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Clinical Heterogeneity in Patients with Long QT Syndrome and Segregation of Single Nucleotide Variants and Clinical Symptoms in 17 Affected Families

Elcin Bora[a] Ayca Yıldız Bulut[a] Tufan Cankaya[a] Tayfun Cinleti[b] Halise Zeynep Genç[c] Emin Evren Ozcan[d] Ebru Ozpelit[d] Ayfer Ulgenalp[a] Ahmet Okay Caglayan[a]

[a]Department of Medical Genetics, Dokuz Eylul University Faculty of Medicine, Izmir, Turkey; [b]Division of Medical Genetics, Department of Pediatrics, Dokuz Eylul University Faculty of Medicine, Izmir, Turkey; [c]Division of Cardiology, Department of Pediatrics, Dokuz Eylul University Faculty of Medicine, Izmir, Turkey; [d]Department of Cardiology, Dokuz Eylul University Faculty of Medicine, Izmir, Turkey

Keywords
Long QT syndrome · Genetic counseling · Dual phenotype · Next-generation sequencing · Clinical heterogeneity

Abstract
Introduction: Long QT syndrome (LQTS) is a disorder of ventricular myocardial repolarization characterized by a prolonged QT interval on the electrocardiogram. It increases the risk of ventricular arrhythmias, which can cause syncope or sudden cardiac death. In this study, we study the genotype-phenotype relationships of patients referred to us with suspected arrhythmia syndrome. ***Methods:*** Seventeen cases and their twenty relatives were evaluated. Next-generation sequencing analysis was performed for 17 LQTS-related genes. ***Results:*** We detected seventeen single nucleotide variants (SNVs) with potential pathogenic significance in 26 of the 36 subjects analyzed. *KCNH2* c.172G>A, *KCNQ1* c.1768G>A, *ANK2* c.4666A>T, c.1484_1485delCT, *KCNH2* c.1888G>A were reported as pathogenic or likely pathogenic in HGMD variant classification database. ***Conclusion:*** Current study pointed out that early diagnosis can be life-saving for patients and their families by taking family history and detailed examination. Also, we highlight the clinical heterogeneity of arrhythmia syndrome through a patient with a dual phenotype.

Introduction

Long QT syndrome (LQTS) is a disorder of ventricular myocardial repolarization characterized by a prolonged QT interval on the electrocardiogram (ECG). It can lead to ventricular arrhythmias known as torsades de pointes. Arrhythmias may result in syncope or sudden cardiac death, especially after increased sympathetic activity such as exercise and emotional stress [Schwartz and Ackerman, 2013]. The LQTS is the phenotypic description. It may be congenital or acquired (most often due to medications and electrolyte disturbances).

The diagnosis of LQTS can be established by >3.5 score from the Schwartz criteria or corrected QT (QTc) interval of at least 500 ms in repeated 12-lead ECGs in the absence

karger@karger.com
www.karger.com/msy

Correspondence to:
Ayca Yıldız Bulut, aychayldz@gmail.com

Karger

of a secondary cause for QT prolongation [Schwartz and Ackerman, 2013]. Identification of pathogenic variant in a confirmed LQTS gene is also diagnostic. The prevalence of congenital LQTS is 1 in 2,000 live births. This prevalence is derived from a prospective study of over 44,000 infants with a disease-causing genetic variant and prolonged QT interval [Schwartz et al., 2009]. Besides, LQTS can show reduced penetrance of the clinical symptoms, so there are disregarded asymptomatic pathogenic variant carriers who are at risk of sudden death.

Congenital LQTS was first described in 1957 by Jervell and Lange-Nielsen in one family with an autosomal recessive form of the disease. They established a relationship between syncope, congenital deafness, and QT interval prolongation on ECG [Jervell and Lange-Nielsen, 1957]. Subsequently, the more common form of autosomal dominant LQTS was described by Romano and Ward [Ward, 1964; Romano, 1963]. Fifteen types of LQTS have been defined based on their genetic causes. Type 1 LQTS is caused by *KCNQ1* gene variants and it accounts for 30 to 35 percent of cases of the LQTS. Type 2 LQTS is caused by *KCNH2* gene variants and it accounts for 25–40% of LQTS [Ackerman et al., 2011]. Type 3 LQTS is caused by *SCN5A* gene variants and it accounts for 5–10% of LQTS. Type 1, 2, and 3 LQTS genes account for at least 75 percent of all LQTS. Minor LQTS susceptibility genes are *ANK2*, *KCNE1*, *KCNE2*, *KCNJ2*, *CACNA1C*, *CAV3*, *SCN4B*, *AKAP9*, *SNTA1*, *KCNJ5*, *CALM1*, *CLM2*, and they contribute to other 5–10% of LQTS. Also, the pathogenic loss-of-function variants in *SCN5A* are associated with Brugada syndrome. Because of cardiac conduction defects, it can cause rapid polymorphic ventricular tachycardia, ventricular fibrillation, and sudden death. We aimed to study the genotype-phenotype relationships of patients referred to us with suspected arrhythmia syndrome due to their ECG findings, symptoms, or family history of sudden cardiac deaths.

