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Molecular Approach of Hereditary Arrhythmias, Long QT Syndrome, and Arrhythmogenic Right Ventricular Cardiomyopathy

ORIGINAL INVESTIGATION

ABSTRACT

Background: Hereditary cardiac arrhythmias result from mutations in various genes encoding ion channels. One major channelopathy is long QT syndrome, which has excellent genetic and clinical heterogeneity. Arrhythmogenic right ventricular cardiomyopathy, another hereditary arrhythmia type, also shows high genetic heterogeneity and variable expressivity. Next-generation sequencing is an effective tool to reveal the disease's underlying genetic etiology.

Methods: In this study, we performed clinical exome sequencing or gene panel including cardiac arrhythmia and cardiomyopathy-associated genes by next-generation sequencing in 13 unrelated patients.

Results: Five pathogenic or likely pathogenic mutations, including three novel mutations, were found in the total cases.

Conclusion: This research shows a strong genetic heterogeneity in the disease. In addition, the study revealed that patients with QT interval prolongation on electrocardiogram might also have mutations in genes that are not associated with long QT syndrome, such as *MYLK2* and *DSG2*. Therefore, our data helped expand the molecular scope of long QT syndrome. It is necessary to study with a broad perspective to elucidate the underlying molecular etiology in patients with hereditary cardiac arrhythmias.

Keywords: Arrhythmia, ARVC, clinical exome sequencing, genetics, LQTS

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INTRODUCTION

Inherited cardiac arrhythmias are a group of disorders with significant developments in the genetics field. Cardiac ion channelopathies constitute the most important group of inherited arrhythmias, and these disorders are caused by mutations in genes encoding ion channels.

One major channelopathy is long QT syndrome (LQTS), which is mainly inherited in an autosomal dominant manner.[1] Long QT syndrome has a prevalence of 1 : 2000 in healthy live births.[2] Spontaneous syncope, palpitations, seizures, and sudden death secondary to ventricular arrhythmia are clinical findings observed in the disease. The rate of symptomatic patients is reported to be 50% until the age of 12 years and 90% until the age of 40 years.[3] A characteristic finding is delayed ventricular repolarization, manifested as a lengthening of the QT interval and ventricular tachyarrhythmia (usually torsades de pointes) on the surface electrocardiogram (ECG). LQTS consists of 17 different subtypes resulting from mutations in 15 genes with autosomal dominant inheritance. The most frequently detected subtypes of the disease are LQT1, LQT2, and LQT3 and are associated with mutations in the *KCNQ1*, *KCNH2*, and *SCN5A* genes, respectively.[4]

Another hereditary arrhythmia is arrhythmogenic right ventricular cardiomyopathy (ARVC), which presents with ventricular arrhythmias and sudden cardiac death, especially in young people.[5] Although autosomal dominant inheritance is the most common inheritance pattern in ARVC patients, autosomal recessive inheritance can also be seen. Variable expressivity and incomplete penetrance are

typical in cases. Fifteen genes related to disease have been identified so far. Five of these genes (*DSC2*, *DSG2*, *DSP*, *JUP*, and *PKP2*) are responsible for about 50% of cases.[6]

Molecular diagnosis is an essential and helpful tool in diagnosing and managing hereditary arrhythmias with high genetic and clinical heterogeneity. Known mutations and genes associated with LQTS and ARVC are increasing with the widespread use of molecular methods. In addition, genetic screening of family members provides early diagnosis of asymptomatic individuals with the cardiac risk that would require prophylactic treatment.[7] To our knowledge, this is the first study from Turkey to include a group of patients studied to elucidate the molecular etiology of LQTS.

METHODS

Patients

This study included all 13 patients referred to medical genetics clinic between 2018 and 2020. The Ethical Committee approved the study. Written informed consent for the publication was obtained from the patient or their family involved in the study. All patients in our cohort were unrelated and mainly were referred to us with long QT (QTc > 460 ms for males and >470 ms for females) or borderline long QT on ECG. In addition to QT prolongation, one patient (case 1) had T-wave abnormality and another patient (case 8) had first-degree AV block on ECG. Electrocardiograms and family histories of all patients were examined in detail. We performed clinical exome sequencing (cases 2, 3, 4, and 9) or gene panel, including cardiac arrhythmias and cardiomyopathy-associated genes for all patients. The panel contains 128 genes associated with arrhythmia and cardiomyopathy.

