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# Spectrum of mutations and their phenotypic manifestations in children and adults with long QT syndrome

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**Aim.** To determine the spectrum of mutations in the genes responsible for the long QT syndrome (LQTS) and study their phenotypic manifestations in patients with LQTS in different age groups.

**Material and methods.** The study included 35 unrelated probands with a clinical diagnosis of LQTS: 23 adults (8 men) and 12 children (9 boys). There were following clinical features: syncope — 54%, positive family history for SCD — 29%, implanted cardioverter defibrillator (ICD) — 46%. All participants underwent 12-lead electrocardiography (ECG), 24-hour Holter monitoring, genealogical analysis, echocardiography and cardiac MRI. The genetic study was performed by next-generation sequencing (NGS) using the MiSeq system (Illumina). The quantitative comparison of two unrelated groups was carried out using the nonparametric Mann-Whitney U-test. The differences were considered significant at $p<0,05$.

**Results.** In the examined group of 35 probands, 23 genetic variants of pathogenicity class IV and V (hereinafter referred to as) were identified. The molecular genetic variant of the disease was verified in 66% of probands. At the same time, the detection of mutations in the group with early manifestation (children) was significantly higher: 83% (10 out of 12 children) vs 57 % in adults (13 out of 23). Rare genetic variants of uncertain significance (VUS, class III pathogenicity) were detected in 4 probands (11%).

In the groups of children and adults with LQT1, LQT2 and LQT3, the sex distribution deviated from the 1:1 ratio. Among children, two-thirds were boys, among adults — the same proportion was represented by women. Disease manifestation time, QTc duration and adverse events risk depended on the genetic type of LQTS, intragenic localization of mutations and sex. In children, all 4 missense mutations in the *KCNQ1* gene were located in transmembrane domain, and in adults, 4 mutations were in the transmembrane domain and three — in the C-terminal domain of the protein. LQT1 in boys was characterized by early manifestation, while QTc did not exceed 500 ms and there were no adverse outcomes. Two women out of 7 adults with LQT1 with mutations in the transmembrane domain had na ICD (QTc >520 ms). All patients with LQT2 (4 children, 4 adults) had QTc >500 ms. At the same time, 2 children and 3 women had an ICD. LQT3 was diagnosed only in the children subgroup (2 boys, with QTc of 510 ms and QTc of 610 ms); one of them died suddenly despite beta-blocker therapy. Four adult patients, carriers of class III pathogenicity variants, had QTc <500 ms and delayed disease manifestation (after 30 years). Three of them had episodes of clinical death with subsequent resuscitation and implantation of cardioverter defibrillator.

**Conclusion.** The average diagnostic efficiency of mutation identification using NGS in patients with clinically manifest LQTS was 66%. At the same time, mutations were more common in the children's group. In genotype-positive probands, the risk of adverse outcomes correlated with sex, age and the genetic variant of disease. The greatest number of adverse outcomes was observed in carriers of mutations in both *KCNH2* (LQT2) and *SCN5A* (LQT3) genes. Variants with unknown clinical significance were identified in 4 probands (11%), which potentially allowed to confirm the diagnosis after functional tests.

**Keywords:** long QT interval syndrome, spectrum of mutations, *KCNQ1* gene, *KCNH2* gene, *SCN5A* gene, *CACNA1C* gene, *ANK2* gene, risk stratification.

**Relationships and Activities:** none.

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Long QT syndrome (LQTS) is a genetically determined disease characterized by an increase in QT interval on an electrocardiogram (ECG), a high risk of developing life-threatening ventricular tachyarrhythmias, syncopal conditions and sudden cardiac death (SCD) at a young age. LQTS is mainly caused by functional changes in potassium, sodium and calcium ion channels, which are most often caused by defects in the genes encoding the pore-forming α-subunits and regulatory β-subunits of ion channels, as well as some other proteins. The prevalence of clinically pronounced LQTS is at least 1 per 2 thousand people [1].

Modern approaches to the diagnosis of LQTS, risk assessment of SCD and choice of treatment tactics in patients with such pathology are largely based on information on molecular genetic nature of the disease. Determination of the genetic cause allows to minimize genotype-specific triggers of life-threatening arrhythmias [2], to exclude drugs that prolong the QT interval, and to individualize treatment and prescribe optimal antiarrhythmic drugs for a particular type of LQTS [1]. The clinical significance of genetic testing in patients with LQTS is also confirmed by the fact that asymptomatic mutation carriers without preventive measures and appropriate therapy have a significantly increased risk of serious cardiac events by the age of 40 [3]. To date, algorithms for risk stratification and management of patients with different genetic variants of LQTS have been proposed, which significantly help in clinical practice [2-5]. At the same time, it has been shown that the prognosis of the disease depends on the sex of the patient and changes with age: the most malignant course of the syndrome and increased risk of SCD is more often observed in adult women, and among children — in boys [6, 7]. However, in our opinion, little attention has been paid to the question of modulating effect of sexual and age characteristics on phenotypic manifestation of specific mutations, depending on their intragenic localization. In this regard, the goal of our study was to determine the spectrum and prevalence of mutations, as well as to study their phenotypic manifestations in patients with LQTS of different age groups.

