Gene-Targeted Analysis of Clinically Diagnosed Long QT Russian Families

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Summary

Long QT syndrome (LQTS) has great genetic heterogeneity: more than 500 mutations have been described in several genes. Despite many advances, a genetic diagnosis still cannot be established in 25-30% of patients. The aim of the present study was to perform genetic evaluation in 9 Russian families with LQTS; here we report the results of 4 positive probands and their relatives (a total of 16 individuals). All subjects underwent clinical examination, 12-lead ECG, and Holter monitoring. Genetic analysis of the 14 genes mainly involved in LQTS was performed using a next-generation sequencing approach. We identified two new mutations (KCNQ1 gene) and 6 known mutations (AKAP9, ANK2, KCNE1 and KCNJ2 genes) in 4 out of 9 probands, some of which have already been described in association with LQTS. Segregation analyses suggest a possible causative role for KCNQ1 p.(Leu342Pro), AKAP9 p.(Arg1609Lys), KCNE1 p.(Asp85Asn), and KCNJ2 p.(Arg82Gln) variations. Our study confirmed the high genetic heterogeneity of this disease and highlights the difficulties to reveal clear pathogenic genotypes also in large pedigrees. To the best of our knowledge, this is the first genetic study of LQTS patients from Russian families. (Int Heart J 2017; 58: 81-87)

Key words: LQTS, Cardiology, Channels, Channelopathy, Next generation sequencing

A common cause of severe ventricular arrhythmia is the syndrome of long QT interval, in which violation of potassium or sodium channels due to genetic mutation slows repolarization of the myocardium, lengthening the QT interval on the electrocardiogram. Symptoms occur suddenly and often without warning.

Congenital long QT syndrome (henceforth LQTS) is a genetically determined disease occurring in one case per 3000-5000 members of the general population.1 The disease is hereditary in approximately 85% of cases, while about 15% of cases are a result of new spontaneous mutations.11 LQTS is usually inherited in an autosomal dominant manner,12 most mutations have been found in genes encoding the alpha or beta subunits of plasma membrane ion channels crucial for heart beat regulation. Although more than 500 mutations have been described in 14 genes (Table I), a genetic diagnosis cannot be established in 25-30% of patients.

Presentation of the disease is mainly monogenic and penetrance, and ranges from 25 to 90%.13 Approximately 10% of patients with LQTS bear at least two mutations associated with the condition;12 polygenic or composite varieties usually have a more severe phenotype.

The length of the QT interval, ECG characteristics, clinical features, and prognosis may vary significantly in individuals with different genotypes,10 depending on homozygous/heterozygous mutations, combinations of different mutations and polymorphisms, as well as environment, which may influence the clinical manifestations of existing genotypes.

Nine Russian families with LQTS were recruited for the genetic evaluation; here we present the results of clinical and genetic characterization of 4 Russian probands with LQTS in which we found variations probably related to the phenotype. We identified two new genetic variations and 6 known mutations, some of which have already been described in association with LQTS. We also performed a family segregation study and new mutations were further characterized for their putative pathogenic potential by in-silico evaluation.

Methods

Study subjects: Nine probands and their families were examined in clinics of Krasnoyarsk city. All patients were enrolled after genetic counseling to explain the risks and benefits of genetic testing. They were invited to sign specific consent forms

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This work was supported by grant from the Autonomia Provincia di Trento (grant number S503/2016/238507).

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Received for publication May 25, 2016. Revised and accepted August 1, 2016.

Released in advance online on J-STAGE December 21, 2016.

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to make their clinical and genetic data available for research and publication.

The work described in this article was carried out in accordance with The Declaration of Helsinki and was approved by the Krasnoyarsk State Medical University Ethics Committee (protocol No 49/2012).

Probands and relatives underwent clinical examination, 12-lead ECG, Holter monitoring, and blood sampling at the Federal Cardiological and Vascular Center (Krasnoyarsk) and the Department of Internal Medicine I and Department of Pediatrics, Krasnoyarsk State Medical University “V.F.Voino-Yasenetsky”.

