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# CLINICAL CHARACTERISTICS OF PATIENTS WITH VARIOUS GENETIC TYPES OF LONG QT SYNDROME

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**The aim** of the study is to evaluate clinical characteristics, including adverse events and outcomes, in patients with various genetic types of long QT syndrome (LQTS).

**Material and methods.** We examined 24 patients with a clinical diagnosis of LQTS, observed in the for 5 years. The clinical and instrumental study included registration of electrocardiography (ECG), Holter monitoring, collection of a genealogical history with an ECG assessment of all family members and identification of cases of sudden cardiac death (SCD) in the family or the presence of a family form of the disease, echocardiography and cardiac magnetic resonance imaging to exclude structural changes in the myocardium. The search for mutations in the coding sequences of genes associated with the development of channelopathy and other hereditary heart rhythm disorders was carried out by next generation sequencing (NGS).

**Results.** Mutations in 4 genes associated with LQTS (*KCNQ1*, *KCNH2*, *CACNA1C*, *ANK2*) were detected in 18 out of 24 (75.0%) patients. Mutations in the *KCNQ1*, *KCNH2* and *CACNA1C* genes were detected in 14 (58.0%) patients. In 4 out of 24 (17%) patients, two or more variants of clinical significance (VUS) were detected in the genes associated with LQTS and hereditary arrhythmias, 6 patients had no genetic changes. The most severe form of the disease with pronounced clinical manifestations and episodes of clinical death with subsequent resuscitation measures, as well as a significant increase in the QTc interval exceeding 500 ms, was observed in patients with LQT2 and multiple mutations. Implantation of a cardioverter-defibrillator (CD) was required in 14 (58.3%) patients, including 11 (78.56%) - for secondary prevention of SCD and 3 (21.4%) - for primary prevention.

**Conclusion.** A comparative analysis between different genetic types of LQTS (LQT1; LQT2; patients with multiple VUS) showed that in patients with LQT1 syndrome, despite the early manifestation of the disease and the presence of syncope conditions, life-threatening arrhythmias, SCD and the frequency of CD implantation were significantly less often recorded than in other LQTS. The most severe form of the disease with pronounced clinical manifestations, episodes of clinical death with subsequent resuscitation and CD implantation was observed both in the group of probands with LQT2 and in patients with several nucleotide variants (VUS), one of which was in the *CACNA1C* or *ANK2* genes.

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Congenital long QT syndrome (LQTS) is a life-threatening arrhythmia syndrome, a major cause of sudden cardiac death (SCD) in young adults. It is characterized by the long QT interval on the electrocardiogram (ECG), the occurrence of syncope or cardiac arrest, mainly triggered by emotional or physical overexertion [1]. The incidence of the disease is now generally estimated to be about 1:2000-2500 [2].

LQTS is caused by functional changes in ion channels, mostly caused by defects in the genes encoding the pore-forming  $\alpha$ -subunits (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNJ2* and *CACNA1C*) or the regulatory  $\beta$ -subunits (*KCNE1*, *KCNE2* and *SCN4B*) of ion channels. 75% of

mutations are concentrated in one of the three genes: about 35% in *KCNQ1*, about 30% in *KCNH2*, and about 10% in *SCN5A*. Another 5-10% of mutations can cause multisystem syndromes, including long QT interval and the occurrence of malignant ventricular arrhythmias: Timothy syndrome (mutations in the *CACNA1C* gene), Andersen-Tawil syndrome (mutations in the *KCNJ2* gene), and Ankyrin B syndrome (mutations in the *ANK2* gene). The list of genes causing LQTS has now been expanded to 17 genes [3].

The clinical features are best studied in the first three genetic types of LQTS. Each type has a characteristic ST-T ECG pattern, a typical trigger for arrhythmias, and a different response to treatment with beta-blockers [4, 5].

The duration of the QTc interval is a significant risk factor for cardiac arrhythmias. According to the recommendations of the Heart Rhythm Society/European Heart Rhythm Association experts (HRS /EHRA, 2011), the values of the 99th percentile of the QTc duration should be considered abnormally prolonged depending on sex [6]. This corresponds to a QTc > 460 ms for male and female patients in the prepubertal phase, a QTc > 470 ms for males, and a QTc > 480 ms for females in the postpubertal phase. A QTc duration  $\geq 500$  ms is considered extremely unfavorable in both males and females and is associated with the risk of ventricular torsades de pointes (TdP) [6]. However, prolongation of the QTc interval on ECG does not always occur. For example, in a study by Silvia G. Priori et al. [7] QT interval values within normal limits were found in 36% of patients with LQT1, in 19% of patients with LQT2, and in 10% of patients with LQT3.

A QTc duration is modulated by LQTS genotype and sex. Female patients with LQTS2 and male patients with LQT3 with a QTc  $\geq 500$  ms are at greatest risk for arrhythmic events between birth and 40 years of age in the absence of therapy [8].