## Material and methods

The study included 35 unrelated probands diagnosed with LQTS based on the ESC Guidelines 2015 [8] and the modified Schwartz PJ, et al. (2011) [9]. The adult group included 23 patients over 18 years of age (median 31 [21; 35]; 8 men) seen at the National Scientific and Practical Center "Cardiology". The group of children is represented by 12 probands (median age 7 [5; 9] years, 9 boys) who were treated at the National Scientific and Practical Center "Pediatric Surgery". Prior to enrollment, written informed consent was obtained from all adult study participants and parents/guardians of probands from the children's group. The study was carried out in accordance with the Good Clinical Practice standards and the principles of the Helsinki Declaration.

The clinical and instrumental study included ECG registration in 12 leads, daily ECG monitoring, genealogical history collection with ECG assessment of all family members and detection of SCD cases. To exclude myocardial structural abnormalities, echocardiographic examination was carried out according to the current recommendations.

The search for mutations in the coding sequences of genes connected to the development of channelopathy and other hereditary heart rhythm disorders was carried out by high-throughput sequencing (NGS) on the genetic analyzer MiSeq (Illumina). Sample preparation of samples was carried out using a TruSight™ Cardio Sequencing Panel (Illumina) set, which includes 174 genes associated with diseases of the cardiovascular system. Verification of the identified mutations was performed by direct Sanger sequencing. The sequencing results were annotated using the ANNOVAR software [10]. The pathogenicity of new and previously described genetic variants was assessed according to the recommendations of the American College of Medical Genetics (ACMG2015) [11]. Pathogenic (Class V) and probably pathogenic (class IV) genetic variants were considered to be diagnostically significant. Variants with uncertain clinical significance (VUS, class III) in genes associated with inherited rhythm disorders were also included in the data analysis [12].

**Table 1**

## Genetic variants of pathogenicity class IV and V identified in Belarusian patients with LQTS

| Code | Gender | Age[a], years | Gene (exon) | Replacement in DNA, rs | Replacement in protein | Pathogenicity class | Critical events |
|---|---|---|---|---|---|---|---|
| | Children's patients | | | | | | |
| 682 | M | 7 | *KCNQ1* | c.IVS96+1G>A rs762814879 | Мутация сплайсинга | V | SCD in family |
| 2H | M | 6 | *KCNQ1* (5) | c.394C>T rs1994722719 | p.Arg132Cys | V | – |
| 602 | F | 3 | *KCNQ1* (7) | c.641C>T rs12720459 | p.Ala214Val | V | Syncope in women in family |
| 5H | M | 9 | *KCNQ1* (9) | c.1233delA | p.Lys411Asnfs*8 | V* | – |
| 4H | M | 10 | *KCNH2* (7) | c.1496T>G rs794728370 | p.Leu499Arg | V | SCD in family |
| 1H | M | 11 | *KCNH2* (7) | c.1682C>T rs121912504 | p.Ala561Val | V | – |
| 6H | M | 7 | *KCNH2* (7) | c.1868C>T rs199472950 | p.Thr623Ile | V** | VT/ICD |
| 684 | F | 12 | *KCNH2* (7) | c.1928G>A | p.Cys643Tyr | V* | Syncope, SCD in family, resuscitation, ICD |
| 3H | M | 6 | *SCN5A* (10) | c.1231G>A rs72549410 | p.Val411Met | V** | – |
| 722 | M | 12 | *SCN5A* (28) | c.4931G>A rs28937316 | p.Arg1644His | V | – |
| | Adult patients | | | | | | |
| 566 | F | 24 | *KCNQ1* (5) | c.379G>A rs120074179 | p.Val127Met | V | Syncope, SCD in family, VT/ICD |
| 609 | F | 12 | *KCNQ1* (6) | c.535G>C rs120074181 | p.Gly179Arg | V | VT/VF, resuscitation, ICD |
| 656 | F | 35 | *KCNQ1* (7) | c.592G>A rs199472756 | p.Gly198Arg | V | – |
| 713 | M | 21 | *KCNQ1* (7) | c.641C>T rs12720459 | p.Ala214Val | V | Syncope |
| 639 | F | 25 | *KCNQ1* (12) | c.1555C>T rs199472787 | p.Arg519Cys | IV | Syncope |
| 640 | F | 18 | *KCNQ1* (13) | c.1621G>A rs199472796 | p.Val541Ile | IV | – |
| 635 | M | 19 | *KCNQ1* (16) | c.1999G>A rs776119582 | p.Val667Met | IV | – |
| 564 | F | 24 | *KCNH2* (3) | c.371T>A | p.Met124Lys | V* | Syncope, VT/ICD |
| 655 | F | 34 | *KCNH2* (6) | c.1424A>G rs199472907 | p.Tyr475Cys | V | Syncope, SCD in family, ICD |
| 589 | F | 35 | *KCNH2* (8) | c.2131A>G rs199473532 | p.Ile711Val | IV | Syncope |
| 720 | F | 43 | *KCNH2* (12) | c.2775dupG rs794728455 | p.Pro926AlafsX14 | V | Syncope, SCD in family, ICD |
| 628 | F | 14 | *CACNA1C* (14) | c.2053C>T | p.Arg685Trp | IV* | – |
| 610 | F | 31 | *CACNA1C* (19) | c.2573G>A rs786205753 | p.Arg858His | V | VT/ICD |

