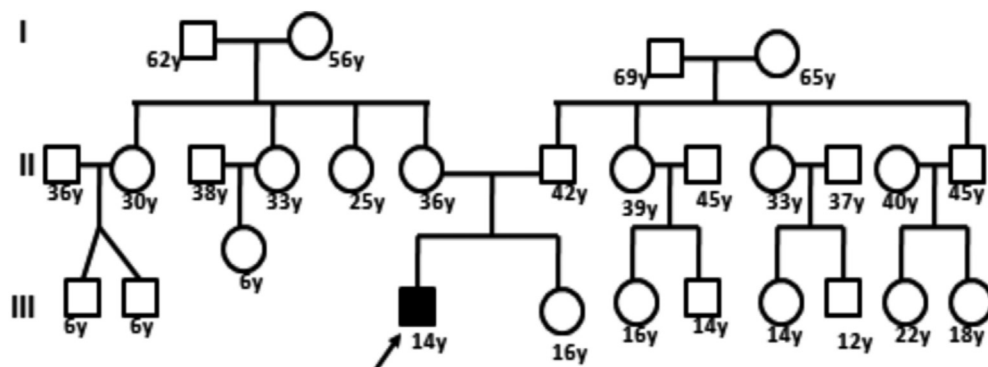


Fig. 22 – Pedigree of Family-14.

QTc. No such variations were observed in mother of the proband. However, carrier status of the father cannot be ruled out.

Family-15

The proband is a 10 year old female who was suffering from episodes of syncope from 9 months of age. She was diagnosed as cLQTS with prolonged QTc on ECG. She neither had a family history of consanguinity or sudden deaths but her parents and sibling's blood samples were collected for genetic analyses (Fig. 23).

Compound heterozygosity

No variations were observed in the proband's parents but the patient was found to be carrying risk alleles for –SCN5A H558R and 98297G > A polymorphisms. The presence of these risk alleles may be altering the functioning of the sodium ion channels.

Relatives at risk

The patient's 9 years old sibling was found to carry the same haplotype as the proband (Table 8) which include the risk allele of SCN5A H558R and 98297G > A polymorphisms posing the sibling at a risk of electrophysiological/ion-channel disorders. Hence, follow up is necessary in such cases and predictive testing is warranted in LQTS.

Family-16

The patient is a 53 year old male diagnosed with acquired LQTS. This is a rare case expressing Dilated, Hypertrophic cardiomyopathy and aLQTS. On being treated for cardiomyopathy, he presented with symptoms of syncope and prolonged QTc of 511 ms an indicator of aLQTS due to drugs (Fig. 24). His treatment was altered and sample collected for molecular analyses. None of the family members were available for sample collection.

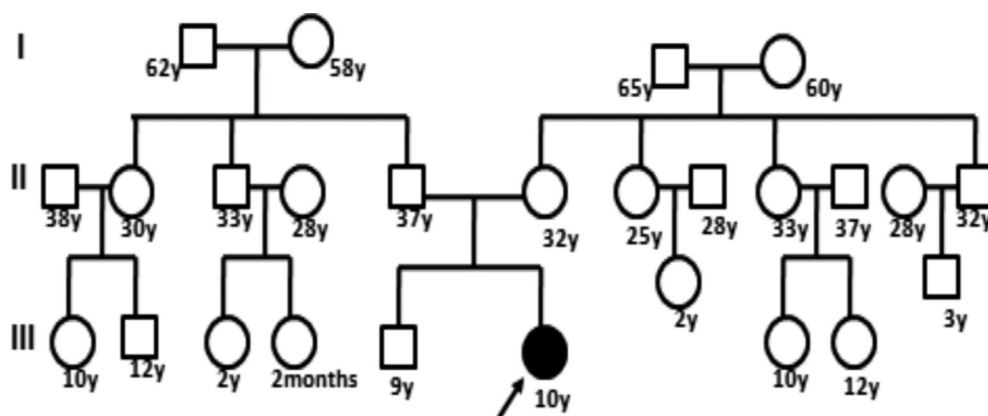


Fig. 23 – Pedigree of Family-15.

Table 8 – Common haplotype group – risk identification in sibling of family-15.			
KCNE1 S38G	SCN5A H558R	SCN5A 98297G > A	Identified in
G	A	G	Proband and sibling