A diagnosis of LQTS involved analyzing the length of the QT interval using the Bazetta formula against the standards of the European Agency for the Evaluation of Medical Products. T-wave morphology was also observed as an indicator of syndrome type. The clinical probability of disease was scored in points on the basis of Schwartz criteria.\(^7\)

Demographic details and information on personal and family medical history were recorded and used to describe families, to determine the inheritance pattern, and to draw pedigrees.

**Molecular genetic analysis using NGS:** Genetic testing was performed at MAGI Laboratory (MAGI Non-Profit Human Medical Genetics Institute, Rovereto, Italy).

Targeted resequencing for 14 genes known to be involved in long-QT syndrome (LQTS) was used to identify pathogenic variants.

### Table I. Genes Involved in LQTS

<table>
<thead>
<tr>
<th>Type (OMIM ID:)</th>
<th>Gene (OMIM ID:)</th>
<th>RefSeq number</th>
<th>Gene Name</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQTS1 (192500)</td>
<td>KCNQ1 (607542)</td>
<td>NM_000218, NM_181798</td>
<td>Potassium voltage-gated channel subfamily Q member 1</td>
<td>40-50%</td>
</tr>
<tr>
<td>LQTS2 (613868)</td>
<td>KCNH2 (152427)</td>
<td>NM_172056, NM_172057, NM_001204798, NM_000238</td>
<td>Potassium voltage-gated channel subfamily H member 2</td>
<td>35-45%</td>
</tr>
<tr>
<td>LQTS3 (603830)</td>
<td>SCN5A (600163)</td>
<td>NM_001099405, NM_001160161, NM_001160160, NM_001099404, NM_000335, NM_198056</td>
<td>Sodium voltage-gated channel alpha subunit 5</td>
<td>2-8%</td>
</tr>
<tr>
<td>LQTS4 (600919)</td>
<td>ANK2 (106410)</td>
<td>NM_001148, NM_001127493, NM_020977</td>
<td>Anklyn 2</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>LQTS5 (613695)</td>
<td>KCNE1 (176261)</td>
<td>NM_000219, NM_001127669, NM_001127670, NM_001127668, NM_0011270405, NM_001270403, NM_001270402, NM_001270404</td>
<td>Potassium voltage-gated channel subfamily E regulatory subunit 1</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>LQTS6 (613693)</td>
<td>KCNE2 (603796)</td>
<td>NM_172201</td>
<td>Potassium voltage-gated channel subfamily E regulatory subunit 2</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>LQTS7 (170390)</td>
<td>KCNJ2 (600681)</td>
<td>NM_000891</td>
<td>Potassium voltage-gated channel subfamily J member 2</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>LQTS8 (601005)</td>
<td>CACNA1C (114205)</td>
<td>NM_001167625, NM_001129844, NM_001129834, NM_001129838, NM_000719, NM_001167624, NM_001129839, NM_001167623, NM_001129841, NM_001129840, NM_001129827, NM_001129836, NM_001129846, NM_001129835, NM_001129830, NM_001129843, NM_199460, NM_001129833, NM_001129829, NM_001129832</td>
<td>Calcium voltage-gated channel subunit alpha 1C</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>LQTS9 (611818)</td>
<td>CAV3 (601253)</td>
<td>NM_001234, NM_033337</td>
<td>Caveolin 3</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>LQTS10 (611819)</td>
<td>SCN4B (608256)</td>
<td>NM_174934, NM_001142349, NM_001142348</td>
<td>Sodium voltage-gated channel beta subunit 4</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td>LQTS11 (61820)</td>
<td>AKA9 (604001)</td>
<td>NM_005751, NM_147185</td>
<td>A-kinase anchoring protein 9</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td>LQTS12 (612955)</td>
<td>STT4A (601017)</td>
<td>NM_003098</td>
<td>Syntrophin alpha 1</td>
<td>&lt; 0.2%</td>
</tr>
<tr>
<td>LQTS13 (613485)</td>
<td>KCNJ5 (600734)</td>
<td>NM_000890</td>
<td>Potassium voltage-gated channel subfamily J member 5</td>
<td>&lt; 0.1%</td>
</tr>
<tr>
<td>LQTS14 (616247)</td>
<td>CALM1 (114180)</td>
<td>NM_006888</td>
<td>Calmodulin 1</td>
<td>Very Rare</td>
</tr>
</tbody>
</table>
in LQTS (Table I) was performed by NGS using an Illumina commercial kit “TruSight One sequencing panel” on the Illumina MiSeq platform (http://www.illumina.com/products/trusight-one-sequencing-panel.ilmn). In-solution target enrichment was performed according to the manufacturer’s protocol for TruSight One sequencing panel kit (Illumina) starting from 5 ng of genomic DNA for each sample.

