Received: 28 November 2023 / Accepted: 24 January 2024 / Published online: 28 February 2024

DOI 10.34689/SH.2024.26.1.001

UDC 614.2(517)

MUTATIONS IN CARDIAC ION CHANNEL GENES IN KAZAKHSTANI PATIENTS WITH LONG QT SYNDROME

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Abstract

Introduction. Cardiac arrhythmias are the most common among the group of cardiovascular diseases (CVD), and have a risk of sudden cardiac death (SCD). Long QT syndrome (LQTS) is a heritable disease characterized by prolongation of the QT interval on an electrocardiogram (ECG), which often leads to syncope and SCD. Currently, identification of mutations in cardiac ion channel genes in patients with LQTS and recognition of genetic causes of the syndrome are actual in cardiology.

Aim. To identify cardiac ion channel mutations in genes associated with long QT syndrome in Kazakhstani patients.

Materials and methods. This study was designed as a cohort study. At present, our study has identified three patients with LQTS. Nevertheless, the recruitment of additional patients with LQTS for the study is ongoing. Illumina TruSight Cardio panel was used for genetic screening. The cardiopanel consists of 174 genes associated with cardiac disorders including LQTS. After a targeted sequencing, data analysis was carried out using the programs SureCall version 2.0.7.0 (Agilent Technologies, Santa Clara, California, USA), ANNOVAR, GTK, bwa, bowtie, bow tie 2, VarScan, etc.

Results. Clinically significant variants were found in patients with LQTS. Namely, in genes SCN5A (c.G5296A:p.E1766K) and KCNH2 (c.C662T:p.A221V). Both variants are pathogenic and cause CVDs, specifically LQTS. In addition, c.G3785A mutation (p.R1262Q), a variant of uncertain significance in SCN5A gene was detected in one patient. Although there is insufficient data to determine the role of the variant in development of the disease.

Conclusions. Screening for mutations in cardiac ion channel genes in patients with LQTS revealed clinically significant mutations. This research will be useful for Kazakhstani patients with LQTS in evaluation of required genetic testing and reliable genetic guidance to prevent SCD and distinguish between various arrhythmias.

Keywords: *cardiac arrhythmia, long QT syndrome, ion channel genes, mutation, sequencing.*

Абстракт

МУТАЦИИ В ГЕНАХ ИОННЫХ КАНАЛОВ СЕРДЦА У КАЗАХСТАНСКИХ ПАЦИЕНТОВ С СИНДРОМОМ УДЛИНЕННОГО ИНТЕРВАЛА QT

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Введение. Сердечные аритмии являются наиболее распространенным заболеванием в группе сердечно-сосудистых заболеваний (ССЗ) и имеют риск внезапной сердечной смерти (ВСС). Синдром удлиненного интервала QT (СУИQT) является наследственным заболеванием, характеризующимся удлинением интервала QT на электрокардиограмме (ЭКГ), что часто приводит к обмороку и ВСС. В настоящее время идентификация мутаций в

генах сердечных ионных каналов у пациентов с СУИQT и определение генетических причин синдрома актуальны в кардиологии.

Цель. Определить мутации сердечных ионных каналов в генах, связанных с СУИQT у пациентов из Казахстана.

Материалы и методы. Это исследование разработано в форме когортного исследования. На данный момент наше исследование выявило трех пациентов с СУИQT. Тем не менее, дальнейший набор пациентов для исследования продолжается. Для генетического скрининга использовалась панель – Illumina TruSight Cardio panel. Кардиопанель состоит из 174 генов, связанных с сердечно-сосудистыми нарушениями, включая СУИQT. После таргетного секвенирования был проведен анализ данных с использованием ряда программ SureCall версии 2.0.7.0 (Agilent Technologies, Санта-Клара, Калифорния, США), ANNOVAR, GTK, bwa, bowtie, bow tie 2, VarScan и т.д.

Результаты. Клинически значимые варианты были обнаружены у пациентов с СУИQT. А именно, в генах SCN5A (c.G5296A:p.E1766K) и KCNH2 (c.C662T:p.A221V). Оба варианта являются патогенными и вызывают СС3, в частности, СУИQT. Кроме того, у одного пациента была обнаружена мутация c.G3785A (p.R1262Q), вариант неопределенной значимости в гене SCN5A. Однако, на сегодняшний день недостаточно данных для определения роли варианта в развитии заболевания.

Выводы. Скрининг мутаций в генах сердечных ионных каналов у пациентов с СУИQT выявил клинически значимые мутации. Это исследование будет полезным для казахстанских пациентов с СУИQT для оценки необходимого генетического тестирования и надежного генетического консультирования для предотвращения ВСС и идентификации определенной аритмий.