The association between genotype and trigger of arrhythmias is well known [9]. Thus, most events in patients with LQT1 occurred during physical exertion or stress, with swimming being a very specific trigger. Patients with LQT2 are extremely sensitive to stress and sudden auditory stimuli such as an unexpected loud noise or phone call. Most LQT3 events occurred when patients were sleeping or resting. The inability of the QTc interval to adapt to a higher heart rate leads to a high risk of post-depolarization, which can lead to the development of TdP [10]. This can be very dangerous for patients with LQT1, in whom a sudden increase in heart rate with impaired QTc shortening contributes to the R-on-T phenomenon and the occurrence of ventricular tachycardia (VT)/ventricular fibrillation (VF).

There is clear evidence of the efficacy of treatment with beta-blockers depending on the LQTS genotype. Thus, patients with LQT1 respond well to treatment with beta-blockers [1]. At the same time, noncompli-

ance with the treatment regimen is the most important cause of arrhythmic events occurring during treatment [11]. Compared with LQT1, patients with LQT2 are more likely to experience life-threatening events (6-7%) despite treatment with beta-blockers [12], yet they remain the first-line agent for this type of LQTS. Arrhythmias have been reported to occur more frequently (10-15%) in patients with LQT3 treated with beta-blockers [12], as bradycardia-dependent arrhythmias are more common in this type of LQTS. Therapy with a sodium channel blocker such as mexiletine shortens the QTc interval more effectively and improves prognosis in patients with LQT3 [1].

Overall, integration of LQTS genotype information with clinical features improves risk stratification for life-threatening arrhythmias and is an example of successful genotype-specific treatment of patients with LQTS. However, the relationship between genotype and phenotype in rare genetic variants of this disease is poorly understood.

The aim of this study was to evaluate clinical characteristics, including adverse events and outcomes, in patients with different genetic types of LQTS.

## MATERIALS AND METHODS

The study included 24 patients with a primary diagnosis of LQTS. The clinical and instrumental study included ECG registration in 12 leads, daily ECG monitoring, endocardial electrophysiological study when indicated, taking a genealogical history with ECG evaluation of all family members to identify cases of SCD in the family or the presence of a family form of the disease.

The following parameters were assessed by ECG: heart rate, corrected QT interval (QTc), morphology, and T-wave change. The QT interval and the preceding RR interval were measured manually in at least three consecutive cardiac cycles, and mean values were calculated. The end of the T wave was defined as the intersection of the tangent line drawn along the maximum slope of the descending T wave with the isoelectric T-P line; the U waves were not included if they were different from the T [13]. QTc was

**Table 1.**

### Genotypes and phenotypes of LQTS

Genotype	ECG pattern	Frequency of asymptomatic carriers	Risk factors for arrhythmic events	Beta-blocker therapy efficacy
LQT1	Prolongation of the QT interval with a high shaped T wave [4]; impaired adaptation of the QTc interval to tachycardia [5].	30% [6].	Physical activity or stress, swimming [7].	High
LQT2	Prolongation of the QT interval with a clear and typical notch on the T wave. Normal adaptation of the QTc interval to tachycardia.	19%.	Stress, auditory stimuli (sudden noise, phone call, sudden awakening) at rest; female.	Decreased*.
LQT3	Horizontally elongated ST segment with a biphasic T wave with a late onset.	10%.	During sleep or at rest; male.	Low*.

Note: \* - in comparison with LQT1 [8].

calculated according to the formula of Bazett. LQTS diagnostic criteria were evaluated using the modified scale of R.J.Schwartz et al. (2011) [14].

To exclude structural myocardial abnormalities, echocardiographic examination was performed according to current recommendations at PHILIPS IE -33.

Daily ECG was used to evaluate the mean heart rate per day, the value of the averaged, maximum, and minimum QT intervals, the presence/absence of an alternate T wave, and concomitant arrhythmias. The ventricular genesis of the arrhythmia was confirmed by daily ECG data, ECG registration during an attack, and in some cases by endocardial electrophysiological study.

A physical stress test on a Shiller ERGOLIM/LODE ergometer with ECG monitoring in 12 leads was performed when QTc values at rest were uncertain to clarify the diagnosis [14]. The test was performed according to the standard protocol until the heart rate reached 170 beats per minute or fatigue occurred. The ECG was recorded for 5 minutes after the end of exercise, and the QTc value was determined after 4 minutes of the recovery period.

The search for mutations in the coding sequences of genes associated with the development of channelopathies and other inherited cardiac arrhythmias was performed by high-throughput sequencing (NGS) on a MiSeq Gene Analyzer (Illumina). Samples were prepared using the TruSight Cardio Sequencing Kit (Illumina). Annotation of sequencing results was performed using ANNOVAR software [15]. Interpretation of pathogenicity of new and previously described genetic variants was performed according to the 2015 American Society for Medical Genetics recommendations [16]. Pathogenic (class V) and probably pathogenic (class IV) genetic variants were considered diagnostically significant. Variants of uncertain clinical significance (VUS, pathogenicity class III) in genes associated with inherited arrhythmias were included separately in the data analysis.