Materials and Methods

We recruited 17 suspected arrhythmia syndrome cases and their 20 relatives in this prospective study for segregation analysis. The suspected arrhythmia syndrome cases were referred to us because of their ECG findings, symptoms, or family history of sudden cardiac deaths. They were evaluated by an expert cardiologist who classified the suspected arrhythmia syndrome phenotype. The QT intervals were measured from electrocardiographic lead II and corrected for heart rate according to Bazett's formula. Informed consent was obtained from all individuals or their guardians before the genetic analysis.

Genetic Testing

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood leukocytes using the High Pure PCR Template Preparation Kit from Roche Applied Science. Next-generation sequencing (NGS) capture-based panel testing (Celemics, South Korea) was performed for 17 potassium, sodium, and calcium channel proteins and some cardiac membrane protein genes (Table 1). The panel included the most common genes associated with LQTS and Brugada syndrome (Table 1). The library runs with NextSeq500 machine (Illumina, USA). NGS analysis covered all exonic and flanking intronic regions corresponding to the 3′ and 5′ extremes of the exons. NGD data were analyzed by Seq software (Genomize, Turkey). Human Reference Genome "hg19/GRCh37" was used for the alignment of the reads. The ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), VarSome web tool (https://varsome.com/), and Franklin web tool (https://franklin.genoox.com) were used during variant interpretation. More than 95% of the targeted regions were read in ×20 depth. Classification of genetic variants was made according to the ACMG recommendations [Richards et al., 2015].

Results

We have been referred to 16 suspected arrhythmia syndrome cases. Fourteen of these individuals had clinical symptoms. Eleven of them had ECG evidence of arrhythmia disorders and an initial diagnosis of LQTS. Two of the 14 patients also had a family history of sudden death. Besides, 2 patients consulted us because of a family history of suspected arrhythmia syndrome without clinical findings.

We have also reported another patient due to the known pathogenic variation of the *KCNH2* gene in the family (patient 5). His brother had motor mental retardation and epilepsy, and pathogenic variation in the *KCNH2* gene was incidentally identified by WES analysis. Thereafter, his family consulted us for genetic counseling. We confirmed the WES results by NGS analysis. We showed the brother had heterozygous c.1888G>A variation in the *KCNH2* gene. The family was informed about LQTS and recommended genetic testing. Proband and his father were shown to have the same variation. They were referred for cardiologic examination.

A congenital arrhythmia syndrome NGS panel was performed on seventeen index patients. Besides, 20 family members of probands got genetic testing either with NGS or Sanger sequencing within segregation analysis.

Ten of 17 probands were women. The probands' mean age was 16.9 years. Eleven of them had long QT intervals on ECG examination, with an average QTc of 451 ms.

Our study detected seventeen single nucleotide variants (SNVs) with potential pathogenic significance in 26

Table 1. Genes and related phenotypes in the congenital arrhythmia syndromes NGS panel

Gene	Ensemble database isoform	Protein name	OMIM phenotype (inheritance pattern)
AKAP9	ENST00000356239	A-kinase anchor protein 9	LQTS 11 (AD)
ANK2	ENST00000357077	Ankyrin-2	LQTS 4 (AD)
CACNA1C	ENST00000347598	Voltage-dependent L-type calcium channel subunit alpha-1C	LQTS 8 (AD) Brugada syndrome 3 (AD)
CACNB2	ENST00000324631	Voltage-dependent L-type calcium channel subunit beta-2	Brugada syndrome 4 (AD)
CAV3	ENST00000343849	Protein caveolin-3	LQTS 9 (AD)
GPD1L	ENST00000282541	Glycerol-3-phosphate dehydrogenase 1-like protein	Brugada syndrome 2 (AD)
KCNE1	ENST00000337385	Potassium voltage-gated channel, Isk-related family, member 1	LQTS 5 (AD) Jervell and Lange-Nielsen syndrome 2 (AR)
KCNE2	ENST00000290310	Potassium voltage-gated channel, Isk-related family, member 2	LQTS 6 (AD)
KCNE3	ENST00000310128	Potassium voltage-gated channel, Isk-related family, member 3	Brugada syndrome 6 (AD)
KCNH2	ENST00000262186	Potassium voltage-gated channel, subfamily H (eag-related), member 2	LQTS 2 (AD)
KCNJ2	ENST00000243457	Inward rectifier potassium channel 2	Andersen syndrome (AD)
KCNJ5	ENST00000529694	G protein-activated inward rectifier potassium channel 4	LQTS 13 (AD)
KCNQ1	ENST00000155840	Potassium voltage-gated channel, KQT-like subfamily, member 1	LQTS 1 (AD) Jervell and Lange-Nielsen syndrome
PRKAG2	ENST00000287878	5′-AMP-activated protein kinase subunit gamma-2	Cardiomyopathy, hypertrophic 6 (AD) Wolff-Parkinson-White syndrome (AD)
SCN4B	ENST00000324727	Sodium channel subunit beta-4	LQTS 10 (AD)
SCN5A	ENST00000413689	Sodium channel protein type 5 subunit alpha	LQTS 3 (AD) Brugada syndrome 1 (AD)
SNTA1	ENST00000217381	Alpha-1-syntrophin	LQTS 12 (AD)

AD, autosomal dominant.

of all 36 subjects analyzed (Table 2). Among the detected pathogenic, likely pathogenic, and uncertain significance SNVs, 3 SNVs (23%) in the *KCNH2* gene, 3 SNVs (23%) in the *KCNQ1* gene, 3 CNVs (23%) in the *AKAP9* gene, 2 CNVs (15.3%) in the *ANK2* gene, and one (7.7%) each in the *KCNJ5* and *GDP1L* genes were detected (Table 3).