Ключевые слова: *сердечная аритмия, синдром длинного QT, гены ионных каналов, мутация, секвенирование.*

Түйіндеме

ҰЗАРТЫЛҒАН QT СИНДРОМЫ БАР ҚАЗАҚСТАНДЫҚ ПАЦИЕНТТЕРДЕГІ ЖҮРЕКТІҢ ИОНДЫҚ КАНАЛ ГЕНДЕРІНДЕГІ МУТАЦИЯЛАРЫ

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Кіріспе. Жүрек аритмиясы жүрек-қан тамырлары аурулары (ЖҚА) тобында ең көп таралған ауру болып табылады және кенеттен жүрек өлімі (КЖӨ) қаупін тудырады. Ұзартылған QT синдромы (LQTS) - электрокардиограммадағы (ЭКГ) QT аралығының ұзаруымен сипатталатын тұқым қуалайтын ауру, бұл жиі естен тануға және КЖӨ -не әкеледі. Қазіргі уақытта LQTS пациенттеріндегі жүректегі иондық канал гендеріндегі мутацияларды анықтау және синдромның генетикалық себептерін анықтау кардиологияда өзекті мәселе болып табылады.

Мақсаты. Қазақстандық пациенттерде LQTS-мен байланысты жүректің иондық канал гендердегі мутацияларын анықтау.

Материалдар мен әдістер. Бұл зерттеу когортты зерттеу түрінде жасалған. Осы уақытқа дейін біздің зерттеуіміз LQTS бар үш науқасты анықтады. Дегенмен, пациенттерді зерттеуге одан әрі іліктеу жалғасуда. Генетикалық скрининг жасау мақсатында Illumina TruSight Cardio панелі қолданылды. Кардиопанель жүрек-қан тамырлары ауытқуларымен байланысты 174 геннен тұрады, соның ішіне LQTS те кіреді. Таргетті секвенирлеуден кейін бірқатар бағдарламалар SureCall 2.0.7.0 (Agilent Technologies, Санта-Клара, Калифорния, АҚШ), ANNOVAR, GTK, bwa, bowtie, bow tie 2, VarScan және т. б. қолдана отырып, деректерді талдау жүргізілді.

Нәтижелері. LQTS бар науқастарда клиникалық маңызды генетикалық варианттар табылды. Атап айтқанда, SCN5A (c.G5296A:p.E1766K) және KCNH2 (c.C662T:p.A221V) гендерінде. Жоғарыда аталған екі генетикалық вариант патогенді болып келеді, және де ЖҚА ішінде LQTS тудырады. Сонымен қатар, бір пациентте c.G3785A (p.R1262Q) мутациясы анықталды, бұл SCN5A геніндегі белгісіз маңыздылық вариантына жатады. Алайда, бүгінгі күнге дейін варианттың ауру дамуындағы рөлін анықтау үшін деректер жеткіліксіз болып келеді.

Қорытынды. LQTS пациенттеріндегі жүректегі иондық канал гендеріндегі скрининг клиникалық маңызды мутацияларды анықтады. Бұл зерттеу қазақстандық LQTS пациенттері үшін қажетті генетикалық тестілеуді бағалаумен қатар, КЖӨ-ін алдын алу және белгілі аритмияны анықтау шаралары үшін пайдалы болып табылады.

Түйін сөздер: *аритмия, ұзартылған QT синдромы, иондық канал гендері, мутация, секвенирлеу*.

Bibliographic citation:

Chamoieva A.E., Zhalbinova M.R., Mirmanova Zh.Z., Rakhimova S.E., Abdrakhmanov A.S., Akilzhanova A.R. Mutations in cardiac ion channel genes in kazakhstani patients with long QT syndrome // *Nauka i Zdravookhranenie* [Science & Healthcare]. 2024, (Vol.26) 1, pp. 7-14. *doi 10.34689/SH.2024.26.1.001*

Чамойева А.Е., Жалбинова М.Р., Мирманова Ж.Ж., Рахимова С.Е., Абдрахманов А.С., Акильжанова А.Р. Мутации в генах ионных каналов сердца у казахстанских пациентов с синдромом удлиненного интервала QT // Наука и Здравоохранение. 2024. 1(Т.26). С. 7-14. *doi 10.34689/SH.2024.26.1.001*

Чамойева А.Е., Жалбинова М.Р., Мирманова Ж.Ж., Рахимова С.Е., Абдрахманов А.С., Акильжанова А.Р. Ұзартылған QT синдромы бар қазақстандық пациенттердегі жүректің иондық канал гендеріндегі мутациялары // Ғылым және Денсаулық сақтау. 2024. 1 (Т.26). Б. 7-14. *doi 10.34689/SH.2024.26.1.001*