**Note:** [a] — age of manifestation; * — new, previously undescribed mutation, ** — *de novo* mutation; m — male, f — female; IV and V — pathogenicity class of genetic variant according to ACMG2015 criteria.

**Abbreviations:** SCD — sudden cardiac death, DNA — deoxyribonucleic acid, ICD — implantable cardioverter-defibrillator, VT — ventricular tachycardia, VF — ventricular fibrillation.

**Table 2**

**Variants with unspecified clinical significance (class III pathogenicity according to ACMG2015 criteria) identified in group of adult patients**

| Code | Gender | Age[a], years | Gene (exon) | Replacement in DNA, rs | Replacement in protein | Additional option (Class III) | Critical events |
|---|---|---|---|---|---|---|---|
| 613 | F | 39 | *CACNA1C* (8) | c.1186G>A rs762756712 | p.Val396Ile | *KCNH2*: c.49A>T (p.Arg17Trp)* | VT/VF/ICD |
| 607 | M | 33 | *CACNA1C* (40) | c.4942G>A rs370432385 | p.Ala1648Thr | *SCN3B*: c.260C>G (p.Pro87Arg), rs371050389 *DSG2*: c.1442T>C (p.Ile481Thr), rs371854289 | VT/VE/RFA |
| 543 | M | 45 | *ANK2* (14) | c.1397C>T rs786205722 | p.Thr466Met | *SNTA1*: c.787G>T (p.Ala263Ser), rs15057653 | VT/AF, ICD |
| 586 | M | 33 | *ANK2* (38) | c. 9161C>G rs139007578 | p.Ala3054Gly | *KCNE1*: c.253G>A (p.Asp85Asn), rs1805128 | VT/VF, ICD, storms |

**Note:** [a] — age of manifestation; * — new, previously undescribed mutation; m — male, f — female.

**Abbreviations:** DNA — deoxyribonucleic acid, ICD — implantable cardioverter-defibrillator, VT — ventricular tachycardia, VE — ventricular extrasystole, RFA — radio frequency ablation, VF — ventricular fibrillation, AF — atrial fibrillation.

Two unrelated groups were compared for quantitative characteristics using the nonparametric Mann-Whitney U-criterion. The differences were considered statistically significant at $p<0,05$.

## Results and discussion

The paper presents a comparative analysis of the spectrum of mutations between groups of adults and children diagnosed with LQTS, as well as the study of some clinical indicators, including adverse events and outcomes, in patients with different genetic types of LQTS, taking into account gender and age characteristics. The clinical characteristics of the general group were as follows: syncopal conditions were registered in 54% of patients, 29% had a family history of SCD, 46% had an implanted cardioverter defibrillator (ICD) (in 88% of cases — due to SCD or cardiac arrest with successful resuscitation, in 12% — for the purpose of primary prevention of SCD).

During genotyping of 35 probands with a clinical diagnosis of LQTS 23 genetic variant classes IV and V of pathogenicity (next mutation) patients were identified in 4 genes in 23 (66%) patients: *KCNQ1* (LQT1) — 11 mutations, *KCNH2* (LQT2) — 8 mutations, *SCN5A* (LQT3) — 2 mutations, *CACNA1C* (LQT8) — 2 mutations (Table 1). In 10 (83%) of 12 children, mutations were in 3 major genes: in *KCNQ1* — 4 mutations, in *KCNH2* — 4 mutations, in *SCN5A* — 2 mutations. In the adult group, 13 mutations were detected in 3 genes: *KCNQ1* — 7 mutations, *KCNH2* — 4 mutations, *CACNA1C* — 2 mutations (Table 1).

In addition, 4 rare variants (VUS) with a frequency of 0,00001 were identified in the examined group of adult patients, of which 2 were in the gene *CACNA1C* encoding the α-subunit of the calcium channel, and 2 were in the gene *ANK2* responsible for ankyrin synthesis (Table 2).

In these patients, additional genetic variants of VUS were also identified in genes associated with rhythm disturbance and encoding subunits of potassium (*KCNH2*, *KCNE1*) and sodium (*SCN3B*) ion channels, and some other proteins (*SNTA1*, *DSG2*) (Table 2).