Statistical analysis. Two unrelated groups were compared for quantitative characteristics using the nonparametric Mann-Whitney U test. Differences at  $p < 0.05$  were considered statistically significant.

## RESULTS

In 18 of 24 (75%) patients, nucleotide sequence variants of pathogenicity classes III-V were identified according to ACMG2015 criteria in 4 genes directly associated with LQTS (*KCNQ1*; *KCNH2*; *CACNA1C*; *ANK2*). Of these, 14 variants were diagnostically significant mutations in classes IV and V: (*KCNQ1*, 8 mutations; *KCNH2*, 4 mutations; *CACNA1C*, 2 mutations). 4 Nucleotide substitutions in the *CACNA1C* and *ANK2* genes were variants of uncertain significance (VUS, grade III) and were found in combination with other rare variants in genes associated with inherited arrhythmias: 1) *ANK2* и *KCNE1*; 2) *ANK2* и *SNTA1*; 3) *CACNA1C* и *KCNH2*; 4) *CACNA1C*, *SCN3B* и *DSG2*. Six (25.0%) patients with a clinical diagnosis of LQTS lacked any genetic alterations (Table 1).

Table 1 shows the clinical characteristics of the three groups of patients: Group 1, with diagnostically significant pathogenicity mutations of class IV-V ( $n=14$ ), including

subgroups with different genetic types of LQTS (subgroup 1, LQT1; subgroup 2, LQT2; subgroup 3, LQT8); group 2, patients with multiple VUS in genes ( $n=4$ ) associated with arrhythmias; and group 3, genotype-negative patients ( $n=6$ ). The median age at diagnosis was 26.4 [12;43] years in the 14 patients with genetically confirmed LQTS, compared with 37.5 [33;45] years in patients with two VUS ( $p=0.04$ , compared with group 1) and 35.5 [22;46] years in patients without mutations ( $p=0.04$ , compared with group 1). Comparative analysis of sex in these groups showed that in LQT1, LQT2 and LQT8 patients with a single pathogenic mutation, the female sex predominated (85.7%), while in the group with multiple VUS, three out of four were male, and in the genotype-negative patients the sex ratio was 1:1. QTc interval duration exceeded 500 ms in 50% of patients with LQT1 and multiple VUS and in 100% of patients with LQT2. A QTc interval duration  $\leq 460$  ms was observed in 12.4% of patients with LQT1, in 25% of patients with multiple VUS, and in 17% of genotype-negative patients. Syncopal episodes were observed in 87.5% (21 of 24) of patients, and 29.2% (7 of 24) had a family history of SCD. Information about SCD with successful resuscitation or cardiac arrest with implantation of a cardioverter-defibrillator (ICD) was documented in 13 of 24 (54.2%) patients observed, and in 2 (8.3%) cases, the ICD was used for primary prevention of SCD. In LQT2, there was a family history of SCD in 50.0% of cases, which was 4 times higher than in LQT1 (12.4%) and twice higher than in the multiple VUS group (25%). It is noteworthy that in the group of genotype-negative patients, the incidence of SCD in the family history was also quite high, reaching 50.0%. In the same group, all subjects developed SCD followed by successful resuscitation and ICD implantation for secondary prevention of SCD. Life-threatening arrhythmic events (SCD with successful resuscitation, sustained VT, cardiac arrest) occurred 3 times less frequently in subjects with LQT1 compared with other groups.

When comparing the clinical manifestations of the disease in the subgroup of patients with LQT1, only 2 (25%) patients (no. 1 and 7) with pathogenic mutations in exons 5 (p.Val127Met) and 6 (p.Gly179Arg) of the *KCNQ1* gene showed severe disease (Table 2). In both patients, the disease manifested with cardiac arrest due to the development of VT/VF during physical activity. The patients were successfully resuscitated and subsequently received an ICD. Both patients had a history of syncope at 24 and 12 years of age; patient 1 had a family history of SCD in a close relative. Resting ECG showed QTc prolongation up to 520 ms and 620 ms, respectively. Three patients (Nos. 3, 4, and 5) with probable pathogenic mutations (class IV according to ACMG2015 criteria) in the *KCNQ1* gene had a more favorable disease course, without development of hemodynamically significant ventricular arrhythmias with mild prolongation of the QTc interval (450 ms; 465 ms and 480 ms, respectively) according to resting ECG. The diagnosis of LQTS was made on the basis of the results of the exercise test (QTc prolongation at the peak of exercise and in the 4th minute of the recovery phase was recorded up to 485 ms; 500 ms and 516 ms, respectively) and the LQTS probability score according to the score of R.J.Schwartz et al, which was 4.5; 5 and 5 total scores, respectively. Patient

Table 2.