Introduction

Globally, cardiovascular diseases (CVD) are the leading cause of death in the world, and estimated to result in 17.9 million deaths annually as reported by the World Health Organization (WHO). Over the past decade, there has been a notable rise in the prevalence of CVDs in Kazakhstan [15, 27]. Cardiac arrhythmias are the most common among the group of CVDs, and can lead to sudden cardiac death (SCD). Long QT syndrome (LQTS) is a life-threatening cardiac arrhythmia characterized by a prolonged ventricular repolarization, namely a prolonged QT interval on a standard electrocardiogram (ECG). The syndrome is associated with an elevated risk of torsades de pointes (TdP)-triggered seizures, syncope, and SCD due to abnormal heart rhythms [19, 22]. Approximately 1:2000 people suffer from LQTS with a more significant impact in SCD.

The diagnosis of LQTS is established based on the following criteria: a) the presence of a corrected QT interval on a 12-lead ECG, typically defined as >470 ms for males and >480 ms for females [1]; b) the presence of a confirmed pathogenic LQTS mutation, regardless of the duration of QT interval; and c) the assessment of LQTS risk, determined by symptoms, family history, and ECG results in the absence of a secondary cause for QT prolongation [19]. Nevertheless, roughly 5–10% of individuals in the general population show a QTc > 460 ms in screening ECGs [21].

In practical terms, a baseline QTc value ≥ 500 ms is considered distinctly abnormal. If observed in the absence of one or more risk factors for QT prolongation, it should strongly induce clinical suspicion for congenital LQTS (cLQTS). Consequently, the observation warrants a Class I recommendation to proceed with LQTS genetic testing [1, 9].

LQTS is a genetic disorder. The key genes KCNQ1, KCNH2 and SCN5A develop three types of LQTS: LQTS type 1 (LQT1), LQTS type 2 (LQT2), and LQTS type 3 (LQT3), respectively [9,26]. Above-mentioned primary genes KCNQ1 (LQT1), KCNH2 (LQT2), and SCN5A (LQT3) constitute roughly 75% of confirmed LQTS cases, while the lesser-known genes collectively contribute about 5% [26]. Despite the rarity of the syndrome, studies using mutational analyses have identified over 450 mutations across 10 genes linked to different types of LQTS [12]. It is important to mention that LQTS is typically inherited in an autosomal-dominant (AD) manner [23]. Sporadic de novo mutations occurring in the germline cells might explain approximately 5% to 10% of LQTS cases. Moreover, multisystem syndromic disorders linked to either QT or QTU prolongation: ankyrin B syndrome (previously known as LQT4), Andersen-Tawil syndrome (ATS, formerly LQT7), and Timothy syndrome (TS, formerly LQT8) are listed below (Table 1).

Table 1.

Current genetic basis of LQTS subtypes (adapted from Giudicessi J.R., Wilde A.A., Ackerman M.J. [9]).

Gene	LQTS subtypes	OMIM	Protein	Functional effect	Mode of inheritance	Frequency
KCNQ1	LQT1	192500	Kv7.1	Reduced I_{Ks}	AD; AR	~30–35%
KCNH2	LQT2	613688	Kv11.1	Reduced I_{Kr}	AD	~25–30%
SCN5A	LQT3	603830	Nav1.5	Increased INa	AD	~5–10%
ANK2	LQT4/ABS	600919	Ankyrin B	Aberrant ion channel / transporter localization		
KCNE1	LQT5	613695	MinK	Reduced I_{Ks}	AD	<1%
KCNE2	LQT6	613693	MiRP1	Reduced I_{Kr}	AD	<1%
KCNJ2	LQT7/ATS1	170390	Kir2.1	Reduced I_{K1}	AD	<1%
CACNA1C	LQT8 / TS	601005	Cav1.2	Increased $I_{Ca,L}$ (slowed VDI)	Sporadic; AD mosaicism	Very rare
CAV3	LQT9	611818	Caveolin 3	Increased I_{Na}	AD	<1%
SCN4B	LQT10	611819	Nav1.5 /β4-subunit	Increased I_{Na}	AD	<1%
AKAP9	LQT11	611820	Yotiao	Reduced I_{Ks}	AD	<1%
SNTA1	LQT12	612955	Syntrophin-α1	Increased I_{Na}	AD	<1%
KCNJ5	LQT13	613485	Kir3.4	Reduced $I_{K,Ach}$	AD	<1%
CALM1	LQT14	616247	Calmodulin 1	Increased $I_{Ca,L}$ (defective CDI)	Sporadic	~1–2%
CALM2	LQT15	616249	Calmodulin 2	Increased $I_{Ca,L}$ (defective CDI)	Sporadic	<1%
CALM3	LQT16	114183	Calmodulin 3	Likely increased $I_{Ca,L}$ (defective CDI)	Sporadic	<1%