Table 2. Characteristics of identified variants classified

	Variant	Variant type	RefSNPs (rs) number	MAF	DAMN score/ Mutation Tester	VarSome/ ClinVar	Protein name and location
1	KCNH2 c.172G>A (E58K)	Missense	rs199473413	–	0.9988 – disease causing	Likely pathogenic	Rapid delayed rectifier potassium canal (Ikr) N terminal PAS domain
2	KCNQ1 c.1768G>A (A590T)	Missense	rs199472813	<0.01	0.9984 – disease causing	Likely pathogenic	Slow delayed rectifier potassium canal (Iks) C-terminal assembly domain
3	ANK2 c.4666A>T (K1556*)	Nonsense novel	–	–	0.9966 – disease causing	Pathogenic	
4	KCNQ1 c.1484_1485delCT	Frame shift-indel	rs39708090	–	No data	Pathogenic	Slow delayed rectifier potassium canal (Iks) C terminus of protein
5	KCNH2 c.1888G>A (V630I)	Missense	–	<0.01	0.9984 – disease causing	Likely pathogenic	Potassium voltage-gated channel subfamily H member 2 Intra-membrane "pore-forming" domain
6	KCNH2 c.1066C>T (R356C)	Missense	rs199472984	<0.01	0.9991 – disease causing	Likely benign/VUS	
7	KCNQ1 c.1538C>A (T513 N)	Missense	–	–	0.9944 – disease causing	VUS	Slow delayed rectifier potassium canal (Iks) C-terminal assembly domain
8	GPD1L c.400A>C (I134V)	Missense	–	–	0.9809 – disease causing	VUS	Glycerol-3-phosphate dehydrogenase 1-like protein
9	ANK2 c.4811G>A (R1604K)	Missense	rs776426910	<0.01	0.5527 – disease causing, polymorphism	Likely benign	Between death 1 and Repeat A domain
10	KCNJ5 c.630G>A (M210I)	Missense	rs138295501	<0.01	0.9824 – disease causing	VUS	G protein-activated inward rectifier potassium channel 4, cytoplasmic domain
11	AKAP9 c.4023A>T (Q1341H)	Missense	rs765475242	<0.01	0.8014 – disease causing	VUS	A-kinase anchor protein 9 Coiled coil
12	CACNA1C c.5586C>A (N1862)	Silent	rs1051978823	–	0.687 – polymorphism	Likely benign	Voltage-dependent L-type calcium channel subunit alpha-1C, cytoplasmic domain
13	AKAP9 c.11519T>C (I3840T)	Missense	rs145675748	<0.01	0.8826 – polymorphism	VUS	A-kinase anchor protein 9
14	CACNA1C c.911T>C (I304T)	Missense	rs201756421	<0.01	0.707 – polymorphism	Likely benign	Voltage-dependent L-type calcium channel subunit alpha-1C Repeat

Table 2 (continued)

	Variant	Variant type	RefSNPs (rs) number	MAF	DAMN score/ Mutation Tester	VarSome/ ClinVar	Protein name and location
15	AKAP9 c.1113A>G (Q371Q)	Missense	rs760101767	<0.01	0.5437	VUS	A-kinase anchor protein 9
16	KCNH2 c.3457C>T (H1153Y)	Missense	rs199473035	<0.01	0.9975 – disease causing	VUS	Rapid delayed rectifier potassium canal (Ikr) C terminal
17	ANK2 c.9679A>C (T3227P)	Miissense	rs140604600	<0.01	0.6844 – polymorphism	VUS	

All SNVs were in the heterozygote state, except one homozygous c.911T>C variation in the *CACNA1C* gene. Missense *KCNH2* c.172G>A (family 1) and *KCNQ1* c.1768G>A (family 2) variants were reported as pathogenic and likely pathogenic in the HGMD variant classification database, respectively. *ANK2* c.4666A>T (family 3) and *KCNQ1* c.1484_1485delCT (family 4) variants directly introduced a stop codon and were classified as pathogenic in HGMD, ClinVar, and VarSome databases. Families 1, 2, 3, and 4 are described in more detail below. Other detected variants, *KCNQ1* c.1538C>A, *KCNJ5* c.630G>A, *AKAP9* c.4023A>T, *GDP1L* c.400A>C, *ANK2* c.9679A>C, *AKAP9* c.1113A>G, and *KCNH2* c.3457C>T, were reported as uncertain significance according to ACMG classification. *CACNA1C* c.911T>C, *CACNA1C* c.5586C>A, *KCNH2* c.1066C>T, and *ANK2* c.4811G>A variants were reported as likely benign according to ACMG classification. Phenotype and genotype data of the patients with potential pathogenic significant variants are shown in Table 2.