## Phenotypic manifestations of genotype in patients with LQTS, n (%)

Patient groups	Sex m/f	Age at diagnosis	SCD history	Family form	Syncope	QTc <460 ms	QTc 460-499 ms	QTc ≥500 ms	SCD / CA	ICD	Associated diseases
Group 1. DS IV-V classes	2/6	23.5[12; 35]	1 (12,4)	4 (50)	6 (75)	1 (12,4)	3 (37,5)	4 (50)	2 (25)	2 (25)	LQTS1
	0/4	34.0[24; 43]	2 (50,0)	4 (100)	4 (100)	-	-	4 (100)	1 (25)	3 (75)	LQTS2
	0/2	22.5[14; 31]	-	1 (50)	2 (100)	-	1 (50)	1 (50)	1 (50)	1 (50)	LQTS8
Group 2. Few VUS, n=4	3/1	37.5[33; 45]	1 (2,5)	1 (2,5)	3 (75)	1 (2,5)	2 (50)	1 (2,5)	3 (75)	3 (75)	LQTS
Group 3. Genetically negative, n=6	3/3	35.5[22; 46]	3 (50)	3 (50)	6 (100)	1 (17)	5 (83)	-	6 (100)	6 (100)	-

Note: hereinafter SCD - sudden cardiac death; CA - cardiac arrest; ICD - implantable cardioverter-defibrillator; DS - diagnostically significant.

Table 3.

## Clinical and genetic characteristics of patients with LQTS (continued)

	N	Patient code	AM	Sex	SCD history	Family form	Syncope	QTc <sub>max</sub> ms <sup>#</sup>	Schwartz score	Other arrhythmias	Events / outcomes	Substitution in the DNA (substitution in the protein)	Pathoge- nicity
LQT1 (мутации в гене KCNQ1)	1.	566	24	f	+	+	+	520	6.0	SB, SAB	PM→ICD	c.379G>A (p.Val127Met)	V (PS)
	2.	656	35	f	-	+	-	500	5.5	SVT	-	c.592G>A (p.Gly198Arg)	V (PS)
	3.	640	18	f	-	-	-	480	4.5	SVT	-	c.1621G>A (p.Val54Ile)	IV (PM)
	4.	639	25	f	-	+	+	450	5.0	SVT	-	c.1555C>T (p.Arg519Cys)	IV (PM)
	5.	635	19	m	-	-	+	465	5.0	-	-	c.1999G>A (p.Val667Met)	IV (PM)
	6.	713	21	m	-	-	+	630	5.5	SVT	-	c.641C>T p.Ala214Val	V (PS)
LQT2 (мутации в гене KCNH2)	7.	609	12	f	-	+	+	620	6.0	SVT	VT/VF. CPR, ICD	c.535G>C (p.Gly179Arg)	V (PS)
	8.	666	34	f	-	-	+	440	4.0	SVT	-	c.1233delA (p.Lys411Asnfs*8)	IV* (PV)
	9.	564	24	f	-	+	+	594	5.5	SVT	VT/ICD	c.371T>A (p.Met124Lys)	V* (PS)
	10.	720	43	f	+	+	+	513	6.0	PVC, SVT	ICD	c.2775dupG (p.Pro926AlafsX14)	V (PV)
LQT8 (мутации в гене CACNA1C)	11	589	35	f	-	+	+	505	4.5	-	-	c.2131A>G (p.Ile711Val)	IV (PS)
	12.	655	34	f	+	+	+	623	5.5	SVT	ICD	c.1424A>G (p.Tyr475Cys)	V (PS)
	13.	628	14	f	-	-	+	440	4.0	PVC, SVT. SVT	-	c.2053C>T (p.Arg685Trp)	IV* (PM)
	14.	610	31	f	-	+	+	580	7.5	SVT	VT/ICD	c.2573G>A (p.Arg858His)	V (PS)

Table 3.