LQTS, Long QT Syndrome; OMIM, Online Mendelian Inheritance in Man; AD, autosomal dominant; AR, autosomal recessive; ABS, Ankyrin-B syndrome; ATS, Andersen Tawil syndrome; TS, Timothy syndrome; CDI, calcium-dependent inactivation; VDI, voltage-dependent inactivation.

The proper function of the heart relies on the synchronized activation and deactivation of inward depolarizing (sodium (Na^+) and calcium (Ca^{2+}) channels) as well as outward repolarizing (potassium (K^+) channels) currents governing by five primary phases of the cardiac action potential (AP). Genetic (inherited) or acquired defects that enhance the depolarizing Na^+/Ca^{2+} currents (I_{Na} and $I_{Ca,L}$) or diminish the repolarizing K^+ currents (I_{Ks}, I_{Kr}, and I_{K1}) can lead to an elongated ventricular cardiac AP [8, 9, 14]: KCNQ1-encoded I_{Ks} ($K_v7.1$) potassium channel, KCNH2-encoded I_{Kr} ($K_v11.1$) potassium channel, or SCN5A-encoded I_{Na} ($Na_v1.5$) sodium channel [5,22]. As a result, this elongation is evident through a prolonged QT interval on the surface 12-lead ECG. Dysfunction of ion channels, so-called channelopathy is caused by mutations in genes coding pore-forming α-subunit of ion channels, consequently causing life-threatening cardiac arrhythmias.

The aim of the research is to identify cardiac ion channel mutations in genes associated with long QT syndrome in Kazakhstani patients by performing a targeted next generation sequencing (NGS).

Materials and methods

Patient material

The research was performed in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committees of the National Laboratory Astana, Nazarbayev University and the National Research Cardiac Surgery Center (NRCSC), Astana, Kazakhstan. Informed written consent was obtained from all participants.

Currently, three patients have been diagnosed with LQTS in our study. Although further recruitment of study participants is still being in process. The clinical data of patients including demographics (age, gender, and ethnicity), diagnosis, abnormalities, and family anamnesis were also collected.

Genomic DNA (gDNA) was isolated from whole blood using QIAamp D NA Mini Kit: DNA purification from blood or body fluids (Qiagen). The concentration of DNA was measured by using NanoDrop 2000 spectrophotometer (Thermo Scientific). The qualitative and quantitative analysis of DNA concentration was performed via electrophoresis using a 1% agarose gel in the Electrophoretic bath apparatus (BioRad) and Qubit 2.0 Flourometer (Thermo Fisher Scientific), respectively.

Preparation of DNA libraries

The preparation of DNA libraries was carried out in accordance with the protocol “Illumina DNA Prep with Enrichment”. DNA libraries were sequenced using the Illumina Truesight Cardio gene panel. The quality control of the DNA libraries was performed on BioAnalyzer 2100 with the Agilent DNA 1000 Kit (Agilent Technologies). Additionally, the check of DNA library concentrations was performed on Qubit 2.0 Flourometer (Thermo Fisher Scientific) by using Qubit TM ds High Sensitivity Assay kit.

Target enrichment and sequencing

Illumina TrueSight Cardio panel was applied for targeted sequencing of samples. The targeted sequencing was performed on Illumina MiSeq platform. The cardiopanel consists of 174 genes associated with cardiac disorders, including LQTS. Particularly, the panel includes following genes: potassium voltage-gated channel, KQT-like subfamily, member 1 – *KCNQ1*, potassium voltage-gated channel, subfamily H, member 2 – *KCNH2*, potassium inwardly rectifying channel, subfamily J, member 5 – *KCNJ5*, potassium voltage-gated channel, Isk-related family, member 1 – *KCNE1*, potassium voltage-gated channel, Isk-related family, member 2 – *KCNE2*, potassium voltage-gated channel, Isk-related family, member 3 – *KCNE3*, sodium voltage-gated channel, alpha subunit 5 – *SCN5A*, calcium channel, voltage-dependent, L-type alpha 1C subunit – *CACNA1C*, caveolin 3 – *CAV3*, sodium channel voltage-gated, type IV, beta – *SCN4B*, ankyrin – *ANK2*, A-kinase anchor protein 9 – *AKAP9*, syntrophin, alpha 1 – *SNTA1*, T-box transcription factor – *TBX3*. Calmodulin 1 – *CALM1*, Calmodulin 2 – *CALM2,* Calmodulin 3 – *CALM3*.