Among the examined probands, the proportion of genotype-positive patients with mutations of classes IV and V was 66% (23 out of 35). Comparative analysis of this index between groups of children (10 of 12) and adults (13 of 23) showed that the detection of diagnostically relevant mutations in the group with early manifestation (children) was higher (83%) than in adults (57%), and for major genes *KCNQ1*, *KCNH2*, *SCN5A*, the difference was statistically significant (83% vs. 48%, $p<0,05$). A combination of several VUS in genes associated with LQTS and other hereditary arrhythmogenic diseases was detected in 4 patients (11%).

The distribution of 27 genetic variants of classes III-V (Tables 1, 2) identified in the general group of probands is shown in Figure 1. In the gene *KCNQ1*, (LQT1) was 11 (41%) pathogenic mutations in the gene *KCNH2* (LQT2) — 8 (30%), in the gene *SCN5A* (LQT3) — 2 (7%). 4 (15%) nucleotide variants (VUS class III pathogenicity), 2 of which were localized in the gene *CACNA1C* and 2 — in the gene *ANK2,* combined with VUS in other genes.

A comparative analysis of mutation distribution depending on the age of patients showed significant differences between groups of children and adults. In children, all 10 identified mutations were con-

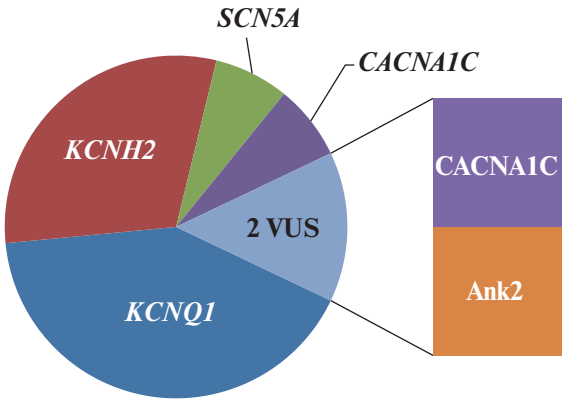


**Figure 1.** Distribution of genetic variants of pathogenicity classes III-V in the general group of patients with LQTS.

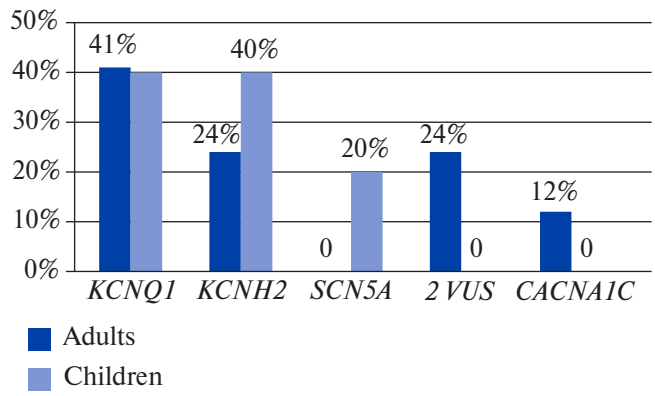


**Figure 2.** Comparative analysis of the distribution of genetic variants of pathogenicity classes III-V between adults and children.

