Original Article

Mutation Screening in *KCNQ1, HERG, KCNE1, KCNE2* and *SCN5A* Genes in a Long QT Syndrome Family

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Abstract

Introduction: Long QT syndrome (LQTS), an inherited cardiac arrhythmia, is a disorder of ventricular repolarisation characterised by electrocardiographic abnormalities and the onset of torsades de pointes leading to syncope and sudden death. Genetic polymorphisms in 5 well-characterised cardiac ion channel genes have been identified to be responsible for the disorder. The aim of this study is to identify disease-causing mutations in these candidate genes in a LQTS family. Materials and Methods: The present study systematically screens the coding region of the LQTS-associated genes (*KCNQ1, HERG, KCNE1, KCNE2* and *SCN5A*) for mutations using DNA sequencing analysis. Results: The mutational analysis revealed 7 synonymous and 2 non-synonymous polymorphisms in the 5 ion channel genes screened. Conclusion: We did not identify any clear identifiable genetic marker causative of LQTS, suggesting the existence of LQTS-associated genes awaiting discovery.

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Introduction

Long QT syndrome (LQTS), a form of life-threatening cardiac arrhythmia, is a rare but significant clinical disorder, with a prevalence of 1 in 10,000 to 15,000 individuals.[1] LQTS is a disorder of ventricular repolarisation characterised by electrocardiographic abnormalities, predominantly a prolongation of the QT interval and ventricular tachyarrhythmia, particularly torsades de pointes leading to syncopes, seizures and sudden death.[2] Congenital LQTS is an inherited heterogenous disorder caused by mutations at various loci, giving rise to a prolonged QT interval. There exists the more common autosomal dominant Romano-Ward syndrome (RW)[3] and the less common autosomal recessive Jervell and Lange-Nielsen syndrome (JLN),[4] the latter which is associated with sensorineural deafness.[5]

Congenital LQTS is a genetically heterogeneous disorder associated with mutations in 5 well-characterised cardiac ion channel genes, of which 4 encode for the potassium channels (*KCNQ1*, *HERG*, *KCNE1* and *KCNE2*) and 1 encodes for the sodium channel (*SCN5A*).[6-8] The genes were mapped to chromosome 11p15.5 (LQT1),[9] 7q35-36 (LQT2),[6] 3p21-24 (LQT3),[10] 21q22 (LQT5)[11] and 21q22 (LQT6).[12] The LQT4 locus was mapped to chromosome 4q25-27.[13] Several lines of evidence show that polymorphisms in LQTS-associated genes may modify arrhythmia susceptibility in potential gene carriers.[14,15]

In this study, we report the genetic profile of the 5 LQTS candidate genes in a LQTS family. We did not identify any clear identifiable genetic marker underlying the molecular pathogenesis of LQTS, suggesting the presence of undiscovered disease-causing genes.

Materials and Methods

Sample Collection and DNA Isolation

The study subjects comprised a total of 13 members in a three-generation family (Fig. 1). The proband (Patient 1) was a 52-year-old woman diagnosed with LQTS at the National Heart Centre, Singapore. All the subjects underwent detailed clinical and cardiovascular examination, including 12-lead electrocardiography (ECG) and 24-hour Holter recording. Subjects were classified as affected in

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cases with documented syncopes or a QT_C interval exceeding 0.5 s (Table 1). The ECG records of the deceased subject showed evidence of prolonged QT interval (Fig. 2) and torsades de pointes (Fig. 3). The genomic DNA extracted from peripheral leukocytes were used as templates in amplifying the exons in the coding region of *KCNQ1, HERG, KCNE1, KCNE2* and *SCN5A* genes. Mutational analysis of the *SCN5A* gene was performed only for the proband and we did not screen the family members as no coding change variant was detected in the proband.