Data analysis and variant classification

The sequenced samples were sent for further bioinformatics analysis. Sequence data processing was conducted on genetic variants in genes predisposed to cardiovascular diseases. Several programs have been used for sequence data analysis: SureCall version 2.0.7.0 (Agilent Technologies, Santa Clara, California, USA), ANNOVAR, GTK, bwa, bowtie, bow tie 2, VarScan, etc.

The sequencing data were compared with available online international genomic databases ExAC, SIFT, ESP, Genbank, NCBI, EP 6500, 1000 Genomes, MutationTaster, SNPedia, Ensemble, ClinVar, etc.

The clinical significance of genetic variants was interpreted in accordance with the guidelines developed by the American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology (AMP) in 2015 [17]. Variant interpretations were made on InterVar (https://wintervar.wglab.org/) platform. The obtained genetic variants were filtered and classified into five categories of ACMG/AMP recommended standards: pathogenic (P), likely pathogenic (LP), a variant of uncertain significance (VUS), likely benign (LB) or benign (B) [16].

Results

Targeted sequencing and step-by-step filtering of annotated variants identified 119 genetic variants in patient № 001, 125 – in patient № 007, and 132 – in patient № 011. Among them, patients № 001 and 007 have disease-causing variants with pathogenicity status.

Patient № 001. Mutation in the SCN5A gene (c.G5296A:p.E1766K) was detected in 23 years old man (Kazakh). He was diagnosed with LQTS at the age of 15, then experienced syncopal event at 19. Family anamnesis of the patient states that an uncle on his mother's side died of heart disease at the age of 28. Holter monitoring on 12-lead ECG was performed. The main rhythm is sinusoidal. The average heart rate was 64 bpm. The maximum heart rate is 125 bpm. The minimum heart rate was 37 bpm. The P-wave is normal. There are two premature ventricular contractions. No pauses. No ST segment change. The maximum corrected QT interval (QTc) was 561 ms (Figure 1). The systolic function at rest is not impaired. Ejection fraction (EF) is 62%.

SCN5A, p.E1766K (rs137854601) is classified as a pathogenic variant by ClinVar and a likely pathogenic according to ACMG/AMP classifications. The variant is nonsynonymous single nucleotide variant (SNV) and located in exon 27 of the SCN5A gene (Table 2).

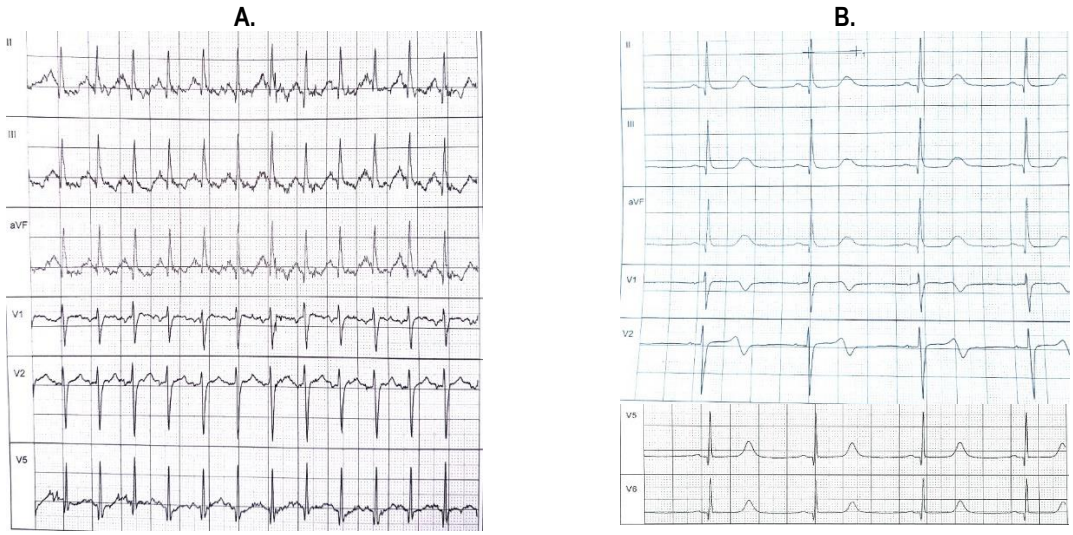

Figure 1. Electrocardiogram of patient №. 001 (diagnosed with LQTS);
A) tachycardia (at a heart rate of 128 bpm) on ECG; B) prolonged QT interval on ECG, QTc max = 561 ms.