## Clinical and genetic characteristics of patients with LQTS (continued)

	N	Patient code	AM	Sex	SCD history	Family form	Syncope	QTc <sub>max</sub> <sup>#</sup> ms	Schwartz score	Other arrhythmias	Events / outcomes	Substitution in the DNA (substitution in the protein)	Pathogenicity
Few VUS	15.	613	39	f	+	+	+	500	6.5	SVT, SAB	VF / CPR, ICD	CACNA1C: c.1186G>A (p.Val396Ile) KCNH2: c.49A>T (p.Arg17Trp)	III III*
	16.	607	33	m	-	-	-	460	4.0	PVC, SVT	VT, RFA	CACNA1C: c.4942G>A (p.Ala1648Thr) SCN3B: c.260C>G (p.Pro87Arg) DSG2: c.1442T>C (p.Ile481Thr)	III III III
	17.	586	33	m	-	-	+	440	5.0	SVT	VT / AF, ICD	ANK2: c.1397C>T (p.Thr466Met) KCNE1: c.253G>A (p.Asp85Asn)	III III
	18.	543	45	m	-	-	+	375	5.5	SVT	VT / VF, ICD, ES	ANK2: c.1397C>T (p.Thr466Met) SNTA1: c.787G>T (p.Ala263Ser)	III III
Genetically negative	19.	574	30	f	+	+	+	500	6.5	PVC, VT	VT, ICD	not found	
	20.	597	22	m	+	+	+	465	6.0	SVT	VT, ICD, ES	not found	
	21.	644	46	f	-	-	+	460	6.5	SVT	VT / VF, CPR, ICD	not found	
	22.	647	46	m	-	-	+	477	5.5	SVT	VT/VF, CPR, ICD	not found	
	23.	629	40	f	+	+	+	478	6.0	SVT	VT, CPR, ICD	not found	
	24.	631	29	m	-	-	+	456	5.5	SVT	VF, CPR, ICD	not found	

Note: AM - age at manifestation; # - on ECG; SB - sinus bradycardia; SAB - sinoatrial block; VT - ventricular tachycardia; NVT - non-sustained ventricular tachycardia; VF - ventricular fibrillation; PVC - premature ventricular complex; SVT - supraventricular tachycardia; ES - electrical storm; CPR - cardiopulmonary resuscitation; V - pathogenic mutation; IV - probably pathogenic mutation, III - variant of uncertain significance (VUS); DNA - deoxyribonucleic acid; PM - pacemaker; ICD - implanted cardioverter-defibrillator; RFA - radiofrequency ablation; AF - atrial fibrillation; \* - new mutations.

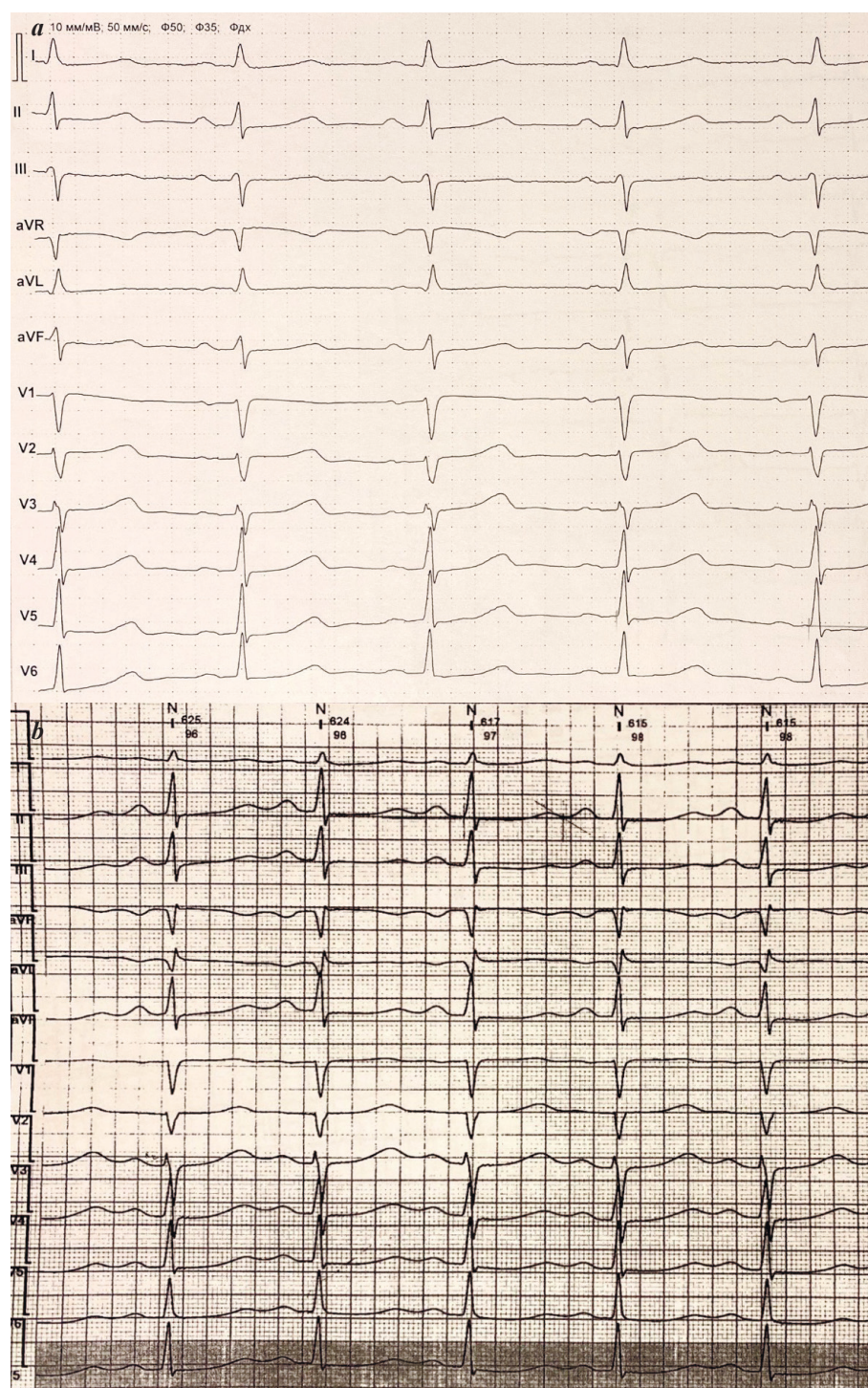
no. 8 was found to have a new deletion with a frameshift and premature stop c.1233delA/p.Lys411Asnfs\*8 in the *KCNQ1* gene. Syncope occurred in the patient at the age of 34 years. On a resting ECG (Fig. 1a), the duration of the QTc interval did not exceed 440 ms. During exercise tests at the peak of exercise and in the 4th minute of the recovery phase, QTc prolongation was recorded up to 475 ms and up to 535 ms, respectively (Fig. 1b). The LQTS probability score according to the score of R.J.Schwartz et al. was 4. Based on the above data, a diagnosis of LQTS was made. The patient was prescribed therapy with a beta-blocker (propranolol 40 mg ½ b.i.d) and syncope did not recur during treatment.