Each 3-plex sample library pool was sequenced using a 150 bp paired-end reads protocol on the MiSeq sequencer (Illumina, San Diego, CA) according to the manufacturer’s protocol.

**Data analysis:** Raw read data in fastq format, generated by Illumina MiSeq reporter software (version 2.5), were analyzed to generate the final set of sequence variants using an in-house pipeline with the following modules: mapping, duplicate read removal, indel realignment, quality calibration, coverage analysis, variant calling, and annotation. In brief, the sequencing reads were mapped to the genome build hg19 using the Burrows-Wheeler Aligner (BWA) with default settings (version 0.7.5a-r405). Next, duplicate fragments were marked and eliminated with the MarkDuplicates GATK Tool (version v2.5-2-gf57256b). The BAM alignment files generated were refined by local realignment and base quality score recalibration using RealignerTargetCreator and IndelRealigner GATK Tools. Statistical and coverage analysis of final BAM files were performed using SAMTools (Sequence Alignment/Map Tools) and BEDTools. Reads aligned to the designated target regions (coding exons and 15 bp flanking of gene-disease subpanel, Table I) were collected for variant calling and subsequent analysis. The following data per sample was generated by coverage analysis: average read depth, low coverage target regions (< 10X); % of target bases with coverage ≥ 10X. Sequence variant calling was performed using three SNP and genotype calling tools: GATK UnifiedGenotyper, Varscan (version v2.3), and BCFTools of SAMTools (version 0.1.19-44428cd). The output data from the three variant callers was united/pooled and converted into a standard vcf file using a custom script. Called variants were annotated using Annovar software.

Target region coverage less than 10 reads was additionally analyzed by Sanger sequencing.

**Variant filtering and prioritization:** To identify mutations previously reported as pathogenic, we consulted the Human Gene Mutation Database (HGMD) (http://www.biobase-international.com/product/hgmd); allele frequencies were checked in the ExAC database (exac.broadinstitute.org/) and Exome Variant Server (EVJS) database (http://evs.gs.washington.edu/EVJS/). To exclude polymorphisms, we also searched the public database dbSNP (http://www.ncbi.nlm.nih.gov/ SNP/). New nucleotide variations were assessed for pathogenicity using the PolyPhen 2 algorithm (Polyorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph2) considering the HumVar trained model, SIFT algorithm (Sorting Intolerant From Tolerant; http://sift.bii.a-star.edu.sg/) and MutationTaster (http://www.mutationtaster.org). When possible, wild-type amino acid properties were compared with the variations (http://www.russelllab.org/aas/aas.html).

Variants were selected for subsequent study on the basis of the following criteria: 1) variants in target region; 2) previously reported in HGMD and HumsVar database; 3) present in dbSNP137, EVS and 1000 Genome Project with allelic frequency not more than 0.03.

The following criteria were applied to evaluate the pathogenic nature of the variant set selected: 1) known mutation; nonsense, frameshift, essential splice site (affecting conserved consensus motif); start and stop loss variants were considered as most likely to be disease causing; 2) missense variants having an allelic frequency less than 0.01 in dbSNP138 and with predicted deleterious effects were considered potentially pathogenic variants.

**Sanger validation and segregation analysis:** All mutations were validated by Sanger sequencing in probands and then the genotype-phenotype correlation of each mutation was evaluated by family segregation study using a Beckman Coulter CEQ™ 8000 Genetic Analysis System (Beckman Coulter, Milano, Italy).

Electropherograms of amplified fragments were analyzed using ChromasPro 1.5 (Technology Pty. Ltd., Australia) and Sequencher 5.0 (Gene Codes®; Ann Arbor, MI, USA) software and compared to GenBank reference sequences with the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov).