Table 2.

Clinically significant genetic variants of patients diagnosed with LQTS in our study.

Patient ID (gender)	Age, years	Family history of CVD/SCD (yes/no)	Mutations	Geno type	Clin Var	ACMG / AMP classification	ID	OMIM
№. 001 (M)	23	Yes/no	***SCN5A: p.E1766K c.*** 5296 ***G>A***	hetero zygous	Pathogenic	**Likely pathogenic**	rs137854601	600163
№. 007 (F)	16	Yes/yes	***KCNH2: p.A221V c. 662 C>T***	hetero zygous	Pathogenic / Likely pathogenic	**Pathogenic**	rs121912504	152427
№. 011 (F)	47	Yes/yes	***SCN5A: p.R1262Q c.3785 G>A***	hetero zygous	Conflicting interpretations of pathogenicity	Uncertain significance	rs765907469	600163

No information on 1000Genome and ExAC databases. The SIFT score is 0.001, and the Mutation Taster score equals to 1.0. The genotype is heterozygous (0/1). The allele depth of the variant is 179 (Ref) and 31 (Alt), the total depth of readings is 210. The quality of the genotype is 99. According to 21 clinical diagnostic laboratories, this variant has a pathogenic status by ClinVar carrying a different phenotype of heart diseases LQT1, LQT3, congenital heart disease, Brugada syndrome, etc.

Patient № 007. Missense variant in KCNH2 gene (c.C662T:p.A221V) was found in a 16 years old girl (Kazakh). (Figure 2).

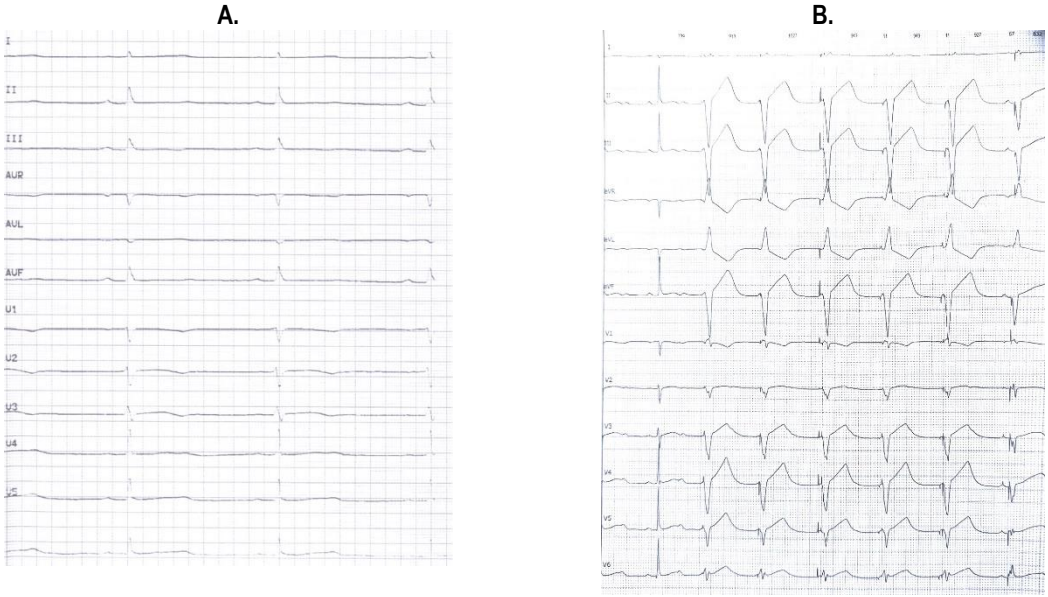

Figure 2. Electrocardiogram of patient № 007 (diagnosed with LQTS, being treated with automatic ICD)
A) prolonged QT interval on ECG (QT max= 672 ms at a heart rate of 50 bpm); B) P-controlled heart stimulation on ECG.

In addition to LQTS, the patient was diagnosed with paroxysmal unstable ventricular tachycardia, and ventricular extrasystole. Associated diagnosis: Idiopathic epilepsy. The girl is being treated with an automatic implantable cardioverter-defibrillator (ICD) by St. Jude Medical Fortify Assure in 2020. Chronic heart failure (CHF) II (NYHA). In family anamnesis, the mother of the patient died of cardiac arrest at young age (32 y.o.). Holter monitoring on 12-lead ECG was performed. The main rhythm is sinusoidal. Episodes of unstable ventricular tachycardia have been recorded. During the observation period, the minimum QT interval was 207 ms at a heart rate of 181 bpm, and the maximum was 672 ms at a heart rate of 50 bpm (Figure 2).