All patients with LQT2 had QTc duration  $\geq 500$  ms with syncopal states. Two of them had a family history of SCD in close relatives. In patients no. 9 and 10 with class V mutations in the *KCNH2* gene, the disease manifested with cardiac arrest due to the development of VT / VF with successful resuscitation and subsequent ICD implantation. Patient No. 12 experienced syncope at the age of 34 years against a background of emotional stress triggered by her identical twin sister's SCD during pregnancy. The ECG showed prolongation of the QTc interval up to 623 ms. The LQTS probability score according to score of R.J.Schwartz et al. was 5.5. Considering the SCD in a twin sister of the same age and the prolongation of the QTc interval up to 623 ms, it was decided to implant an ICD in the patient. Genetic testing revealed a pathogenic p.Tyr475Cys (rs199472907) mutation in the *KCNH2* gene. This mutation is represented as VUS in the ClinVar database and is registered as diagnostically significant in other databases (HGMD, LOVD). The results of segregation analysis confirmed the pathogenic significance of this variant: the mutation was found in a sister with SCD and the daughter of a deceased woman with clinical manifestations of LQTS.

Class IV-V mutations in the *CACNA1C* gene were found in two patients (no. 13 and 14). In patient no. 14 with the pathogenic c.2573G > A (p.Arg858His) mutation in exon 8 of the *CACNA1C* gene, a severe

clinical picture with recurrent syncope and cardiac arrest requiring resuscitation and subsequent ICD implantation was observed (Table 2). The ECG showed prolongation of the QTc interval up to 550 ms and a negative T wave with a broad base in leads V2-V5 (Fig. 2). Over the course of 8 years, the patient developed spindle-shaped VT (TdP) three times, which was stopped by an ICD; the ICD was replaced twice, and she currently continues to be treated with a beta-blocker (nadolol 80 mg q.d.).

In patient no. 13 with a single, previously undescribed c.2053C > T, (p.Arg685Trp) variant pathogenic by predictors in silico and localized in the region of other pathogenic



**Fig. 1.** Resting ECG (a) of patient #8, female 34 years old, recorded QTc 440 ms, ECG during exercise test (b) at the 4th minute of the recovery period recorded QTc 531 ms.

mutations in the *CACNA1C* gene, the disease manifested at age 21 years with a presyncopal state and episodes of unstable monomorphic VT. No changes in T-wave morphology and prolongation of the QTc interval (QTc 420–440 ms) were registered in the ECG series. During cycle ergometry, prolongation of the QTc interval was registered up to 495 ms at the peak of exercise and up to 485 ms in the recovery phase. No sustained VT paroxysms were elicited during diagnostic endocardial electrophysiology study administration. Considering the absence of syncope, paroxysms, or stable VT, it was decided to treat the patient conservatively. Beta-blocker therapy (metoprolol 100 mg/day) was prescribed. No syncopal states were observed during the follow-up examination, and no QTc prolongation was registered in the ECG.

None of the patients with the observed genetic variants, including the pathogenic mutation and the new variant, showed syndactyly, cognitive impairment, facial dysmorphism, or other noncardiac features suggestive of Timothy syndrome.