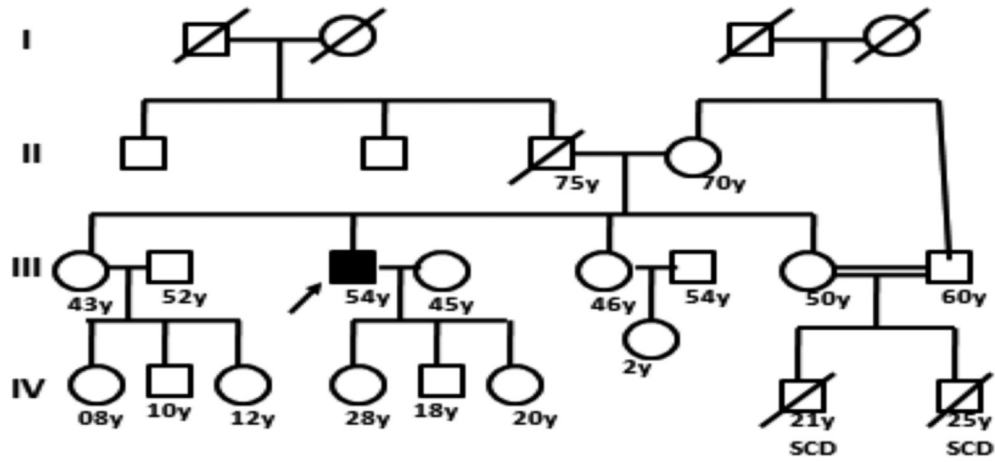


Fig. 24 – Pedigree of Family-16.

Compound heterozygosity

Molecular analyses of the proband revealed compound heterozygosity with the risk alleles being SCN5A H558R and G98297A polymorphisms. The presence of this genetic compound may have led to the manifestation of aLQTS further strengthening the drug responses of patients based on specific genotypes. The study also points to the importance of pharmacogenomics as it is observed that genetic compounds may respond to treatment differently compared to probands with single gene mutations.

Relatives at risk

Both the parents of the proband were found to carry the same haplotype as the patient (Table 9) which include the risk allele of – KCNQ1 S546S and IVS13 + 36A > G, and SCN5A 98297G > A polymorphisms highlighting the role of genetics in aLQTS and the predisposition of segregating alleles in the family members which can end up in aLQTS triggered by drugs/chemicals. This clearly indicates pharmacogenomic relevance of alleles to drug induced LQTS.

Family-17

Family-17 included a 2 year old male proband diagnosed with aLQTS due to the ingestion of pyrethrin (mosquito repellent). Though he did not have a family history of sudden death or consanguinity, his parents' blood sample was collected to identify the genetic parameters involved in the etiology of aLQTS (Fig. 25).

Family-18

Family-18 had a 9 year old male cLQTS proband with a history of syncope and consanguinity (Fig. 26). And the available family members were the proband's mother, maternal grandmother and grandfather.

Compound heterozygosity

The proband was found to be carrying risk alleles for – KCNQ1 S546S and IVS13 + 36A > G, SCN5A H558R and 98297G > A polymorphisms.

Compound heterozygosity

The proband was found to express the risk alleles of KCNQ1 S546S, IVS13 + 36A > G, SCN5A H558R and G98297A polymorphisms. The compound heterozygosity identified in the proband could be the reason for impairment of the potassium and sodium ion-channels leading to cLQTS. Genetic analyses of family members neither revealed any variations nor were

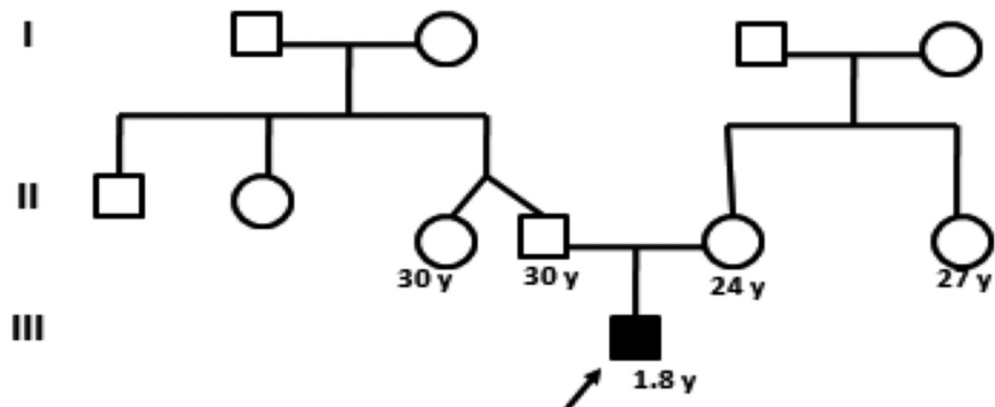


Fig. 25 – Pedigree of Family-17.

KCNQ1 S546S	KCNQ1 IVS13 + 36A > G	SCN5A 98297G > A	Identified in
A	A	G	Father and Mother

found to carry the same haplotype as the proband. However, history of parental consanguinity was reported thus, cLQTS could result not only by single gene mutation but also due to genetic compound and may act in a recessive mode.

Discussion

Screening of 46 LQTS probands and their available family members was carried out for variations in IKs, INa encoding genes and polymorphisms in beta 1 and 2 – adrenergic receptors, tumor necrosis factor-alpha and atrial natriuretic factor encoding genes. Of the 46 families screened, 18 have been described above with probands revealing novel variations or compound heterozygosity. Relatives carrying the same haplotype as the proband were also identified which may help in predictive testing.