DNA Sequencing and Variant Classification

Peripheral blood samples were collected into EDTA tubes. DNA of patients was extracted by using the QIAcube automated DNA isolation system (Qiagen Inc. Mississauga, ON, Canada). A NanoDrop 1000 (ThermobFisher Scientific Inc., MA, USA) spectrophotometer was used for DNA concentration and quality measurement. The sequencing was performed on the Illumina NextSeq platform (Illumina Inc., San Diego, CA, USA) using the clinical exome solution by Sophia Genetics kit. Targeted sequencing for 128 genes was performed by next-generation sequencing (NGS) using an Illumina commercial kit "TruSight One sequencing panel" on the Illumina MiSeq platform. Sequences were aligned to the human reference genome hg19/GRCh37. The data analyses were performed using Sophia DDM software (Sophia Genetics, Saint-Sulp). We excluded variants with a minor allele frequency of more than 0.01 in the EXAC. We searched the ClinVar, OMIM, and HGMD databases and *in silico* prediction tools (Mutation taster, SIFT, Polyphen2). According to the recent ACMG/AMP guideline, standardized variant interpretation was performed. Sanger sequencing was used for the validation of novel variants or family screening.

HIGHLIGHTS

- Molecular genetic approaches in hereditary cardiac arrhythmias are significant and essential in the early and definitive diagnosis of the patient and family screening.
- The most commonly mutated gene was *KCNQ1* (30%). Three novel variants in the *MYLK2*, *DSG2*, and *KCNQ1* genes were identified.
- Patients with QT interval prolongation on electrocardiogram may also have mutations in genes not associated with long QT syndrome, such as *MYLK2* and *DSG2*. This research expands the spectrum of mutations and provides new insights for genotype-phenotype correlations of arrhythmias.

RESULTS

There are 13 unrelated probands between the ages of 2 and 31 years in our patient group. Of which, 11 heterozygous, 1 compound heterozygous, and 1 hemizygous variant were observed. The majority of changes were missense variants (11/13, 85%). Also, we detected frameshift variants in 2 patients. Pathogenic or likely pathogenic variants were detected in 5 of the total cases (5/13, 38%). Three novel variants in the *MYLK2*, *DSG2*, and *KCNQ1* genes were identified. The most commonly mutated gene was *KCNQ1* (4/13, %30). The list of all genomic variants defined is reported in Table 1. Three of these cases had other affected individuals in their families (cases 2, 4, and 8). Case 2 and his mother had the same variant in the *DSG2* gene, but his mother was asymptomatic with no ECG abnormalities. Also, the exact genomic change was detected in the mother and grandmother of case 4 by Sanger sequencing, and the variant was classified as likely pathogenic with increased segregation data. The pathogenic *KCNQ1* mutation defined in case 8 was also detected in her father and brother, who had QT prolongation on ECG. No mutation was detected in the patient's mother with a normal ECG.

DISCUSSION

The primary aim of the clinician in hereditary arrhythmia patients is to prevent sudden death events. The second important objective is to identify the potentially affected individuals in the family. Therefore, genetic tests are significant and essential in the early and definitive diagnosis of the patient and family screening. In the present study, the mutation spectrum of 13 LQTS and ARVC patients is reported.

LQTS is characterized by significantly genetic and clinical heterogeneity.[2] Due to the clinical heterogeneity, different degrees of QT prolongation may be observed in patients with the same mutation in the same family, and even in some individuals, no symptoms may have appeared yet. In addition, the disease shows significant genetic heterogeneity, and it will be possible to identify new genes with the widespread use of genetic testing. Next-generation sequencing technology is very useful to explain the underlying molecular genetic mechanism of the disease.[8] Although about 15 genes associated with LQTS have been identified, NGS analyses are beneficial because of the possibility of detecting mutations in different genes in patients with LQT phenotype.

