

## Identification of the Gene Causing Long QT Syndrome in an Israeli Family

Michal Tenenbaum MSc<sup>1,5\*</sup>, Shahar Lavi MD<sup>4\*</sup>, Nurit Magal PhD<sup>1,2</sup>, Gabrielle J. Halpern MD<sup>1</sup>, Inbal Bolocan MSc<sup>1,2</sup>, Monther Boulos MD<sup>4</sup>, Michael Kapeliovich MD<sup>4</sup>, Mordechai Shohat MD<sup>1,2,3</sup> and Haim Hammerman MD<sup>4</sup>

<sup>1</sup>Department of Medical Genetics and <sup>2</sup>Felsenstein Medical Research Center, Rabin Medical Center (Beilinson Campus), Petah Tikva, Israel

<sup>3</sup>Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

<sup>4</sup>Intensive Cardiac Care Unit, Rambam Health Care Campus, Haifa, Israel

<sup>5</sup>Maccabi Health Services Megalab, Rehovot, Israel

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### Abstract

**Background:** Long QT syndrome is an inherited cardiac disease, associated with malignant arrhythmias and sudden cardiac death.

**Objectives:** To map and identify the gene responsible for LQTS in an Israeli family.

**Methods:** A large family was screened for LQTS after one of them was successfully resuscitated from ventricular fibrillation. The DNA was examined for suspicious loci by whole genome screening and the coding region of the LQT2 gene was sequenced.

**Results:** Nine family members, 6 males and 3 females, age (median and interquartile range) 26 years (13, 46), who were characterized by a unique T wave pattern were diagnosed as carrying the mutant gene. The LQTS-causing gene was mapped to chromosome 7 with the A614V mutation. All of the affected members in the family were correctly identified by electrocardiogram. Corrected QT duration was inversely associated with age in the affected family members and decreased with age.

**Conclusions:** Careful inspection of the ECG can correctly identify LQTS in some families. Genetic analysis is needed to confirm the diagnosis and enable the correct therapy in this disease.

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### Subjects and Methods

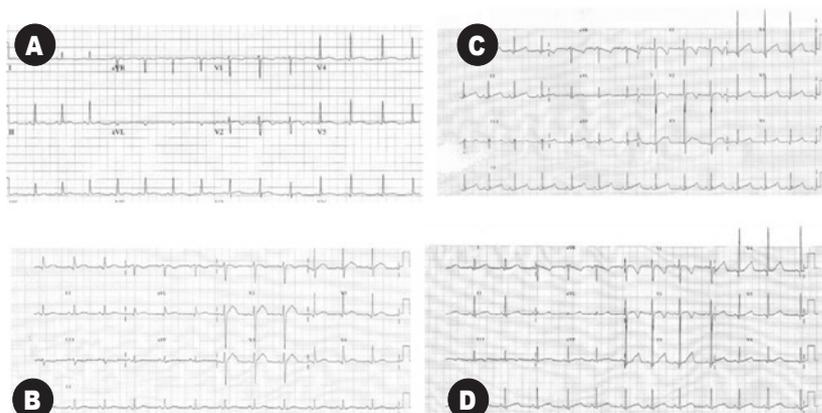
An apparently healthy 24 year old woman was found unconscious and without pulse while watching television. Cardiopulmonary resuscitation was initiated by a family member, and ECG tracing later on showed ventricular fibrillation. She was successfully defibrillated and transferred intubated to our hospital. Initial laboratory tests showed mild hypokalemia and hypomagnesemia, and on the ECG, QT interval was slightly prolonged [Figure 1A]. She was treated with potassium and magnesium supplements, but had several short episodes of Torsade de pointes during her hospital stay which resolved with temporary pacing. She subsequently made a full recovery and underwent implantation of a defibrillator before discharge. During 4 years of follow-up she was treated with beta blockers and there were no recurrent events.

After written informed consent was obtained, ECG was performed in 14 family members and DNA was isolated using the PUREGENE™ Genomic DNA Purification Kit. The DNA was examined for suspicious loci by whole genome screening on the ABI PRISM 3100 Genetic Analyzer by 400 fluorescence markers from ABI PRISM Linkage Mapping Set V2.5. The coding region of the LQT2 gene, *KCNH2*, was sequenced. Automated "Dye terminator" cycle sequencing was performed directly on purified polymerase