Family 1

The index patient was a 40-year-old female. She was referred to our clinic after she had a cardiac arrest during anesthesia for a myomectomy operation. She did not have any known diseases or syncope attacks before. It was learned that she had a family history of sudden death (shown in Fig. 1). Her sister, her mother, and her mother's sister had died suddenly at the ages of 30, 44, and 39, respectively. She was taken for a cardiac examination. Her initial twelve-lead electrocardiography showed long QTc interval and negative U waves. After the pretest genetic counseling, the patient agreed to undergo genetic testing. In a NGS analysis of the proband, we detected a pathogenic, heterozygous, c.172G>A (E58K) variant in the *KCNH2* gene. Family members were called for a segregation study. After obtaining informed consent, family members who agreed to take the test were taken for sequence analysis with NGS or Sanger. Proband's 17-year-old son and nephew were shown not to carry pathogenic *KCNH2* gene variant. Proband's 45-year-old brother and 53-year-old uncle carried the same pathogenic *KCNH2* gene variant (Table 2). The test result was delivered with counseling, and they were referred to the cardiology department for follow-up. Unfortunately, other family members could not be reached for genetic testing.

Family 2

The index case was of a 51-year-old male. He applied to our clinic due to his family history. He had been followed up for hypertrophic septal cardiomyopathy (HCM) by a cardiology clinic. His ECG showed a QTc at 490 ms. His echocardiogram showed EF 55% and a global dilated left atrium. Cardiac MRI showed HCM (16 mm). His brother had sudden cardiac death without a preknown cardiac disease. Furthermore, his father and two uncles have an HCM clinic history (shown in Fig. 2). We performed NGS panel for congenital arrhythmia and cardiomyopathy genes. In the NGS analysis, we detected a pathogenic, heterozygous c.1768G>A (A590T) variant in the *KCNQ1* gene. Family members were called for a segregation study. After obtaining informed consent, family members who agreed to take the test were taken to sequence analysis with NGS or Sanger. Proband's father and 15-year-old nephew were shown not to carry the same variant. Proband's mother, a 22-year-old daughter, 16-year-old daughter, and 9-year-old nephew showed that they carried the same pathogenic *KCNQ1* gene variant. They received cardiologist follow-ups to prevent

Table 3. Proband's and family members' clinical and genetic data

	Age/ sex	Initial diagnosis	Clinical findings	ECG/Holter/ECO	Family history of sudden death	Gene-variant	ClinVar
Patient 1	40/F	LQTS	Cardiac arrest history	ECG: SR, QTc: 489 ms, negative U waves, type 3 long QT	+	*KCNH2* c.172G>A (E58K)	Likely pathogenic
Brother of patient 1	45/M	–	–	QTc: 457 ms	+	*KCNH2* c.172G>A (E58K)	Likely pathogenic
Mother's brother of patient 1	53/M	–	–	Not available	+	*KCNH2* c.172G>A (E58K)	Likely pathogenic
Son of patient 1	17/M	–	–	Normal cardiological examination	+	*Normal*	
Brother' son of patient 1	18/M	–	–	Normal cardiological examination	+	*AKAP9 c.971T>C (I327T)*	Likely benign
Patient 2	51/M	LQTS	HCMP diagnosis and palpitation	ECG: SR, QTc: 490 ms, intraventricular C conduction delay ECO and MRI: cardiomyopathy+	+	*KCNQ1* c.1768G>A (A590T)	Likely pathogenic
Daughter of patient 2	22/F	–	–	Normal cardiological examination	+	*KCNQ1* c.1768G>A (A590T)	Likely pathogenic
Brother's son of patient 2	9/M	–	–	QTc: 460 ms, type 1 long QT ECO: minimal mitral insufficiency, MVP	+	*KCNQ1* c.1768G>A (A590T)	Likely pathogenic
Daughter of patient 2	15/F	-	-	Normal cardiological examination	+	*KCNQ1* c.1768G>A (A590T)	Likely pathogenic
Brother's son of patient 2	15/M	–	–	Normal cardiological examination	+	*Normal*	
Father of patient 2	80/M		–	Hypertrophic CMP, ICD+	+	*Normal*	
Father's brother's daughter of patient 2	45/F	–	Palpitation	β-Blocker treatment+ for palpitation (no other medical data)	+	*Normal*	
Patient 3	16/M	LQTS	Hypertension history	ECG: Normal ECO: Normal	+	*ANK2* c.4666A>T (K1556*)	Pathogenic
Sister of patient 3	12/F	–	–	Normal cardiological examination	+	*ANK2* c.4666A>T (K1556*)	Pathogenic
Mother of patient 3	43/F	–	–	Normal cardiological examination	+	*Normal*	

Table 3 (continued)