Table 1. The List of All Genomic Variants Defined

Case	Sex	Age (Year)	Indication	Gene	Nucleotide Change	Affected Protein	Inheritance	Pathogenity (ACMG Criteria)	Novelty
1	M	10	Borderline LQT, abnormal T-wave morphology	*MYLK2*	c.564dupC	p.Arg189Glnfs*31	Heterozygous	Likely pathogenic	Novel
2	M	20	LQT	*DSG2*	c.938_944delCATCAGG	p.Ala.313Glufs*9	Heterozygous	Pathogenic	Novel
3	F	12	LQT	*KCNQ1*	c.725A>C	p.Asp242Ala	Heterozygous	Likely pathogenic	Novel
4	F	2	LQT	*KCNQ1*	c.877C>T	p.Arg293Cys	Heterozygous	Likely pathogenic	-
5	F	14	LQT	*TGFB3*	c.82A>C	p.Thr28Pro	Heterozygous	VUS	-
6	F	16	LQT	*AKAP9*	c.1709A>G	p.Asp570Gly	Heterozygous	VUS	-
7	F	2	LQT	*KCNQ1*	c.583C>T	p.Arg195Trp	Heterozygous	VUS	-
8	F	16	LQT, 1st degree AV block	*KCNQ1* *JUP*	c.728G>A c.1028G>A	p.Arg243His p.Ser343Asn	Heterozygous Homozygous	Pathogenic VUS	- -
9	M	31	LQT	*DSC2*	c.802A>G	p.Thr268Ala	Heterozygous	VUS	-
10	M	10	LQT	*EMD*	c.352C>T	p.Arg118Cys	Hemizygous	VUS	-
11	F		LQT	*MYOM1*	c.3914T>C	p.Met1305Thr	Heterozygous	VUS	-
12	M		Borderline LQT	*ABCC6*	c.3063C>A c.1540G>A	p.Phe102Leu p.Val514Ile	Heterozygous Heterozygous	VUS VUS	- -
13	M		LQT	*SCN5A*	c.44G>C	p.Arg15Thr	Heterozygous	VUS	-

F, female; LQT, long QT interval; M: male.