Moreover, 12 pauses due to bradyarrhythmia were registered. EF is 64%. The chambers of the heart are not expanded.

According to both ClinVar and the ACMG/AMP classifications KCNH2, p.A221V (rs121912504) is classified as pathogenic variant. There is no available information on 1000 Genome and ExAC databases; however, Mutation Taster indicates a score of 1.0. The genotype of KCNH2 is heterozygous (0/1). The allele depth of the variant is 32 (Ref) and 63 (Alt), with a total read depth of 95. The genotype quality is 99.

Patient № 011.

A variant of uncertain significance in SCN5A gene (c.G3785A:p.R1262Q) was detected in a 47-years-old woman (Kazakh). The patient was diagnosed with congenital LQTS. In family anamnesis, the mother died suddenly at the age of 30. According to echocardiogram results, the chambers of the heart are normal; systolic function of the left ventricle is satisfactory; EF is 66%. Holter monitoring on 12-lead ECG was performed for 1 day. 13h 37min, of which 2h 46 min. was occupied by physical activity, 9h 3min – sleep. During the observation period, the average heart rate was 61 bpm during the day and 53 bpm at night. The minimum heart rate was 45 bpm during sleep. The maximum heart rate reached 83 bpm (submaximal heart rate not achieved, 48% (<80%)). AV conduction is normal. No pauses longer than 2.0 seconds were detected. A single ventricular ectopic activity was registered in the form of one isolated premature ventricular contraction. No diagnostically significant changes in the ST-T segment were detected. The average QTc interval was 485ms (ranging from 442 to 540ms). A significant prolongation of the corrected QT interval from 450 to 540 ms was recorded during 14h 42 min. (Figure 3).

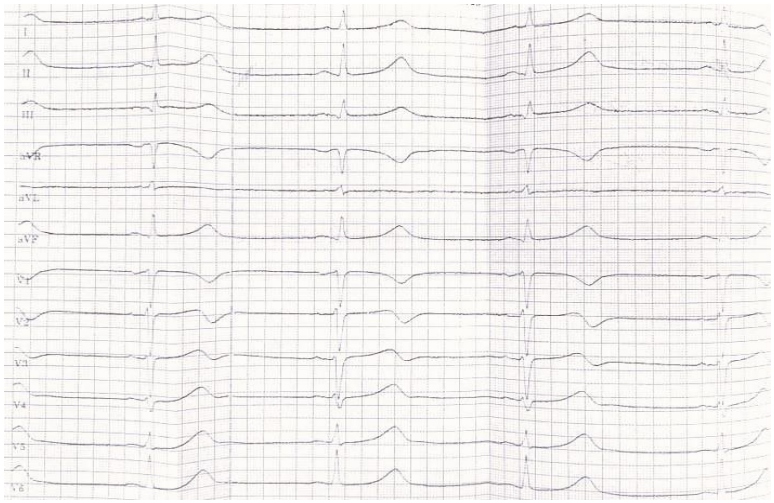

Figure 3. Electrocardiogram of patient № 011 (diagnosed with cLQTS); the average QTc interval = 485ms (ranging from 442 to 540ms).

No pathogenic variants have been found. Nevertheless, genetic variants with different clinical interpretations were identified – variants of uncertain significance, benign and likely benign variants. For example, SCN5A (rs765907469) is classified as a variant of uncertain significance according to ACMG/AMP guidelines. According to ClinVar, the variant has “Conflicting Interpretations of pathogenicity” status (Table 2). The variant has a heterozygous genotype, with a total coverage depth of 257. The genotype quality is 99. However, presently provided data are insufficient to determine the role of this variant in disease development.

Discussion

LQTS is usually characterized by syncope or heart arrest, primarily triggered during physical activity and emotional stress [19]. In most patients, symptoms might not be observed throughout their lives. It has been demonstrated that up to 13% of cases could lead to SCD, with 36% experiencing syncope episodes before the age of 40 [23].

Moreover, SCD and syncope are clinical manifestations of prolonged ventricular arrhythmias.

In fact, approximately 75% of all LQTS cases result from mutations in genes encoding cardiac ion channels, ion channel subunits, or proteins modulating ion channel function. However, a quarter of LQTS cases are challenging to identify based on genotype, especially complicating matters for family members within the risk group.