	Age/ sex	Initial diagnosis	Clinical findings	ECG/Holter/ECO	Family history of sudden death	Gene-variant	ClinVar
Patient 4	13/F	LQTS	No symptoms	ECG: 450 ms, type 3 long QT ECO: LV hypertrophy and mild MVP	–	*KCNQ1* c.1484_1485delCT	Pathogenic
Mother of patient 4	47/F	–	–	Normal cardiological examination	–	*Normal*	
Father of patient	50/M	–	–	Not available	–	*Normal*	
Patient 5	3/M	LQTS	Asymptomatic	Normal cardiological examination	–	*KCNH2* c.1888G>A	Likely pathogenic
Mother of patient 5	39/F	–	–	Not available	–	*Normal*	
Father of patient 5	32/M	–	–	Not available	–	*KCNH2* c.1888G>A	Likely pathogenic
Brother of patient 5	13/M	–	MMR, epilepsy	ECG: sinus tachycardia ECO: normal	–	*KCNH2* c.1888G>A	Likely pathogenic
Patient 6	8/F	LQTS	Dyspnea and palpitation	ECG and Holter: QTc: 450 ms ECO: Minimal MR, TR	–	*KCNH2* c.1066C>T (R356C) *KCNQ1* c.1538C>A (T513 N)	VUS
Mother of patient 6	45/F	–	–	Not available	–	*Normal*	
Father of patient 6	47/M	–	–	Not available	–	*KCNH2* c.1066C>T (R356C) and *KCNQ1* c.1538C>A (T513 N)	Likely benign VUS
Patient 7	15/F	LQTS	Dizziness, chest pain	ECG: normal Holter: 440 ms, long QT	–	*ANK2* c.4811G>A (R1604K)	Likely benign
Patient 8	10/F	LQTS	Palpitation+	ECG and Holter: normal ECO: VSD (minimal muscular), ASD (minimal)	–	*KCNJ5* c.630G>A (M210I)	VUS
Patient 9	5/F	LQTS	Family history	ECG: normal ECO: PFO	+	*CACNA1C* c.5586C>A (N1862)	Likely benign
Patient 10	12/M	LQTS	Sudden death of 3 maternal uncle	ECG: 450 ms, type 2 long QT ECO: normal	+	*AKAP9* c.11519T>C (I3840T)	VUS
Mother of patient 10	41/F	–	–	Normal cardiological examination	+	*AKAP9* c.11519T>C (I3840T)	VUS

Table 3 (continued)

	Age/ sex	Initial diagnosis	Clinical findings	ECG/Holter/ECO	Family history of sudden death	Gene-variant	ClinVar
Patient 11	5/F	LQTS	Asymptomatic	ECG and Holter: QTc: 450 ms, T alternans type 2 long QT ECO: normal	–	*GPD1L* c.400A>C (I134V)	VUS
Patient 12	16/M	LQTS	Sudden cardiac arrest history during exercise	ECG: normal ECO: LV concentric HT, LV dilatation, AR	–	*CACNA1C* homozygous c.911T>C (I304T)	Likely benign
Patient 13	13/F	LQTS	Palpitation and presyncope	ECG: QTC:450 ms, type 3 long QT Holter: randevu+ ECO: PFO	–	*ANK2* c.9679A>C (T3227P)	VUS
Patient 14	11/F	LQTS	Palpitation	ECG: normal ECO: normal	–	Normal	
Patient 15	9/M	LQTS	Sudden cardiac arrest	ECG: QTc:580 ms (post-CPR), 450 ms (following ECG) type3 long QT Holter: 470 ms ECO:LV hypertrophy	-	Normal	
Patient 16	35/F	LQTS	Palpitation, dyspnea and presyncope	ECG: sinus bradycardia, PR: 110 ms QTc: 410 ms ECO: papiller hypertrophy Holter: normal	+	*AKAP9* c.1113A>G (Q371Q, rs760101767)	VUS
Patient 17	34/M	LQTS	Syncope	ECG: QTc: 434 ms	+	*KCNH2* c.3457C>T (H1153Y, rs199473035)	VUS

ECG, electrocardiography; ECO, echocardiogram; MRI, magnetic resonance imaging; QTc, corrected QT interval; MR, mitral regurgitation; TR, tricuspid regurgitation; AR, aortic regurgitation; LV, left ventricle; HT, hypertrophy; VUS, variant of uncertain significance; MMR, motor mental retardation.

possible cardiac events. They were evaluated with 12-lead ECG, Holter, and screening echocardiography. Proband's daughters have normal ECG findings, cardiac chamber size, and systolic function. His 9-year-old nephew had type 2 long QT on the Holter examination. His QTc was 465 ms. Also, we performed a cardiomyopathy NGS panel for proband to explain the paternal history of cardiac thickness. We detected a pathogenic, heterozygous c.927-9G>A (chr11:47367930, rs397516083) variant in the *MYBPC3* gene (ENST00000545968). The proband also had heterozygous c.5203C>T (chrX:32381027) (R1735, rs147904018) variation in the *DMD* gene that was classified as uncertain significance. For the *MYBPC3* gene variation, we offered sanger analysis from the proband's father and children in the family. After the pretest genetic counseling, they did not accept genetic analysis for the *MYBPC3* gene. Proband had intracardiac defibrillator (ICD) and was using β-blocker therapy, and, his 9-year-old nephew, who had long QT intervals, was started on β-blocker medication by the cardiology clinic.