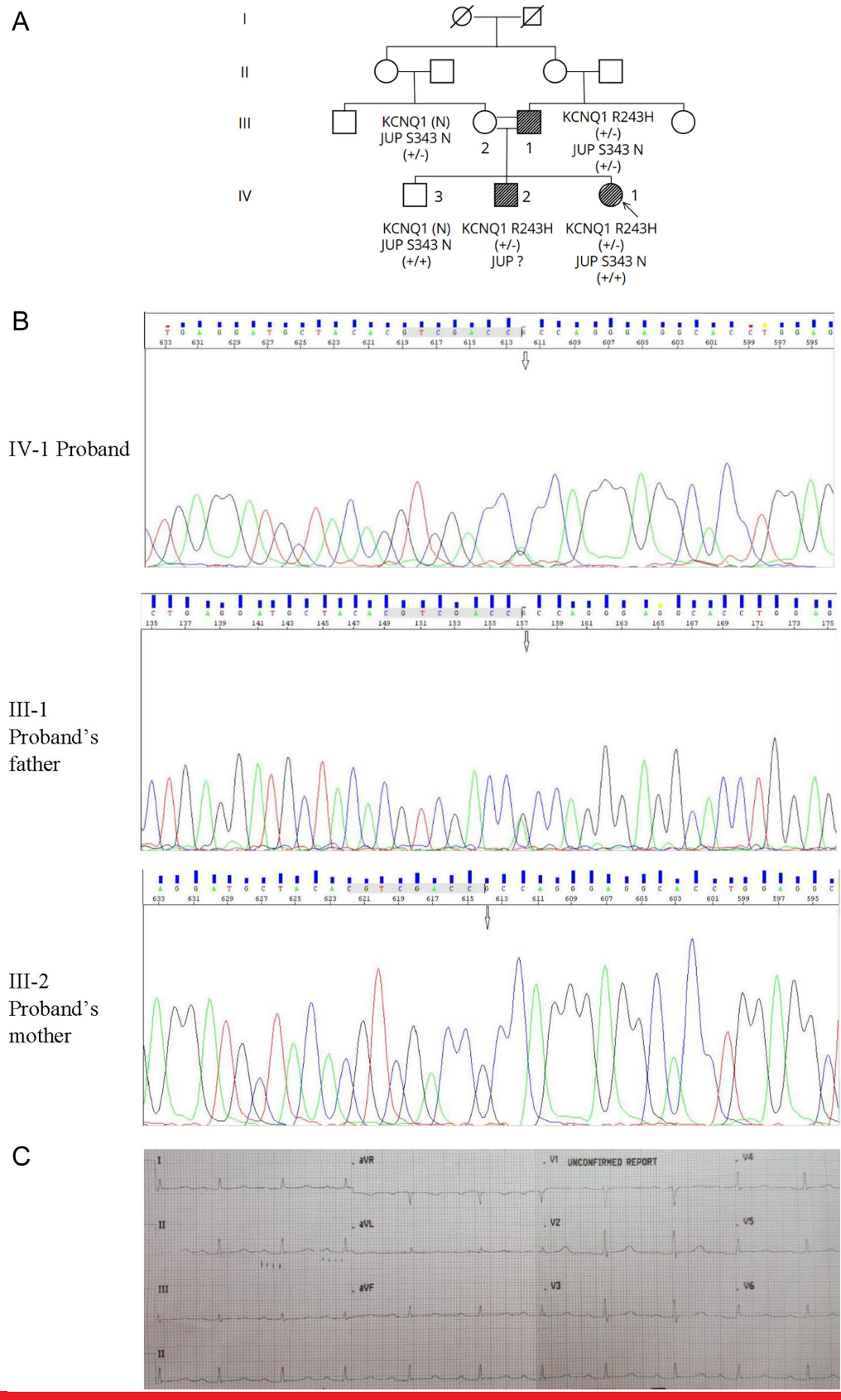

Figure 1. (a) Pedigree of the family with phenotypic and genotypic information. The arrow indicates the proband. Highlighted boxes represent the affected males, and highlighted circles represent the affected females. (b) Results of *KCNQ1* Sanger sequencing of family members. (c) Electrocardiogram findings of case 8. ECG shows sinus rhythm with the first-degree AV block and QT interval prolongation. ECG, electrocardiogram.

The *MYLK2* gene is mainly expressed in the skeletal muscle and encodes myosin light-chain kinase 2.[9] Wang et al[10] reported four heterozygous missense mutations in a family with dual long QT and hypertrophic cardiomyopathy phenotypes. One of these mutations was detected in the *MYLK2* gene, which was suggested to cause inverted T-waves. In addition, the long QT phenotype seen in the family was associated with another gene (*KCNQ1*). Another study suggested that *MYLK2* gene was associated with a digenic form of hypertrophic cardiomyopathy (HCM).[11]

A heterozygous c.564dupC p.(Arg189Glnfs*31) variant in the *MYLK2* gene was detected in case 1, which was referred to our clinic with borderline LQT (QTc 470 ms) and abnormal T-wave morphology on ECG. The novel frameshift variant is classified as pathogenic according to the ACMG guidelines. No variants were detected in any other gene that may be associated with LQTS or HCM in this patient. The patient's ECHO was normal, and there was no family history of cardiomyopathy. This case shows that in patients with *MYLK2* gene mutations, borderline QT interval prolongation or T-wave abnormalities may be seen on ECG rather than HCM findings unless there is another mutation in the cardiomyopathy-related genes. Since there are few studies on the *MYLK2* gene mutations and cardiac effects in the literature, we think the case will contribute to the genotype-phenotype correlation.

The *DSG2* (Desmoglein-2) gene is a member of the desmosomal cadherin family, and its mutations are known to be associated with ARVC.[6] ARVC is an autosomal dominant disease with incomplete penetrance and a highly variable phenotype.[12] In case 2, a heterozygous c.938_944delCATCAGG p.(Ala.313Glufs*9) novel and pathogenic variant in the *DSG2* gene was identified. The patient was referred to our clinic with QT interval prolongation, but a frameshift variant in the ARVC-related *DSC2* gene was unexpectedly detected. There was no ARVC-related finding in ECHO, but the patient will be followed in this regard. This case showed us that patients with mutations in ARVC-related genes might be followed up with the diagnosis of LQT, especially in the early stage of the disease. The same mutation was detected in the patient's mother with no cardiac symptoms. A cardiac examination was offered to the mother, but she refused. The gender-related differences and incomplete penetrance are the most critical reasons explaining the phenotypical variety in the cases.

Another significant case in terms of genotype-phenotype correlation of arrhythmias is a 16-year-old female patient (case 8). ECG demonstrated QT prolongation and first-degree AV block in the patient complained of palpitation. A heterozygous c.728G>A p.(Arg243His) variant in the *KCNQ1* gene classified as pathogenic has been identified by NGS. The patient's father and 21-year-old brother, followed with prolongation of QT interval, had the same mutation in the *KCNQ1* gene. Proband, her father, and brother have been on non-selective beta-blocker therapy for 1 year. No *KCNQ1* mutation was observed in the healthy mother of the patient. An additional homozygous c.1028G>A p.(Ser343Asn) variant in the *JUP* gene was detected in the proband. It is known that homozygous mutations in the *JUP* gene are related to NAXOS disease (cardiomyopathy, ARVC, with skin, hair, and nail abnormalities) (MIM number 601214).[13] The case did not have any skin, hair, or nail findings to consider an ectodermal disorder. In the literature, a homozygous missense mutation was identified in the *JUP* gene by whole-exome sequencing in a case with only ARVC findings without accompanying cutaneous problems.[14] Therefore, cardiac MRI and ECHO were performed to exclude the ARVC, and normal findings were observed. The patient's 26-year-old brother, who was asymptomatic and did not receive any treatment, had only a homozygous variant in the *JUP* gene. Sanger sequencing found this variant to be heterozygous in the consanguineous mother and father. The pedigree information and ECG findings are shown in Figure 1. Considering all this information, we think that the mutation in the *KCNQ1* gene is responsible for the cardiac findings in this family and that the variant in the *JUP* gene may not have any clinical effect.