Polymerase Chain Reaction Amplification

DNA fragments for the potassium channel genes were generated using specially designed primers based on flanking intronic sequences (Lasergene DNASTAR software) and combinations of primers as described in previous studies,[12,16-19] with modifications of the amplification conditions. The primer pairs and amplification conditions of the PCR fragments are available at the NUS Pharmacogenetics Lab website (http://www.med.nus.edu.sg/medphc/PGLab/research/lqts.htm). The exons are numbered according to the GenBank sequences in the National Center for Biotechnology Information (NCBI). Primer pairs published by Wang et al[20] were used to amplify all 28 exons of the SCN5A gene. The amplification reactions were performed in a total volume of 50 µL containing 1 X Master Mix (Promega, Madison, USA), 0.2 µM of each primer (Research Biolabs, Singapore) and 100 ng of DNA. Due to the rich GC content of Exon 1 of *KCNQ1*, FastStart Taq DNA Polymerase (Roche Diagnostics GmbH, Mannheim, Germany) was necessary for amplification of the fragment. Following an initial pre-denaturation step at 94°C for 3 min, the reactions were cycled 35 times through denaturation at 94°C for 1 min, variable annealing temperatures for 1 min and extension at 72°C for 1 min. The reactions were terminated by an additional extension step at 72°C for 10 min. The amplification reactions were performed on the Peltier Thermal Cycler (DNA Engine Dyad; MJ Research Inc, Waltham, MA, USA). The polymerase chain reaction (PCR) products were subjected to 1.6% agarose gel electrophoresis to verify successful amplification of the desired fragments.

DNA Sequencing

Prior to sequencing, unincorporated deoxynucleotide triphosphates and excess primers were removed from the

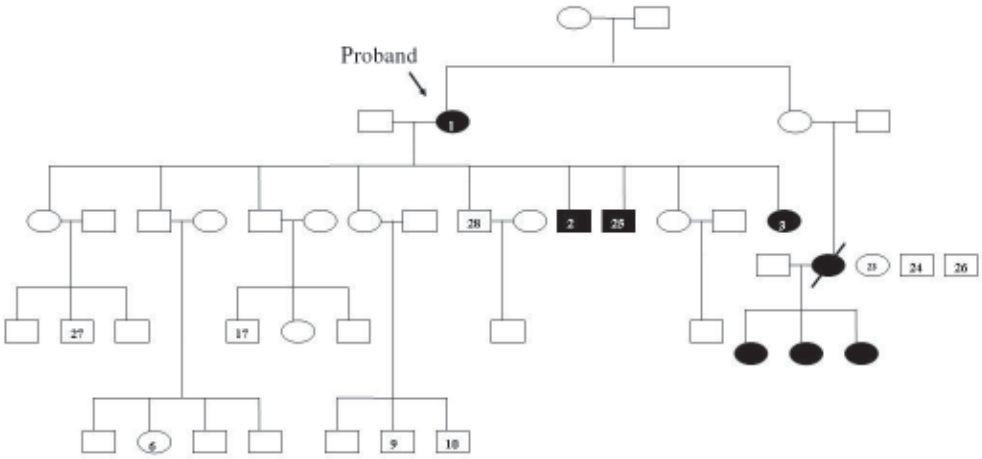

Fig. 1. Pedigree structure of the LQTS family. Squares denote men and circles denote women. Filled symbols denote affected individuals, empty symbols denote non-affected individuals, and slash symbol denotes deceased individual.

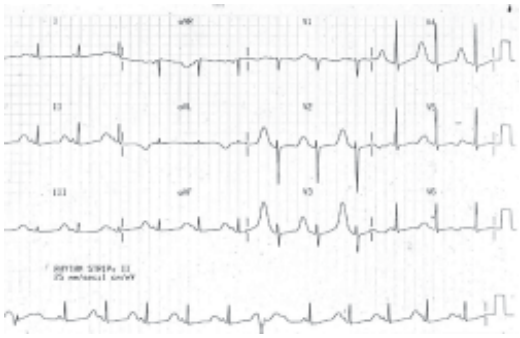

Fig. 2. Twelve-lead ECG of the deceased subject.

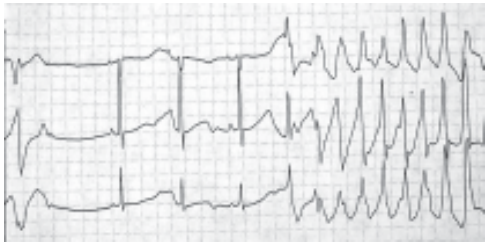

Fig. 3. Onset of torsades de pointes in the deceased subject.