Family 3

The index patient was a 16-year-old man. He was referred to our clinic because of his family history of sudden cardiac death. He did not have any symptoms, and his ECG and ECO were normal. His blood pressure was 140/90. It was learned that his paternal uncle and cousin had hypertension at the ages of 37 and 25,

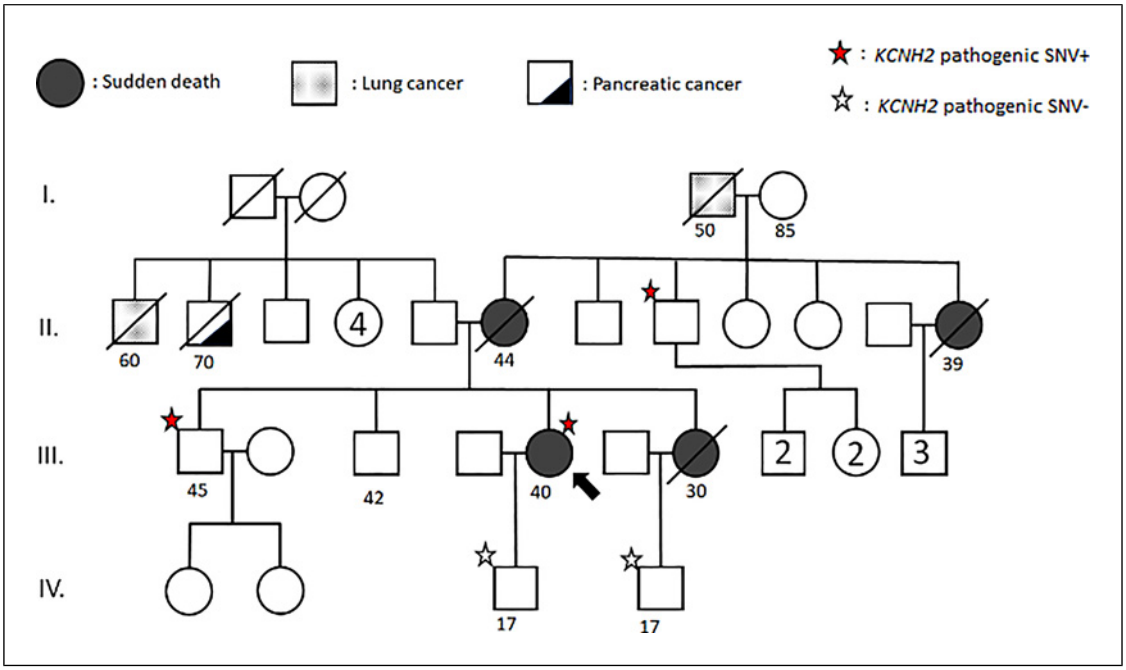

Fig. 1. Pedigree of family 1.

respectively. His father died in the daytime at the age of 35. He did not have any known diseases or syncope attacks before. His father's father and paternal uncle had a history of sudden death at 52 and 35, respectively. They did not have any known cardiac disease before. After informed consent was obtained from his mother, we performed NGS panel for congenital arrhythmias. In NGS analysis, we detected novel, pathogenic, heterozygous c.4666A>T variant in the *ANK2* gene. He was referred to the pediatric cardiology clinic for follow-up. Family members were called for a segregation study. His 12-year-old sister was shown to carry the same variation. His 43-year-old mother did not have any pathogenic variation of panel genes. Other family members did not want to take the genetic testing.

Family 4

The index patient was a 13-year-old female. She was referred to our clinic after a long QTc interval was detected during school ECG screenings. Screening QTc was measured at 450 ms, and she had type 3 long QT. She had no history of fainting, and there was no family history of sudden death, arrhythmia, or hearing loss. Her ECO showed mild left ventricular hypertrophy. Physical examination was normal. We performed NGS panel for congenital arrhythmias and detected pathogenic, frameshift, heterozygous c.1484_1485delCT (chr11:2683280, rs397508090) variant in the *KCNQ1* gene. Also, we showed that her 51-year-old father had the same variant and heterozygous c.11716C>T (R3906W, rs121912706) uncertainly significant variant in the *ANK2* gene. We referred him to the cardiology clinic for examination and follow-up. Other family members were called for genetic counseling and testing.

Discussion

Congenital LQTS is a potentially life-threatening condition. It has been reported that cumulative mortality risk before age 40 in the LQTS type 1, type 2, and type 3 phenotypes is 6–8% [Alders and Christiaans, 2017]. Besides, the clinical manifestations of LQTS are variable. Patients may have no symptoms. In addition, approximately 25% of individuals with a pathogenic variant have a normal QTc (defined as <440 msec) [Goldenberg et al., 2011]. Therefore, the prevalence of individuals with no clinical symptoms who have genotype-positive LQTS may be considered higher than expected. Those asymptomatic variant carriers are at a risk of sudden death. Furthermore, a family history of sudden cardiac death may be the only clue to suspect arrhythmia syndromes in these cases. When a careful clinician questions the family history, it can be