Study Limitations

Due to the relatively small number of our patients, future studies with a large number of cases will be more informative about genotype-phenotype correlation. In addition, family screening of a case could not be performed because they did not accept it.

CONCLUSION

In recent years, significant advances in molecular genetic techniques have improved the understanding of the genetic background of hereditary arrhythmias. Also, the widespread use of NGS methodologies has made it possible to identify people at cardiac risk and prevent sudden cardiac deaths by performing family screenings. This study aims to elucidate the genotype in Turkish patients with LQT or ARVC. Our patient cohort and their NGS data expand the spectrum of mutations and provide new insights for genotype-phenotype correlations of arrhythmias.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Dr. Abdurrahman Yurtaslan Ankara Oncology Research and Training Hospital (approval no: 2021-12\19).

Informed Consent: Written informed consent was obtained from the patients or their parents for the publication of this report.

Peer-review: Externally peer-reviewed.

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REFERENCES

1. Kline J, Costantini O. Inherited cardiac arrhythmias and Channelopathies. *Med Clin North Am*. 2019;103(5):809-820. [CrossRef]
2. Nakano Y, Shimizu W. Genetics of long-QT syndrome. *J Hum Genet*. 2016;61(1):51-55. [CrossRef]
3. Mizusawa Y, Horie M, Wilde AA. Genetic and clinical advances in congenital long QT syndrome. *Circ J*. 2014;78(12):2827-2833. [CrossRef]
4. Wallace E, Howard L, Liu M, et al. Long QT syndrome: genetics and future Perspective. *Pediatr Cardiol*. 2019;40(7):1419-1430. [CrossRef]
5. Cadrin-Tourigny J, Bosman LP, Wang W, et al. Sudden cardiac death prediction in arrhythmogenic right ventricular cardiomyopathy: a multinational collaboration. *Circ Arrhythm Electrophysiol*. 2021;14(1):e008509. [CrossRef]
6. Vimalanathan AK, Ehler E, Gehmlich K. Genetics of and pathogenic mechanisms in arrhythmogenic right ventricular cardiomyopathy. *Biophys Rev*. 2018;10(4):973-982. [CrossRef]
7. Louis C, Calamaro E, Vinocur JM. Hereditary arrhythmias and cardiomyopathies: decision-making about genetic testing. *Curr Opin Cardiol*. 2018;33(1):78-86. [CrossRef]
8. Campuzano O, Sarquella-Brugada G, Mademont-Soler I, et al. Identification of genetic alterations, as causative genetic defects in long QT syndrome, using next generation sequencing technology. *PLoS One*. 2014;9(12):e114894. [CrossRef]
9. Davis JS, Hassanzadeh S, Winitsky S, et al. The overall pattern of cardiac contraction depends on a spatial gradient of myosin regulatory light chain phosphorylation. *Cell*. 2001;107(5):631-641. [CrossRef]
10. Wang J, Guo RQ, Guo JY, et al. Investigation of myocardial dysfunction using three-dimensional speckle tracking echocardiography in a genetic positive hypertrophic cardiomyopathy Chinese family. *Cardiol Young*. 2018;28(9):1106-1114. [CrossRef]
11. Qin X, Li P, Qu HQ, et al. FLNC and MYLK2 gene mutations in a Chinese family with different phenotypes of cardiomyopathy. *Int Heart J*. 2021;62(1):127-134. [CrossRef]
12. Haugaa KH, Leren IS. Prevalence, clinical presentation, and management of channelopathies and cardiomyopathies, long QT syndrome, Brugada syndrome, arrhythmogenic cardiomyopathy and hypertrophic cardiomyopathy. *Curr Cardiovasc Risk Rep*. 2019;13(7):1-7. [CrossRef]
13. Oktem A, Doolan BJ, Akay BN, et al. Autosomal recessive mutations in plakoglobin and risk of cardiac abnormalities. *Clin Exp Dermatol*. 2020;45(5):654-657. [CrossRef]
14. Liu L, Chen C, Li Y, Yu R. Whole-exome sequencing identified a de novo mutation of junction plakoglobin (p.R577C) in a Chinese patient with arrhythmogenic right ventricular cardiomyopathy. *BioMed Res Int*. 2019:9103860. [CrossRef]