The combination of multiple VUS nucleotide variants in different genes associated with cardiac arrhythmias was found in 4 (17%) patients, and in 2 subjects one of the replacements was in the *CACNA1C* gene and in 2 subjects - in the *ANK2* gene. All patients with multiple replacements had a severe disease course (Table 2). The most severe clinical picture with prolongation of the QTc interval up to 500 ms, syncopal episodes, development of VT/VF with successful resuscitation and ICD implantation was observed in a patient with VUS in exon 19 of the *CACNA1C* gene in combination with a new c.49A > T (p.Arg17Trp) substitution in the *KCNH2* gene. A patient with VUS in exon 40 of the *CACNA1C* gene in combination with rare substitutions in the *SCN3B* and *DSG2* genes had frequent episodes of unstable VT and malignant ventricular extrasystoles. Radiofrequency ablation of the ectopic foci was performed,

and beta-blocker treatment (metoprolol 100 mg/day) was prescribed (Table 2).

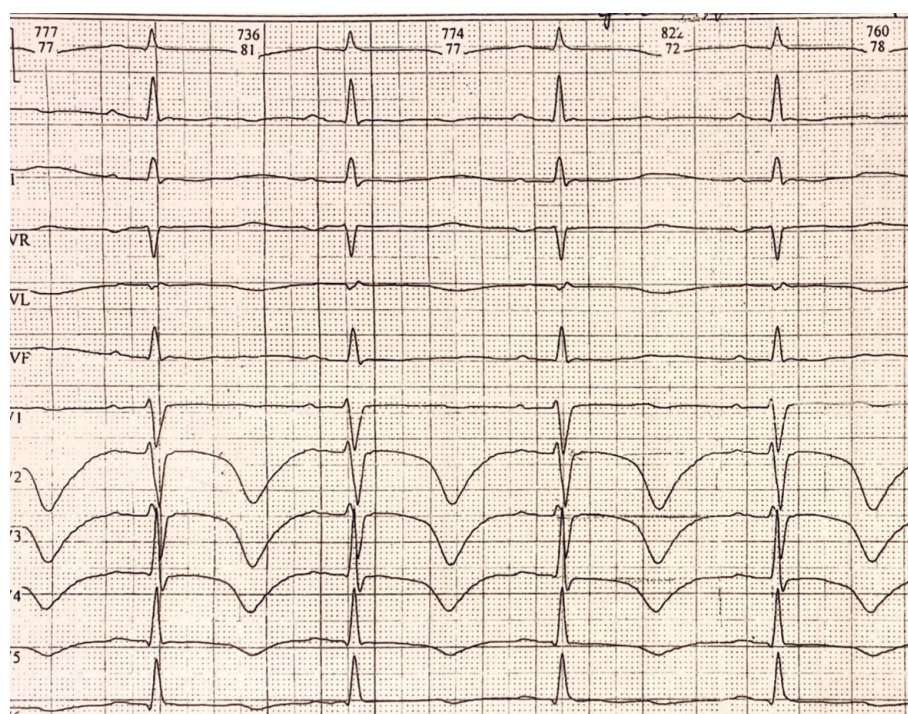
In 2 unrelated male subjects, VUS were identified in exons 15 and 38 of the *ANK2* gene, which encodes an ankyrin family adaptor protein previously associated with the development of LQTS type 4, in combination with additional mutations in the *KCNQ1* and *SNTA1* genes [17]. Both patients had no poor family history and QTc interval prolongation in the ECG series (median QTc of 407.5 [375;440] ms). The LQTS probability score according to R.J.Schwartz et al. was 5.0 and 5.5. Patients had recurrent syncopal states, development of VT / VF requiring resuscitative measures and ICD implantation. One 43-year-old proband with substitutions in the *ANK2* and *SNTA1* genes developed polymorphic VT/VF during the 8-year follow-up period, and ICD replacement was performed three times (Table 2). No episodes of syncopal states and repeated ICD storms leading to resuscitation occurred during the last years.

We found that patients with multiple VUS in genes associated with arrhythmias were at high risk for life-threatening arrhythmic events despite a slight prolongation of the QTc interval (460 [440; 460]) ms, which falls in the “gray zone,” as were patients with mutations in the *KCNH2* gene (LQT2). Thus, during the follow-up period, 5 of 6 patients (80.0%) with multiple VUS and 3 of 4 (75.0%) patients with LQT2 had VT/VF with ICD implantation, whereas only 25.0% of patients with LQT1 had life-threatening arrhythmic events, as mentioned above.

Unexpectedly severe disease courses with development of VT/VF and ICD implantation were recorded in all 6 genotype-negative patients, despite borderline QTc values (median 471 [462.5;477.5]) ms. The LQTS probability score on the score of R.J.Schwartz et al. was 5.5 to 6.0 total scores. Moreover, half of them had a family history of SCD in their relatives, indicating an apparent heritability of the disease (Table 2). It is possible that the absence of genetic abnormalities in these patients was due to the localization of diagnostically significant mutations in the intron region or in other genes that were not included in the study panel, or that they were extensive deletions that are difficult to detect with NGS. The distinguishing feature of this group, as well as of patients with multiple VUS, was the absence of sex differences, as mentioned previously, whereas the clinical manifestations of LQT1 and LQT2 were mainly observed in female patients.