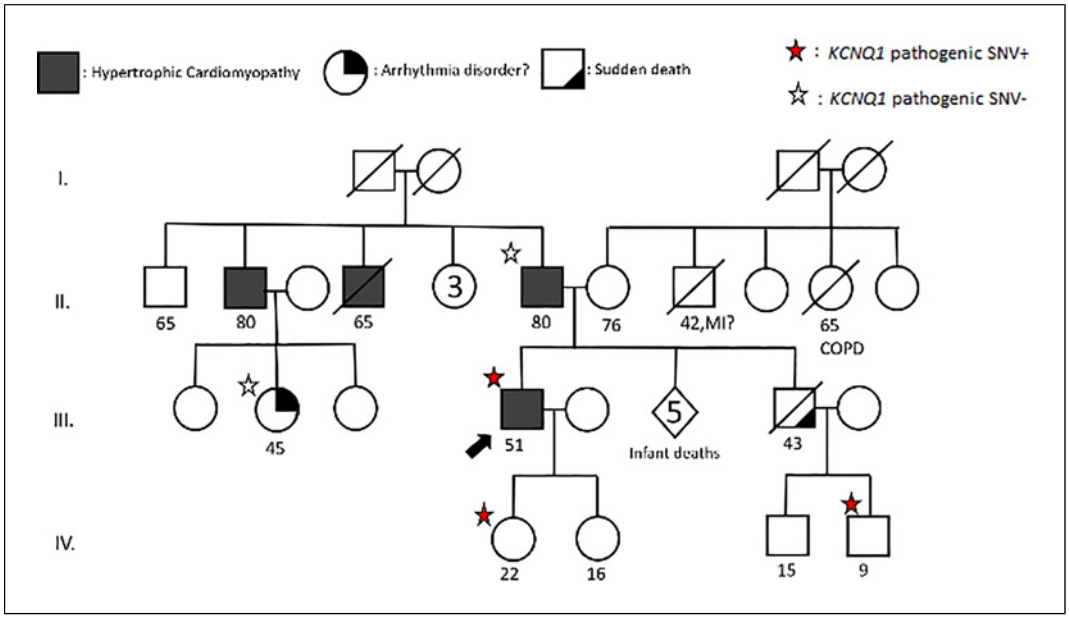

Fig. 2. Pedigree of family 2.

life-saving for the patients and their asymptomatic relatives.

Genetic testing is recommended in all cases diagnosed or clinically suspected [Modell et al., 2012]. Hence, a pathogenic variant can be detected from family screening in clinically diagnosed patients' families. These suspected cases may also be diagnosed.

We have studied 17 probands and their 20 family members with arrhythmia genes using the NGS panel. We detected five pathogenic/likely pathogenic variations and nine uncertainly significant variants among 36 patients.

Patient 1 has a heterozygote c.172G>A (E58K) variant of the *KCNH2* gene. *KCNH2* gene encodes a rapid delayed rectifier potassium canal (Ikr) protein. The c.172G>A missense variant is the loss-of-function variant, affects the N-terminal PAS domain of the protein, and causes LQTS type 2. This variant is reported as likely pathogenic according to the databases ClinVar and VarSome. It is located in the hotspot region, in silico prediction programs verdict seven pathogenic predictions versus two benign predictions and at the same position c.172T>C variation is reported as pathogenic from ClinVar. Also, it segregates truly with the phenotype in the family. We have shown that the proband's brother and maternal uncle carry the same variant. Proband's brother has had a cardiac examination, and his QTc has been detected as 457 ms. The pedigree shows autosomal dominant inheritance of pathogenic variants in this family. Thus, it was classified as pathogenic according to the American College of Medical Genetics and Genomics (ACMG) criteria. A recent study has reported a 51-year-old woman with LQTS who had QTc:490 ms and sinus bradycardia with the same variation [Hasegawa et al., 2014]. Our patient also had a history of sinus bradycardia in her ECG during β-blocker therapy, and an intracardiac defibrillator was placed. Unfortunately, we do not have DNA samples of sudden deaths in the family and cannot reach other family members. This patient shows us the importance of questioning family history. Despite three sudden cardiac deaths under 45 years old, our patient was diagnosed with cardiac arrest. After diagnosis, preventive treatments can protect family members at risk from sudden cardiac events.

Patient 2 has a c.1768G>A (A590T) variation of the *KCNQ1* gene. *KCNQ1* gene encodes voltage-gated potassium channel protein (KvLQT1), a transmembrane protein forming slow delayed rectifier potassium canals (Iks). This missense variant affects the C-terminal α helical region of the channel protein and causes LQTS type 1. A previous functional study has reported that variants at the A590 region impair IK channel surface expression and function [Kinoshita et al., 2014]. UniProt classifies this variant as "disease," and the Database of ClinVar classifies it as "likely pathogenic." Our case has a hypertrophic cardiomyopathy (HCMP) diagnosis and paternal HCMP family history. Previously, *KCNQ1* variants have been

reported to be associated with LQTS, neonatal persistent sinus bradycardia, and familial atrial fibrillation [Lupoglazoff et al., 2004]. However, *KCNQ1* variants have not been associated with HCMP in the literature. One family is reported from China with the HCMP and LQTS phenotypes. That family was shown to carry tetrad heterozygous variants in *KCNQ1, MYH7, MYLK2,* and *TMEM70* genes [Wang et al., 2016]. We examined our proband for CMP genes with NGS analysis and detected c.927-9G>A variation in the *MYBPC3* gene. All family members were called for genetic counseling, and family members who accepted were evaluated with 12-lead ECG, Holter, and screening echocardiography. His daughters do not show the LQTS phenotype despite the pathogenic *KCNQ1* variant. This may be due to variable penetrance, as is known in LOTS. Also, two different variants were detected in our patient, related to two different phenotypes. Cardiomyopathy phenotype can be explained by the *MYBPC3* gene variant, and the family history of sudden death can be explained by the *KCNQ1* gene variant. It should be kept in mind that patients may have a complex phenotype, and there may be more than one pathogenic variant affecting the clinic.