Table 1. Clinical and ECG Characteristics of the LQTS Family

Patient ID	Gender	Age (y)	Symptoms	Treadmill	Holter monitoring	Tilt test	LQT	QT(s)	QT_c(s)
1	F	52	No	Not done	Not done	Not done	Yes	0.56	0.55
2	M	33	Syncope	Not done	Normal	Negative	Yes	0.49	0.52
3	F	30	No	Negative	Normal	Negative	Yes	0.52	0.52
6	F	20	No	Negative	Normal	Equivocal	No	0.36	0.40
9	M	12	No	Not done	Not done	Not done	No	0.40	0.43
10	M	9	No	Not done	Not done	Not done	No	0.32	0.39
17	M	19	No	Negative	Normal	Negative	No	0.36	0.45
23	F	34	No	Negative	Normal	Negative	No	0.48	0.41
24	M	31	No	Negative	Normal	Negative	No	0.40	0.40
25	M	37	-	Negative	Normal	Not done	Yes	0.68	0.58
26	M	33	No	Negative	Normal	Negative	No	0.46	0.50
27	M	17	No	Not done	Normal	Positive	No	0.36	0.43
28	M	39	No	Negative	Normal	Negative	No	0.42	0.39

ECG: electrocardiography; F: female; LQTS: long QT syndrome; M: male

Table 2. Summary of Polymorphisms Identified in the Study Subjects

Gene			LQTS patient ID												
Exon	**Nucleotide change***	**Amino acid change**	**1**	**2**	**3**	**6**	**9**	**10**	**17**	**23**	**24**	**25**	**26**	**27**	**28**
KCNQ1															
1	C435T	I145I	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CT	CC
9	C1343G	P448R	CG	CG	CG	CC	CC	CC	CC	CG	CG	CG	CG	CC	CC
12	G1638A	S546S	GG	GA	GG	GG	GG	GG	GA	GA	GA	GA	GA	GG	GA
HERG															
6	T1467C	I489I	TC	TC	TC	TC	TT	TT	TC	TC	TC	TC	TC	TT	TC
6	T1539C	F513F	TC	TC	TC	TC	TT	TT	TC	TC	TC	TC	TC	TT	TC
7	G1692A	L564L	GA	GA	GA	GA	GG	GG	GG	GG	GA	GA	GA	GG	GA
8	C1956T	Y652Y	CT	CT	CT	CT	CC	CC	CC	CC	CT	CT	CT	CC	CT
KCNE1															
3	G112A	G38S	GA	GG	GG	GG	GG	GG	GA	GG	AA	GG	GG	GG	GG
SCN5A															
17	A3183G	E1061E	GG	-	-	-	-	-	-	-	-	-	-	-	-

* The nucleotide numbering starts from the ATG start codon. NCBI GenBank Accession Numbers: AF000571 (*KCNQ1*), AF363636 (*HERG*), NM_000219 (*KCNE1*), NM_172201 (*KCNE2*) and NM_000335 (*SCN5A*).

12 µL of PCR products using 2 units of Exonuclease 1 (New England Biolabs, Beverly, MA, USA) and 1 unit of Shrimp Alkaline Phosphatase (Promega, Madison, USA) by incubating at 37^{O}C for 15 min, followed by enzyme deactivation at 80^{O}C for 20 min. The sequencing reactions were carried out using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequences of genomic fragments were analysed on the automated ABI Prism Model 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The results were blasted against published GenBank sequences in the NCBI (Table 2).

Results

A systematic survey of the 5 LQTS-associated genes revealed 2 non-synonymous polymorphisms (P448R-*KCNQ1* and G38S-*KCNE1*), 2 synonymous polymorphisms in *KCNQ1*, 4 synonymous polymorphisms in *HERG* and 1 synonymous polymorphism in *SCN5A* (Table 2). No base change was detected in *KCNE2*.