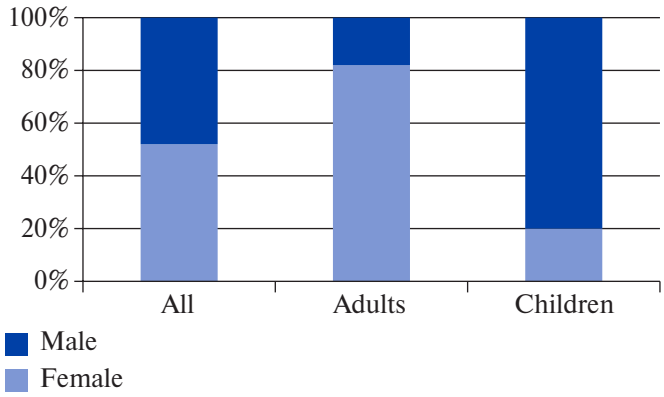


**Figure 3.** Gender distribution among patients with LQTS in the general group and depending on age.

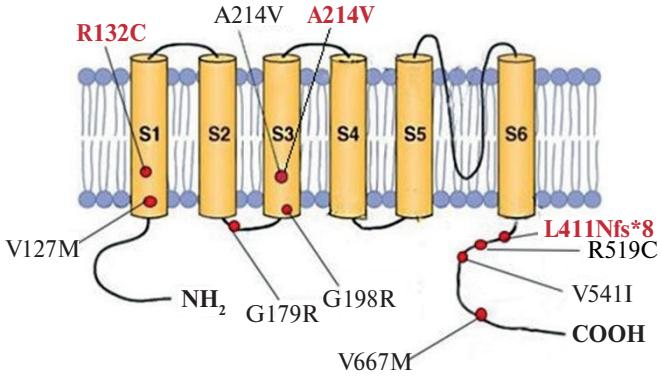


**Figure 4.** Mutations in the *KCNQ1* (LQT1) gene: mutations in children are marked in red font.

centrated in three major genes (*KCNQ1*, *KCNH2* and *SCN5A*), whereas in adults the proportion of mutations in these genes was only 65% (11 out of 17). The proportion of mutations in the gene *KCNH2* in the group of children was 2 times greater (40%) than in adult probands (22%), while the proportion of mutations in the gene *KCNQ1* was the same in both samples (Figure 2).

Mutations in the gene *SCN5A* were found exclusively in a group of children: in 2 out of 10 patients (Table 1, Figure 2). 2 pathogenic mutations of classes IV and V in the gene *CACNA1C* were found in 2 adult probands, one of which was not previously described. In the group of adult patients, a combination of several VUS in different genes was also detected (24%) (Table 1, Figure 2).

A study of the gender distribution in different age groups of patients with a genetically confirmed diagnosis of LQT1-LQT3 detected the following differences: among adult probands, 82% (9 out of 11 patients) were women, and in the group of children, 80% (8 out of 10 patients) were boys. It should be noted that the gender distribution in the general group was 1:1 (Figure 3).

The observed shifts in the sex distribution indicate that this characteristic has a significant impact on the age of manifestation of the clinical manifestations of LQTS of the first three types. Our findings confirm the results of other studies, which also noted the predominance of males in the younger age group and the predominance of females in the older age group [6].

### Gene *KCNQ1* (LQT1)

Table 1 and Figure 4 show the mutations found in Belarusian patients in the *KCNQ1* gene encoding the α-subunit of the potential-dependent potassium channel ($K_V$7.1) responsible for the slow flow of positively charged potassium ions from cells.

82% of mutations in the general group were missense mutations, while this type of mutation was observed in all adults, and in children — only in half of the cases. The remaining genetic variants in the group of children were represented by a mutation

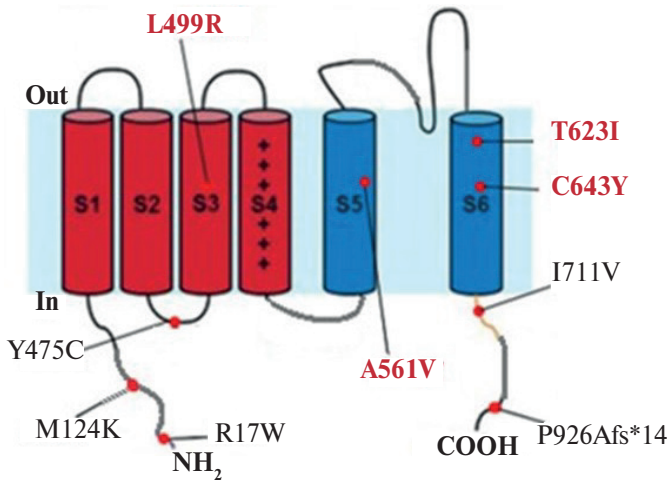


**Figure 5.** Genetic variants in the *KCNH2* (LQT2) gene: mutations in children are marked in red font.

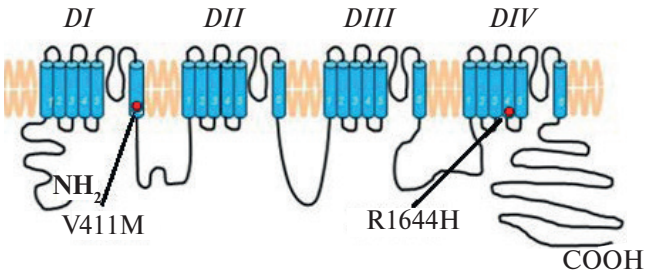


**Figure 6.** Mutations in *SCN5A* (LQT3).

IVS96+1G>A in the second intron leading to splicing failure and a new deletion of c.1233delA (p.Lys411Asnfs*8) in exon 9 with a frameshift and formation of a premature stop codon.

57% of missense mutations in the gene *KCNQ1* in adults and all missense mutations in children were concentrated in exons 5-7 of the gene *KCNQ1*, corresponding to the 1st (S1) and 3rd (S3) segments of the α-subunit transmembrane domain. 43% of mutations in adult patients were concentrated in exons 12, 13, and 16 encoding the C-terminal region of the protein (Table 1, Figure 4).