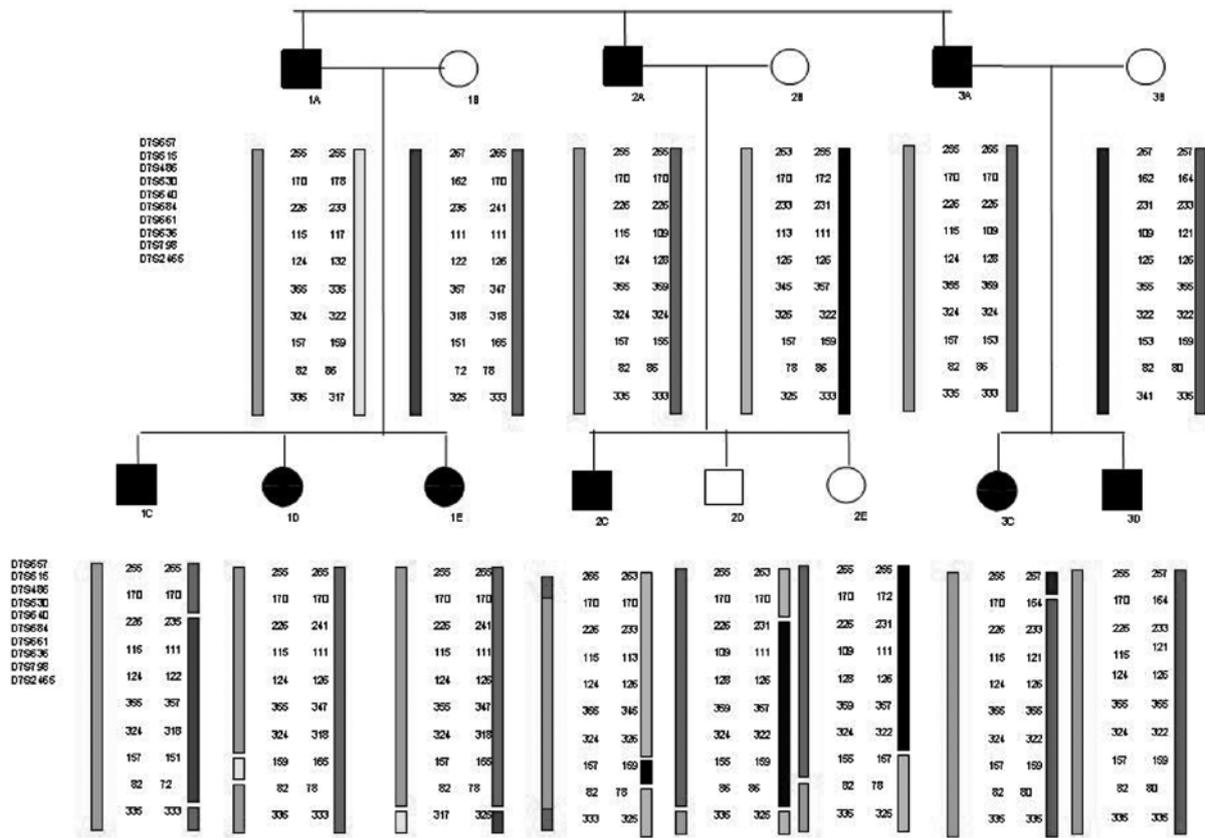
Long QT syndrome is an inherited cardiac ion channel disorder that is manifested by prolonged QT interval and ventricular arrhythmias. The location of the genetic mutation determines to a large extent the phenotype expression. However, there is variable expression of severity and incomplete penetrance [1,2]. Until now eight loci associated with LQTS have been identified (LQT1 through LQT8) with 95% of the known mutations located in LQT1-LQT3 [1,3]. Here we report the identification of a rare disease-causing gene in an affected family and investigate the phenotype associated with this mutation.

\* The first two authors contributed equally to this work

LQTS = long QT syndrome



**Figure 1.** [A] ECG of the proband. [B, C, D] Representative ECGs of family members.



**Figure 2.** The pedigree and haplotypes of the family members for the locus on chromosome 7

chain reaction products (Amersham purification kit, UK) according to the manufacturer's instructions using an ABI PRISM 3100 Genetic Analyzer. Mutation analysis was carried out by comparing the sequences to the *KCNH2* gene sequence of Ensembl genome Browser ([www.ensembl.org](http://www.ensembl.org)), gene number: ENSG0000005118 using Sequencher DNA Analysis software.

**Results**

Nine family members, age (median and interquartile range) 26 years (13, 46), with abnormal ECG were identified. There was no family history of arrhythmias, palpitations or syncope, although the index patient's brother underwent prior evaluation for suspected seizures. The ECG of her brother [Figure 1B] and other family

members revealed prolonged QT intervals with bizarre T waves [Figure 1 C and D, and Table 1] and a pedigree was prepared [Figure 2].

Figure 2 shows two generations of the affected family. In the first generation, three brothers have LQTS (I-1, I-3 and I-5), and in the second generation six family members are affected (II-1, II-2, II-3, II-4, II-7 and II-8). Individual II-7 is the proband.

The QTc and T wave duration were significantly prolonged in the affected compared to the unaffected family members [Table 1]. Patients with long QT were more likely to have biphasic T waves or a second component on the T waves (non-significant). A significant correlation was found between QT duration and age ( $r^2 = 0.69$ ,  $P < 0.05$ ). T wave durations in males were significantly longer compared to females, and there was a trend toward an increase in QTc duration in females compared to males [Table 1].