## DISCUSSION

In the present study, the clinical diagnosis of LQTS was confirmed by genetic testing in 14 of 24 (58.0%) patients in whom mutations in 4 genes



**Fig. 2. ECG of patient #14 after cardiac arrest: QTc 550 ms and wide negative T waves in V2–V5 leads were recorded.**

directly associated with LQTS (*KCNQ1*, *KCNH2*, *CACNA1C*) were identified. Six patients with a preliminary diagnosis of LQTS had no genetic alterations. This study presents the results of sequencing 15 genes directly associated with LQTS and genes responsible for the development of other hereditary, life-threatening cardiac arrhythmias. Most previous studies have been limited to the investigation of mutations in 3 genes (*KCNQ1*, *KCNH2*, *SCN5A*) [8]. For each of these genes, genetic evidence is based on linkage analysis in more than one family and is supported by a wealth of genetic and experimental data collected over decades of research and clinical observation [8, 9]. Mutations in both *KCNQ1* and *KCNH2* genes were detected in 50.0% of Belarusian patients in the studied cohort, while no mutations were detected in the *SCN5A* gene. Pathogenic mutations in the *CACNA1C* gene without other noncardiac manifestations indicative of Timothy syndrome were found in 2 patients.

Four of 24 (17%) patients had multiple VUS nucleotide variants, one of which was in the *CACNA1C* or *ANK2* genes. Additional replacements in these subjects were in genes associated with LQTS or other inherited arrhythmias. Syncopal states and prolongation of the QTc interval > 480 ms on ECG series were recorded in the clinical picture of patients with a history of multiple VUS (except for two patients with a mutation in the *ANK2* gene, in whom the QTc did not exceed 440 ms). Three (75%) patients had a documented SCD with successful resuscitation and ICD implantation, and one of them had a family history of SCD.

The presence of multiple mutations in patients with monogenic myocardial disease is increasingly discussed in the literature. A large study by D. Mullally et al [18] in a large cohort of 403 patients with LQTS also identified patients with multiple mutations (14.1%), in whom the phenotypic manifestations and risk of life-threatening events were assessed compared with the group of patients with a single mutation in one of the genes associated with LQTS. Patients with multiple mutations had a longer QTc interval compared to patients with a single mutation (506±72 ms vs 480±56 ms,  $p=0.003$ ) and had a higher rate of life-threatening events during follow-up (23% vs 11%,  $p < 0.001$ ). Multivariate analysis showed that patients with multiple mutations had a 2.3-fold ( $p=0.015$ ) higher risk of life-threatening events than patients with a single mutation. The results of our study indicate that the combination of multiple VUS, similar to the effect of multiple mutations, may have a cumulative effect that significantly affects the clinical phenotype. However, this observation requires further

research, including clustering of similar cases and segregation analysis.

A comparative analysis between 3 groups (group 1-diagnostically significant mutations of pathogenicity classes IV-V ( $n=14$ ) with different genetic type of LQTS (LQT1; LQT2; LQT8); group 2-patients with multiple VUS; group 3-genotype-negative patients) revealed a difference in adverse outcomes and events between patients depending on the genetic type of LQTS. The lowest incidence of SCD was observed in patients with LQT1, although this group did not differ from other LQTS in terms of frequency of syncope, and the age of manifestation was earliest in all groups. A severe form of the disease with pronounced clinical manifestations, episodes of clinical death followed by resuscitation, and ICD implantation were observed in the group of subjects with LQT2, as well as in patients with multiple VUS.

It should be noted that all genotype-negative subjects also had a severe clinical picture, including syncopal states and SCD followed by resuscitation and ICD implantation. However, the duration of the QTc interval (471 [462.5;477.5] ms) was shorter compared with patients with LQT1 (513.1 [440;630] ms) and LQT2 (553.5 [509;608.5] ms). In genotype-negative patients, disease manifested before beta-blocker therapy, whereas in genotype-positive patients, life-threatening events occurred during beta-blocker treatment, which should be considered when stratifying the risk of adverse events. ICD implantation was required in 14 of 24 (58.3%) patients, including all six genotype-negative patients.

## CONCLUSION

The present study investigated the spectrum of clinical manifestations in patients with different genetic types of LQTS (LQT1; LQT2; patients with multiple VUS). Comparative analysis between these groups showed that patients with LQT1 syndrome were significantly less likely to have life-threatening arrhythmias, SCD, and ICD implantation than other LQTS patients, despite early manifestation of the disease and the presence of syncopal states. The most severe form of the disease with pronounced clinical manifestations, clinical deaths followed by resuscitation, and ICD implantation was observed both in the group of subjects with LQT2 and in patients with multiple nucleotide VUS, one of which was in the *CACNA1C* or *ANK2* genes.

The results confirm the importance of genetic testing of patients with LQTS for disease prognosis and stratification of SCD risk.

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