The QTc value in children with LQT1 ranged from 447 to 528 ms, the clinical course was characterized by the absence of syncope, but all probands had a family history of the disease, with female relatives. The QTc interval duration in adults with LQT1 varied from 450-630 ms. Severe form of the disease was observed only in 2 women (29%) with pathogenic mutations in the 5th (p.VAL127MET) and 6th exons (p.gly179arg) of the gene *KCNQ1*. These patients had QTc values >520 ms, recurrent syncope, and ventricular tachycardia (VT)/ventricular fibrillation (VF), followed by successful resuscitation and ICD implantation. It should be noted that QTc value >500 ms was observed in all adults with mutations in exons 5-7 of the gene *KCNQ1* regardless of gender, but adverse events were observed in women over 24 years of age with QTc >520 and mutations in exons 5 and 6.

All mutations were unique, except for p.Ala214Val localized in S3, which was found in 2 unrelated probands (a 30-year-old male with 6-year-old manifestation, recurrent syncope, nonsustained ventricular tachycardia (NVT), and QTc =630 ms, and a 3-year-old girl with QTc =505 ms).

**Gene *KCNH2* (LQT2)**

Mutations in the gene *KCNH2* (LQT2) encoding the α-subunit of rapidly activating potential-dependent potassium channel ($K_V$11.1) are presented in Table 1 and Figure 5. Mutations in this gene, as well as in the gene *KCNQ1*, lead to a decrease in the repolarizing current from cell and an increase in the action potential duration due to QT prolongation.

7 out of 8 (88%) genetic variants were missense mutations, and duplication leading to the appearance of a premature stop codon was also detected.

All mutations were unique and were concentrated in exons 6-8 in 75% of cases. 23% of the mutations were located in the N- and C-terminal regions and were detected in the group of adults (Figure 5).

All 4 pediatric patients with LQT2 had mutations in exon 7, which corresponds to the 5-6 segments of the transmembrane domain that directly form the pore channel region. Three of the probands were boys, which indirectly indicates an early phenotypic manifestation of these mutations (before age 11) primarily in males. QTc >500 ms was observed in all children and adults with LQT2, regardless of the mutation localization. For 50% of children and 75% of adults, ICD implantation was required. 50% of children and adults have a family history of CHF. Risk factors for adverse events among children with LQT2 were mutation localization in exon 7 of the gene *KCNH2*, and in adult patients — female sex and age older than 28 years.

**Gene *SCN5A* (LQT3)**

Mutations in the gene *SCN5A* encoding the α-subunit of potential-dependent sodium channel ($Na_V$1.5) and responsible for development of LQTS of the third type (LQT3), were detected only in

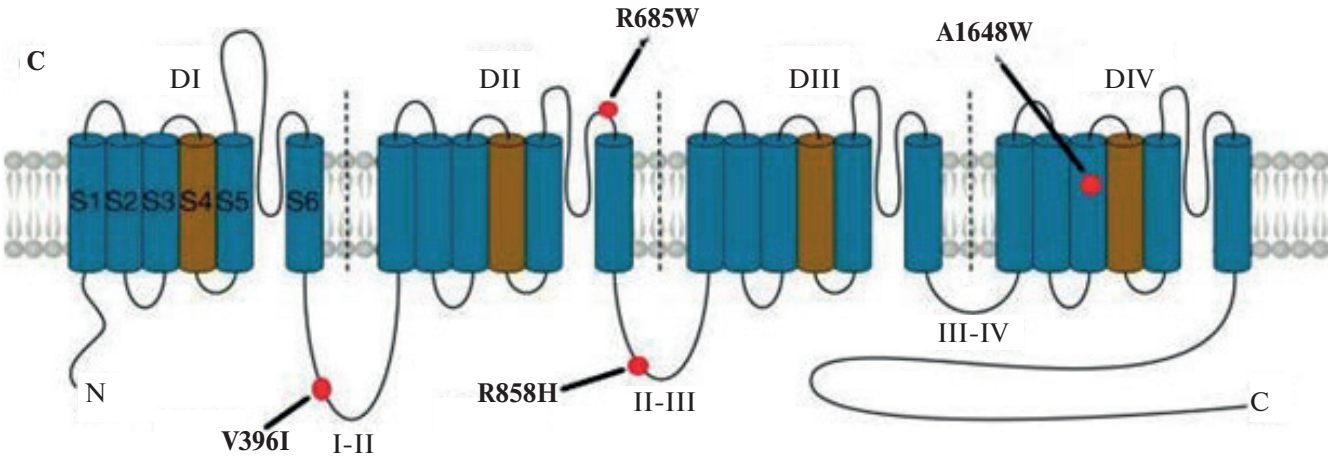


**Figure 7.** Genetic variants in the gene *CACNA1C* (LQT8).

a group of children — in 2 probands (Table 1). The most dangerous of the identified genetic variants was the *de novo* pathogenic mutation c.1231G>A in the gene *c.1231G>A*, which leads to the replacement of an amino acid in transmembrane region of DI-S6 sodium channel (p.VAL411MET, rs72549410) (Figure 6). In the boy, the disease was manifested by syncopal states lasting 5-7 seconds, the first of which occurred at the age of 3, and the value of QTc =595 ms (max. QTc =616 ms). Despite therapy with beta-blockers, the boy had SCD in his sleep against the background of viral infection at age of 9.