The disease-causing gene was mapped to chromosome 7 in the region of the *KCNH2* gene. Sequencing of the *KCNH2* gene revealed a missense mutation in exon 9 in all the affected individuals. We identified the A614V mutation, caused by a nucleotide substitution C → T [Figure 3].

**Table 1.** Comparison between affected and unaffected family members

	Affected females (n=3)	Affected males (n=6)	Unaffected (n=5)
QTc duration (msec)	493 ± 15	452 ± 34	393 ± 6 *
T wave amplitude (mV)	0.52 ± 0.23	0.38 ± 0.18	0.47 ± 0.15
T wave duration (msec)	263 ± 21 **	317 ± 34	240 ± 40 *
Biphasic T waves	3 (100)	2 (33)	0
Second component on T wave	2 (66)	5 (83)	1 (20)

Data are presented as mean ± STD or values and percentage.

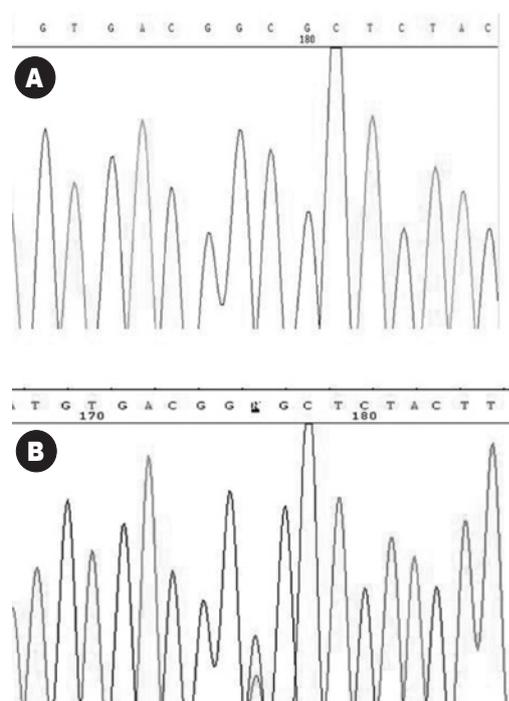
\*  $P < 0.05$  unaffected vs. affected.

\*\*  $P < 0.05$  affected females vs. affected males.

QTc = corrected QT interval

**Discussion**

The diagnosis of LQTS is based on assessment of the ECG, clinical criteria and genetic analysis. Diagnostic criteria for LQT have low sensitivity, but often simple calculation of the QTc interval using the Bazett's formula yields reasonable diagnostic results [4].



**Figure 3.** Sequencing of exon 9 of the *KCNH2* gene by ABI PRISM 3100 Genetic Analyzer. **[A]** Wild-type GCG. **[B]** Mutant GTG.

The diagnostic accuracy is related to the type of LQT and may be affected by other factors. Bifid T waves with a second component are characteristic of the LQT2 genotype and in specific circumstances an accurate diagnosis can be obtained by inspection of the ECG alone [5,6]. Indeed, all affected members in the current study were diagnosed by ECG. Nevertheless, genetic assessment provided better risk stratification and is warranted to identify silent carriers [7,8].

We undertook this study after the proband had a severe arrhythmia due to LQTS. Of the 14 family members examined, 9 had LQT2, each of whom carried the pore mutation A614V. Mutations in the pore region are associated with increased risk for arrhythmic events [9] but the other affected family members have not yet suffered cardiac events. The risk of symptomatic arrhythmia is increased in patients with very prolonged QTc (> 500 msec) and the risk of having arrhythmia at a young age in patients with LQT2 is higher in females, which may explain the arrhythmia in our patient [10]. In the present study we found that in patients with the same HERG mutation, the QTc decreases with age and is longer in females. The genetic identification of the specific mutation in this family helped with better risk stratification [10] and indeed this mutation was reported to be associated with increased risk of arrhythmic events [9].

The first report of the mutation A614V was in 1997 in a Japanese family [11] and until now there have been a few reports of this mutation, from different ethnic origins [11-13]. This mutation causes a change in the protein potassium voltage-gated channel subfamily H member 2, coded by the *KCNH2* gene and located in the pore of the ion channel. The pore region

is responsible for selectivity, conduction and C inactivation of potassium channels [9]. Four *KCNH2* subunits co-assemble with four mirp1-(LOT6) subunits to form a tetrameric protein that is transported to the cell membrane; the electrophysiological characteristics of this mutation were reported [14].

In summary, our findings indicate that in some families with LQTS, identification of affected members can be easily performed by simple inspection of the ECG. More sophisticated genetic analysis is warranted to tailor the best therapy for these patients.

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**Correspondence:** Dr. S. Lavi, Intensive Cardiac Care Unit, Rambam Health Care Campus, P.O. Box 9602, Haifa 31096, Israel.  
Phone: (972-4) 854-2242;  
Fax: (972-4) 854-2176  
email: s\_lavi@rambam.health.gov.il