Discussion

LQTS is a disorder resulting from a prolongation in ventricular repolarisation and increases the risk of developing torsades de pointes and sudden death. The main

criteria for clinical diagnosis of LQTS are a prolonged QT_C value exceeding 450 ms and documented syncopal episodes. However, QT_C measurement on ECG is not always a reliable surrogate marker for prolonged repolarisation. It is not an ideal metric for accurate indication of clinical outcome as not all patients who present the LQTS phenotype exhibit prolonged QT interval and some unaffected individuals have prolonged QT_C value.[21,22] Moreover, QT interval varies with gender, age, concurrent drug administration, electrolyte abnormalities and other diseases. Molecular genetics thus plays a complementary role in defining diagnosis in difficult cases. 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, there is a pressing need to screen the LQTS-associated genes for pathological mutations in high-risk individuals.

In this study, we revealed the spectrum of mutations across the coding region of *KCNQ1, HERG, KCNE1, KCNE2* and *SCN5A* genes in a LQTS family. Genetic polymorphisms in the cardiac ion channel genes under study account for the vast majority of LQTS cases. Both non-synonymous polymorphisms (P448R-*KCNQ1* and G38S-*KCNE1*) are relatively frequent among the normal Chinese population, with allele frequencies of 8.6% and 30.6% respectively,[23] suggesting that they are likely to represent common, benign polymorphisms. P448R-*KCNQ1* was once thought to cause LQTS,[24] but was later discovered to be an ethnic-specific polymorphism present in approximately 14% to 20% of the Asian population.[25-27] Functional studies demonstrated that the current kinetics and expression level of P448R-*KCNQ1* were indistinguishable from that of the wild-type channel.[27] G38S-*KCNE1* was reported to have an allele frequency of 21.9% in the study by Lai et al.[28] The authors regarded A at position 112 to be the original sequence of *KCNE1* and G as the substitution. We also identified several synonymous polymorphisms in this LQTS family (Table 2). We do not exclude the possibility that some of these variants may act in concert in predisposing individuals to arrhythmias in the presence of appropriate precipitating factors. We believe that there exists a genetic contributory marker in a disease-causing gene that is yet to be discovered.

An alternative explanation for the failure in finding a pathological mutation in this study might be that only mutations within the coding sequence were considered. It is increasingly recognised that mutations within the promoter and "nonsense" intronic sequences may have important effects on gene transcription and splice variants. Recently, a mutation in the intronic sequence of *KCNH2* was found to result in the prolongation of the QT interval.[29] Mutations in these regions can provide important insights in gene regulation and expression. However, it is currently impractical to sequence the whole gene unless there is a signal suggesting its involvement. One way to ascertain this will be to perform a family-based whole genome linkage analysis. This has been done previously to identify a causative locus in several well-characterised diseases, such as a novel locus associated with atrial fibrillation.[30] Such an analysis might reveal the presence of a novel locus or further narrow the genes involved and allow for selected whole gene sequencing to identify novel mutations not inclusive of the exonic sequences.

Also, we did not exhaustively evaluate the continuously expanding list of arrhythmia and sudden death candidate genes. There are currently at least 9 genes associated with LQTS.[31] A few other candidate genes not screened in this study include the *KCNQ2* gene (causative of the recessive form associated with deafness),[31] the *KCNJ2* gene known to cause Andersen Syndrome (LQT7),[32] and the gene encoding the scaffolding protein ankyrin B (responsible for causing LQT4).[33]

Regardless of the considerable effort devoted to finding genotype-phenotype relationships, the potential severity of LQTS mutations has been difficult to evaluate. A functional assay involving the expression of mutant channels is necessary for elucidating their electrophysiological properties. This electrophysiological characterisation will shed light on the molecular mechanisms underlying LQTS and allow a more reliable prediction of clinical outcome.

Despite the relevance of genetic polymorphisms of these ion channel genes in clinical cardiology, genotyping-based population screening is not feasible due to the vast number of disease-causing mutations. Moreover, there may be unknown disease-causing genes that await discovery. Therefore, molecular diagnosis is usually limited to family members of clinically diagnosed LQTS patients.

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