**RESULTS**

Genetic analysis was performed on 9 unrelated probands with a clinical diagnosis of LQTS. In this study, we performed NGS analysis for the 14 known LQTS-related genes (Table I) using a TruSight One sequencing panel (Illumina). The average number of mappable reads per sample was 10 M. On average, 96% of the target bases of gene-disease subpanels were covered at least 10X, with a mean coverage of 117X per sample. With this approach we identified 8 heterozygous mutations, two of which are novel mutations, in 4 affected subjects; we discovered mutations in KCNQ1 (NM_000218), KCNQ2 (NM_000238), and KCNQ3 (NM_001148), KCNQ1 (NM_000219), KCNQ2 (NM_000238), and AKAP9 (NM_005751) genes (Table II). Overall, a mutation was found in 44% (4/9) of patients. Segregation study was then performed by extending the genetic analysis to a total of 16 relatives.

Features of genetic variations and results from pedigree analysis of patients are shown in Table II and the Figure. Sanger sequencing chromatograms are shown in the Supplemental Figure.

**Family 1:** The female proband, 20 years of age with onset at 16 years, complained of syncope and premature ventricular beats. Examination showed a heart-rate-corrected QT interval (QTc) > 450 msec and QTcmax of 530 msec. The patient underwent implantation of a cardioverter-defibrillator (ICD). The sister is healthy, without clinical manifestations and normal QTc. The mother has no symptoms of the disease, but a 12-lead ECG showed QTc > 450 msec and QTcmax of 489 msec. Examination of the parents showed that the mother is healthy, has no symptoms and a normal QTc, whereas the father has no symptoms of the disease, but a 12-lead ECG showed QTc > 450 msec.

**Family 2:** The proband first suffered from syncope at 7 years of age. A 12-lead ECG showed a QTc > 450 msec and QTcmax of 489 msec. Examination of the parents showed that the mother is healthy, has no symptoms and a normal QTc, whereas the father has no symptoms of the disease, but a 12-lead ECG showed QTc > 450 msec.

**Family 3:** The 14-year-old female proband sought medical
advice about syncope and premature ventricular contractions during physical activity. A 12-lead ECG showed a QTc > 470 msec and QTcmax of 515 msec. Her brother also has syncope and a QTcmax of 474 msec; he too was diagnosed with LQTS. The proband’s mother (II:3) has no symptoms of the disease, but her QTcmax was 513 msec and she is therefore at high risk (> 4 points according to the Schwartz-Moss scoring system) of developing the disease. The brother of the mother (II:2) is in the same situation, with a QTcmax of 480 msec. The proband’s cousin (III:2) is at intermediate risk with a QTcmax of 469 msec. The grandmother (I:2) and grandfather (I:1) of the proband from the mother’s side were also studied. The grand-

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Genotype</th>
<th>SIFT</th>
<th>Polyphen</th>
<th>Mutation Taster</th>
<th>dbSNP accession N°</th>
<th>MAF dbSNP</th>
<th>MAF ExAC</th>
<th>MAF EVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNQ1</td>
<td>c.1025T&gt;C</td>
<td>p.(Leu342Pro)</td>
<td>Het</td>
<td>D</td>
<td>PrD</td>
<td>DC</td>
<td>Novel</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>KCNQ1</td>
<td>c.1011C&gt;G</td>
<td>p.(Ile337Met)</td>
<td>Het</td>
<td>D</td>
<td>PrD</td>
<td>DC</td>
<td>Novel</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>KCNH2</td>
<td>c.526C&gt;T</td>
<td>p.(Arg176Trp)</td>
<td>Het</td>
<td>D</td>
<td>PoD</td>
<td>P</td>
<td>rs36210422</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ANK2</td>
<td>c.9854T&gt;C</td>
<td>p.(Ile3285Thr)</td>
<td>Het</td>
<td>D</td>
<td>PrD</td>
<td>DC</td>
<td>rs36210417</td>
<td>0.004</td>
<td>0.0082</td>
<td>0.0068</td>
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<tr>
<td>KCNE1</td>
<td>c.253G&gt;A</td>
<td>p.(Asp85Asn)</td>
<td>Het</td>
<td>D</td>
<td>B</td>
<td>P</td>
<td>rs1805128</td>
<td>0.38</td>
<td>0.0091</td>
<td>0.0087</td>
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<tr>
<td>KCNJ2</td>
<td>c.245C&gt;A</td>
<td>p.(Arg82Gln)</td>
<td>Het</td>
<td>D</td>
<td>PoD</td>
<td>DC</td>
<td>rs199473653</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>AKAP9</td>
<td>c.139C&gt;T</td>
<td>p.(His47Tyr)</td>
<td>Het</td>
<td>T</td>
<td>B</td>
<td>P</td>
<td>rs35669569</td>
<td>0.0022</td>
<td>0.0081</td>
<td>0.0096</td>
</tr>
<tr>
<td>AKAP9</td>
<td>c.4826G&gt;A</td>
<td>p.(Arg1609Lys)</td>
<td>Het</td>
<td>T</td>
<td>PoD</td>
<td>P</td>
<td>rs148146011</td>
<td>0.0006</td>
<td>0.0004</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