The p.VAL411MET mutation was described earlier as having arisen *de novo* in three unrelated cases, which indicates its special malignancy and the "hot spot" of mutations. Functional studies have shown that it leads to hyperactivation of sodium channel and prolonged repolarization of action potential.

In the second patient with LQT3, the pathogenic mutation c.4931G>A affected the DIV-S4 region of sodium channel protein (p.ARG1644HIS, rs28937316) (Figure 6) and phenotypically manifested at the age of 12 with a slight QTc prolongation (up to 490 ms). This variant has been reported previously in several patients with LQT3. At p.ARG1644HIS, a stable internal current of Nations is shown, however, this nucleotide variant may be less serious than other changes in this gene. The boy's mother's father and her 34-year-old cousin were diagnosed with LQTS; in the patient's mother, the presence of the p.Arg1644His mutation was not accompanied by phenotypic manifestations. There was no SCD in the family.

A comparative analysis of clinical parameters between patients with different genetic types of LQTS showed that the QTc value was independent of gender and age and was mainly determined by the genetic type of LQTS, as well as by intragenic mutation localization in patients with LQT1. Thus, all probands with LQT2 and LQT3 showed an increase in QTc >500 ms, and in patients with LQT1, only carriers of mutations in exon 5-7 had QTc >500 ms. The probability of adverse outcomes correlated with QTc value and was higher in LQT2 and LQT3 patients compared with LQT1 patients. At the same time, in adult patients with LQT1, the risk factors were female, QTc >520 and mutation localization in exons 5 and 6. All adult patients with LQT2 requiring ICD insertion were also female, and in the pediatric group of patients with LQT2 and LQT3, events requiring ICD implantation were more often recorded in boys.

A combination of several VUS was found in 4 adult patients, while in 2 probands one of the variants of class III pathogenicity was in the gene *CACNA1C* (Figure 7), in 2 — in the gene *ANK2*. All patients with several genetic variants in the genes associated with this pathology had a severe disease course.

**Gene *CACNA1C***

The gene *CACNA1C* encodes the α-subunit of the potential-dependent calcium channel ($Ca_V1.2$), generating L-type calcium currents. This gene has previously been associated predominantly with Timothy syndrome, which occurs as multiple organ dysfunction, including webbing of fingers and toes, congenital heart defects, immunodeficiency, hypoglycemia, cognitive impairment and autism [13]. Recent studies have found more and more evidence of its importance in the development of isolated autosomal dominant LQTS without extracardiac features [14], SQTS [15], as well as seizure states, including epilepsy [16].

The most severe disease pattern with QTc interval prolongation up to 500 ms, syncopal episodes, development of VT/VF with successful resuscitation and ICD implantation was observed in the patient with p.Val396Ile substitution in exon 8 of gene *CACNA1C* (Table 2, Figure 7) combined with a new

substitution p.Arg17Trp (c.49A>T) in gene *KCNH2* (Table 2, Figure 5).

A patient with p.ALA1648THR replacement in exon 40 of the gene *CACNA1C* in combination with rare variants in the genes *SCN3B* and *DSG2* had frequent episodes of NVT and malignant ventricular extrasystole, which required radiofrequency ablation of ectopic foci. A pronounced clinical picture of the disease with QTc prolongation >500 ms, recurrent syncope, cardiac arrest episode and successful resuscitation with ICD implantation was observed in a patient with a pathogenic mutation (class V) of p.Arg858His in exon 19 of the gene *CACNA1C* (Table 1, Figure 7). Over the course of 8 years, the patient developed pirouette-type polymorphic VTs (TdP) three times, which were stopped by ICD.

In a patient with a new, pathogenic by *in silico* predictors, p.Arg685Trp mutation in exon 14 of the gene *CACNA1C* (Table 1, Figure 7), the disease course was more mild, without syncope and significant episodes of ventricular tachyarrhythmias, controlled by drug therapy.

**Gene *ANK2***

VUS substitutions in 15 and 38 exons of the gene *ANK2,* previously associated with the development of type 4 LQTS, in combination with additional variants in the genes *SNTA1* and *KCNE1* were detected in 2 unrelated male probands (Table 2) [17]. The gene *ANK2* encodes an adapter protein from the ankyrin family involved in localization and stabilization of membrane ion carriers and ion channels. Not so long ago, it was established that mutations in the gene *ANK2* lead to other rhythm disorders, including sinus node weakness syndrome, atrial fibrillation, as well as life-threatening ventricular tachyarrhythmias with a high risk of SCD. All this phenotypic variety of cardiac rhythm and conduction abnormalities is united, for the time being, in "ankyrin-B syndrome" [18].