Patient 3 has a novel heterozygote c.4666A>T (K1556*) variant of the *ANK2* gene. *ANK2* gene encodes the Ankyrin-2 protein, which has an important role in the localization and membrane stabilization of ion transporters and ion channels in cardiomyocytes. The c.4666A>T variation is a novel nonsense variation. The novel variant is predicted in silico as deleterious. This induces that the protein is predicted to be 2,402 amino acids shorter than the wild-type protein. It is classified as pathogenic according to the databases of ClinVar and VarSome. Our patient's genotype coincided with his family history. However, he also had a hypertension history and no known kidney disease. A previous study supported the *ANK2* gene and systolic blood pressure relationship by gene-level association analyses [Sung et al., 2015]. However, segregation analysis is required for this family to explain the relationship between genotype and phenotype. Unfortunately, other family members could not be reached. Only her sister was shown to carry the same variation and had normal blood pressure follow-up.

Patient 4 had a two-base pair deletion in eleventh exon of the *KCNQ1* gene. This variant is predicted to result in a frameshift in the amino acid sequence, premature stop codon at the C terminus of the protein, and the nonfunctional truncated potassium voltage-gated channel protein. It is classified as pathogenic according to the ClinVar and VarSome databases. It has been published several times in association with the JLNS. After segregation analysis of JLNS patients, some of these studies have reported that parents who carry heterozygous deletion have a normal QTc interval [Wang et al., 2017]. By contrast, other studies show heterozygous variant carriers with long QT intervals [Wang et al., 2017; Al-Hassnan et al., 2017]. This discrepancy may be explained by variable penetrance and other genes affecting the cardiac conduction system. Our patient undergoes cardiology control due to ECG pathology, and family screening has been initiated through detected variant.

Patient 5 has likely pathogenic c.1888G>A variant in the *KCNH2* gene. After the family screening, we showed that his father and brother had the same variant. His brother's cardiological examination revealed only sinus tachycardia. C.1888G>A (V630I) variation is located in a mutational hotspot. Also, another amino-acid substitution at this position (Val630Ala) is classified as a "disease" (LQTS 2) by UniProt and classified as likely pathogenic according to ACMG criteria. The family was informed about the pathogenic gene variant. We recommended cardiology examination and variant screening for the family.

Patient 10 had a c.11519T>C variant in the *AKAP9* gene, and his 41-year-old mother had the same variant. She had normal ECG findings and no symptoms. Nevertheless, the patient's mother's brother had SCD at 52. Moreover, her 48 and 50-year-old brothers had ICDs for unknown reasons.

Patient 15 had type 3 long QT on ECG and sudden cardiac arrest history, and he got 4 points from the Schwartz scoring system. Besides, we did not detect any pathogenic variants in our NGS panel genes. Our panel contained 13 genes known to be associated with LQTS. Pathogenic variants in other genes may also cause LQTS because approximately 20% of patients with a clinical diagnosis of LQTS do not have any pathogenic variants. Also, we planned to perform a CMP NGS panel due to his hypertrophic LV ECO results and refer the family members to a cardiological examination.

We have detected five pathogenic/likely pathogenic variations in the arrhythmia syndromes NGS panel. Two of them had a family history and cardiac findings. One of them only had a family history. Moreover, the latter was detected during routine school ECG controls. All family members were informed and referred to the cardiology clinic, and the ones who accepted were screened with genetic testing. We have detected 9- and 45-year-old asymptomatic family members with long QT intervals, pathogenic variants, and three asymptomatic variant carriers. Asymptomatic variant carriers started annual ECG and Holter screenings and were informed about drugs and particular exercises to avoid.

This study highlights the importance of genetic analysis in the diagnosis and the clinical heterogeneity of

LQTS. Establishing the diagnosis helps patients and their families receive appropriate genetic counseling. Besides, asymptomatic variant carriers can be detected after the family screening, which may be vital for them in terms of preventive treatment options. Furthermore, these asymptomatic carriers can sometimes be diagnosed only by questioning the family history of sudden death, as in patient 3. An analysis of a larger cohort of individuals with LQTS is necessary to elucidate the clinical spectrum and molecular landscape further.

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Statement of Ethics

Ethics committee approval of our study was obtained from Ýzmir Dokuz Eylul University Non-Interventional Clinical Research Ethics Committee on April 27, 2022, with decision number 2022/16-22. Informed consent for genetic analysis and publication of clinical reports were obtained from the patients or their parents/guardians in compliance with the national ethics regulation.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Elçin Bora, Ayça Yıldız Bulut, Ahmet Okay Çağlayan conceived, designed, and coordinated the study. Ayça Yıldız Bulut, Elçin Bora, Tayfun Çinleti evaluated the patients and carried out pre- and posttest genetic counseling. Halise Zeynep Genç and Emin Evren Özcan performed a cardiological examination of patients and their family members. Ahmet Okay Çağlayan and Ayça Yıldız Bulut performed the genetic tests and analyzed the data. Ayça Yıldız Bulut wrote the manuscript in consultation with Elçin Bora, Ahmet Okay Çağlayan, and Tufan Çankaya. All authors discussed the results and commented on the manuscript.

Data Availability Statement

The authors declare that all data supporting the findings of this study are available in the article.

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