In our research, patient № 001 has a pathogenic mutation in SCN5A (c.G5296A:p.E1766K) gene, consequently the mutation lead to LQT3 accounting for roughly 10% of all cases [7, 18]. The particular genetic variant is not found in 121,222 chromosomes from a control group, yet it is frequently identified in affected individuals with LQTS and BrS conditions. A functional study has demonstrated that this variant has a deleterious effect on protein structure/function and leads to a permanent inward flow of Na^+ ions, which has also been previously noted in other LQTS-associated SCN5A mutations [6]. Furthermore, this variant has been classified as pathogenic by numerous clinical diagnostic laboratories and respected databases.

Besides, 47 years old woman, patient № 011 has a heterozygous variant of uncertain significance in SCN5A gene, although targeted sequencing has not revealed any pathogenic variant. Due to insufficiency of clinical information, well-established functional studies are necessary for proper interpretation of such variants. The Genome Aggregation Database (gnomAD) has noted this variant in 7 out of 273,056 chromosomes from a general population sample [25]. Presently, the evidence is

insufficient to definitively ascertain the impact of this variant in relation to disease. Consequently, this variant is categorized as VUS. The woman was diagnosed with cLQTS, however, despite the all progress in genetic screening methods, the detection of a potential pathogenic variant in a LQTS patient with a clear phenotype is around 75%–80% [26]. Cardiac incidents can manifest from early childhood to the middle stages of life, more prevalent between preteen and the twenties, consequently with the decreasing risk through this period. The typical age range for these events varies slightly depending on the genotype. After the age of 40, cardiac events are rare; if they do occur, they are often induced by the use of drugs that prolongs the QT interval or by hypokalemia, or they might be linked to the LQT3 [27].

Remarkably, we identified a missense pathogenic mutation in KCNH2 gene in patient № 007. As KCNH2 gene encodes a component of a voltage-activated potassium channel found in cardiac muscle, mutations in this gene can cause LQT2. About 30% of LQTS cases are linked to KCNH2 mutations causing LQT2 [11]. The found missense variant in KCNH2 gene leads to misfolding of the KCNH2 protein. This mutation results in the formation of a heterozygous ion channel with a reduced function, comprising both the mutant and wild-type proteins. Additionally, this mutation inhibits the expression of the fully developed or mature KCNH2 protein [4].

Generally, genetic testing for LQTS has both diagnostic and prognostic implications. It is crucial to differentiate pathogenic mutations from rare variants in order to accurately analyze genetic tests for LQTS. The careful examination of identified gene variants is critical before attributing pathogenicity to them, and involving a genetics specialist in this process is a sensible approach. The interpretation should consider the variant's prevalence in population databases, its predicted impact on protein function (based on in vitro and in vivo studies).

In our study, the patient № 007 is being treated with ICD at young age. Despite the fact that the frequency of ICD implantation was highest among LQT3 patients, the highest rate of successful interventions was observed among women with LQT2 who were identified as being at high risk [18, 28]. One of the largest studies on ICD (involving 233 patients, mean age at implantation=30±17 years) state that a notably high proportion of females (77%) and LQT3 patients received ICDs. Over a monitoring period of 4.6 years on average, ~28% of patients experienced at least one appropriate shock, while a quarter encountered adverse events. Notably, more than half of these patients had not previously experienced a cardiac arrest, and a significant portion had not failed β-blocker therapy [24].

We have not functionally characterized each specific mutation, which is a potential study limitation. Moreover, up to 25% of LQTS cases still lack a clear genetic explanation [24]. Thorough clinical observation, particularly carriers of clinically significant variants, will significantly contribute to our comprehension of further risk factors and evaluating their impact on the onset and progression of the disease.

Conclusion

Despite the study limitation in terms of quantity of study participants, genetic screening for mutations in cardiac ion channel genes in LQTS patients revealed clinically valuable mutations. Targeted sequencing of genetic variants associated with cardiac ion channels in individuals with LQTS is planned to continue as further recruitment of the patients to the cohort is still ahead. The study could be beneficial for Kazakhstani patients with LQTS in assessing the need for genetic testing and offering genetic counseling of patient's close relatives to prevent SCD and to differentiate between various types of arrhythmias.

Conflict of interest: *The authors declare that they have no conflicts of interest.*

Contribution of the authors: *Each of the authors made an equal contribution.*

Funding: *This study was carried out within the framework of the projects: AP14869903 "Comparative assessment of genetic variants in individuals who died from sudden cardiac death and cardiological patients: searching for genetic risk markers and diagnosis"; AP19677442 "Modeling of induced pluripotent stem cells (iPSCs) to improve understanding of the mechanisms underlying electrical heart disorders in Kazakhstani patients".*

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