Families 1–4 revealed probands carrying novel variations in KCNQ1 gene along with some risk genotypes of the above screened genes and compound heterozygosity was identified. The proband's parents or siblings were found to carry the same haplotype as the proband predisposing them at a risk of developing the ion-channel disorder later on in life. In a study by Koo et al. [1], a spectrum of mutations were revealed across the coding region of KCNQ1, HERG, KCNE1, KCNE2 and SCN5A genes in a Chinese LQTS family. Several synonymous polymorphisms were also identified in this LQTS family. The possibility of some of these variants acting in concert may predispose individuals to arrhythmias in the presence of appropriate precipitating factors. Mutations associated with JLN syndrome were also reported in Chinese families and other populations [19–23].

Interestingly, three families- 5, 6 and 7 were identified as typical cases of “anticipation” and age related penetrance in which both mother and child were diagnosed with cLQTS. In families- 5 and 6, the children presented with symptoms at an

earlier age but their mothers exhibited symptoms in third or fourth decade of life. Whereas in family-7 the mother and daughter presented with symptoms in their childhood following which the mother was diagnosed as JLNS patient and the daughter was identified with cLQTS. In family-6, proband's brother and maternal uncle were carrying the same haplotype for six alleles of various genes screened as the proband's which may point to similar cardiac events and age related penetrance at later stages.

Family-16 and 17 represents aLQTS probands with variations in IKs and INa encoding genes. Proband – 16 was a typical case wherein prolonged QTc was developed due to drugs whereas proband – 17 was a child who developed aLQTS due to ingestion of a mosquito repellent. The parents of proband – 17 showed the same haplotype as the child revealing the risk of developing a prolonged QTc on being triggered by a similar event pointing to pharmacogenomic implications.

Data point to a positive correlation between duration of the QTc interval and probability of carrying LQTS disease-causing mutations [2]. Hence, QTc duration was examined and it was observed that all the patients carrying mutations had a QTc of >480 ms. This study is in accordance with earlier reports where LQTS infants with disease causing mutations had a QTc of >470 ms which could establish the genotype–phenotype correlations as suggested by Schwartz et al. [2].

It was demonstrated that mutation carriers are at risk of developing cardiac events even when the QTc interval is normal [3]. Hence, in the present study, family members harboring the variations were also predicted to be at a risk of developing cardiac events and a follow up is therefore warranted. Most of the families described had family members carrying the same haplotype as the probands indicating their risk and confirming that family screening/predictive testing is important in identifying and management of LQTS.

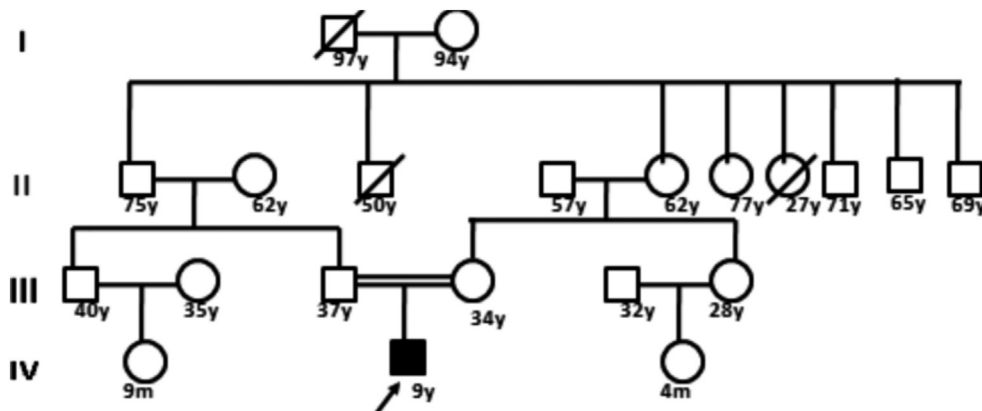


Fig. 26 – Pedigree of Family-18.

Conclusion

In the present study, FDRs carriers of the same haplotype as the probands were predicted to be at a risk to develop cardiac events. Hence, a follow up is therefore warranted. This establishes that family screening/predictive testing is necessary in the identification and management of LQTS. Further, an interesting finding is that in most of the probands exhibiting family history were found to be genetic compounds, which clearly points to the role of cardiac genes and their modifiers in LQTS manifestation.

Author's contributions

S F Q has carried out the molecular, hapmap and In-silico analysis described in this manuscript and has compiled the manuscript. A A has helped to carry out the in-silico analysis described in this manuscript. A V has interpreted the results described in this manuscript. The probands described in this manuscript have been diagnosed for LQT syndrome by M P J, C V, J S and H R at their respective hospitals. K T has been critical in the review and compilation of the manuscript. As the corresponding author, the concept, design and compilation of this manuscript has been carried out by P N.

Conflict of interest

There is no conflict of interest within the authors/no declarations of interest.

Consent

Informed written consent was obtained from the probands and their family members.

Ethics committee approval

The study has been approved by the Institutional Ethics Committee, Dept. of Genetics, Osmania University, INDIA obtained on 24th August 2009.

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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.2015.12.001>.

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