Despite the absence of a burdened family history and QT prolongation on the ECG series (375 and 440 ms), both patients had recurrent syncopal conditions, VT/VF development, requiring resuscitation and ICD implantation. A 43-year-old proband with substitutions in the genes *ANK2* and *SNTA1* developed polymorphic VT/VF, which was stopped by ICD three times during the 8-year follow-up, and ICD replacement was carried out three times. In recent years, episodes of syncopal states and repeated ICD triggers leading to resuscitation measures have not been repeated.

All patients with multiple VUS were characterized by a slight prolongation of the QTc interval falling into the "gray zone", but had rhythm disturbances and a high risk of SCD, the same, in fact, as patients with mutations in the gene *KCNH2* (LQT2). Thus, during the follow-up period, 3 of 4 (75,0%) patients with multiple genetic variants and 3 of 4 patients with LQT2 had VT/FV with ICD implantation, whereas patients with LQT1 only had life-threatening arrhythmic events in 25,0% of cases, as mentioned above.

A distinctive feature of the group of adult patients with multiple VUS was some predominance of men, whereas the clinical manifestations of LQT1 and LQT2 were observed mainly in female patients.

**Study limitation.** The results of this study should be considered in light of several limitations, the main one being the relatively small patient sample due to the low prevalence of LQTS. An increase in the number of groups under consideration would allow to obtain more accurate results. Nevertheless, the presented data are in good agreement with those already described in the literature. The second limitation concerns assumptions about diagnostic significance of combination of several VUS in genes associated with malignant arrhythmias in patients with LQTS. Conducting family cascade screening of first-degree relatives of such probands in the future will help assess the diagnostic reliability of the identified variants.

## Conclusion

To date, despite huge leaps in understanding of the LQTS pathogenesis due to identification of molecular genetic causes, there are still gaps in knowledge about phenotypic manifestations of genetic defects, including the role of age and sex factors in clinical manifestation of LQTS symptoms is not fully understood. Prognosis and risk stratification of SCD in patients with LQTS is mainly based on QT interval prolongation on ECG, history of syncopal episodes due to torsade de pointes VT or cardiac arrest, and cases of SCD in blood relatives. An independent prognostic factor for development of adverse outcomes is the presence of pathogenic mutations in the genes associated with this pathology.

Comparative analysis of SCD predictors (age of manifestation, syncope, cardiac arrest, type and localization of mutation) in groups of children and adults of Belarusian patients with LQTS of the first three types confirms a significant modulating effect of age and gender on phenotypic realization of the disease. During the study, it was found that in the groups of younger and older probands the sex distribution deviated from the ratio of 1:1 and was exactly the opposite — among children, two-thirds were boys, among adults — women. Based on this fact, it can be assumed that the age of manifestation of clinical manifestations of LQTS of the first three types significantly depends on patient gender. In males, signs of the disease appear in childhood more often than in women.

The results of our study showed that the spectrum of mutations also has age-related features. In the group of children with LQTS, all identified mutations were concentrated in three major genes: 40% each in the genes *KCNQ1*, *KCNH2* and 20% in the gene *SCN5A*. In adult probands, the proportion of mutations in these genes was only 65%: 41% in the gene *KCNQ1* and 24% in the gene *KCNH2*. There were no mutations in the gene *SCN5A* in this group. In 4 adult patients, a combination of several VUS was found in the genes associated with rhythm disturbances, while in 2 probands, one of the variants was in the gene *CACNA1C* and in 2 other probands — in the gene *ANK2*.

In the course of studying the association between age of manifestation and intragenic localization of mutations, it was revealed that in children all missense mutations in the gene *KCNQ1* were concentrated in exons 5-7 corresponding to transmembrane domain region of α-subunit, while in adults almost half of the mutations resulted in amino acid substitutions in the C-terminal region of the protein. Based on these data, it can be assumed that mutations in the gene *KCNQ1* affecting the C-terminal region of the protein have a later phenotypic manifestation. Relating to LQT2, all pediatric patients with this type of LQTS had mutations in exon 7 that predominantly disrupted the protein in pore region. This fact indirectly indicates an early phenotypic manifestation of these mutations, at least in males. All patients with multiple VUS substitutions had a severe course of the disease, but the disease manifestation in these probands was statistically significantly later than in carriers of mutations in the major genes (*KCNQ1*, *KCNH2*, and *SCN5A*).

Due to the above, monitoring of the main clinical and electrophysiological markers of SCD risk in prolonged QT interval syndrome should preferably be performed taking into account age and gender. This will optimize treatment and avoid adverse outcomes of this type of canalopathy, as well as timely prevention of life-threatening arrhythmias in persons with latent and nonsyncopal forms of the disease taking into account these parameters. A comparative approach to study of LQTS phenotypic manifestations, taking into account the age of patients, allows a better assessment of the prognostic significance of identified mutations.

**Relationships and Activities:** none.

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