In-silico evaluation of the 8 genetic variations identified in 4 probands and their relatives. Het indicates genotype heterozygous; WT, wild-type; T, SIFT score system: tolerated; D, SIFT score system: damaging; B, Polyphen 2 score system: benign; PoD, Polyphen 2 score system: possibly damaging less confident prediction; PrD, Polyphen 2 score system: probably damaging more confident prediction; P, Mutation Taster score system: polymorphism; DC, Mutation Taster score system: disease-causing; MAF, Mutation Taster score system: minor allele frequency; and NA, not available.

Figure. Family pedigree illustrating cosegregation of gene mutations and LQTS phenotypes. * Documented clinical evaluation; E+ and E-, positive and negative to genetic test respectively; ● Obligate carrier [AKAP9 p.(His47Tyr)]; | Asymptomatic/presymptomatic carrier. Schwartz diagnostic scoring system: ≤ 1 point, low probability of LQTS; 2 to 3 points, intermediate probability of LQTS; ≥ 4 points, high probability of LQTS.
mother is healthy with a normal QTc. The grandfather has syncope, tachycardia, and QTc > 450 msec.

**Family 4:** A female infant of healthy parents first manifested syncope at 10 years of age. A 12-lead ECG showed a QTc > 450 msec and QTcmax of 475 msec, i.e., LQTS. Both parents are healthy and without symptoms; a 12-lead ECG showed normal QTc.

**Discussion**

Long QT Syndrome is considered a very heterogeneous hereditary condition. In the present genetic study, in 9 LQTS patients we investigated the presence of variations in 14 genes known to be associated with the condition. In 4 out of 9 probands, we identified two new genetic variants in the KCNQ1 gene and another 6 variants already reported in AKAP9, KCNJ2, KCNH2, KCNE1, and ANK2 genes (Table II).

In family 1 we found p.(Arg82Gln) in the KCNJ2 gene that encodes the potassium channel Kir2.1. Mutations in this gene are involved in Andersen-Tawil Syndrome (ATS) which is characterized by different phenotypes involving multiple body districts and organs. In our study, the proband only had severe heart abnormalities that included cardiac arrhythmias. The proband’s mother carries the same mutation but she only showed a subclinical form of the disease with an intermediate penetrance of this mutation was also noted by Schwartz-Moss according to the Schwartz-Moss system. The incomplete penetrance of this mutation can be found in the literature and many authors report clinical variability and low penetrance. Since we identified the p.(Arg176Trp) in two healthy individuals from two different families, our results seem to support the hypothesis that this variation is likely benign. The proband is on beta-blocker therapy with good clinical effect. Other members of the family with LQT responded well to beta-blockers.

In family 2, only one variation, p.(Arg1609Lys) in the AKAP9 gene, was found in the proband and her father. Although listed in dbSNP (rs148146011, MAF 0.0006), at the time of this report no one has yet shown any association with disease phenotypes. Although the condition was subclinical in the father and only detected by ECG, segregation study in our family suggests that it can cause LQTS. AKAP9 was shown to possess two binding sites for KCNQ1 in its C-terminus (residues 1574-1643); of note, the p.(Arg1609Lys) variant is located in this domain. Only one mutation in this site, namely p.(Ser1570Leu), has been reported to be associated with LQTS and its pathogenicity was supported by functional studies confirming that the variant acts by reducing the interaction with KCNQ1. Taken together, these observations seem to point out a pathogenic role also for this rare missense variation.

The proband has been on non-selective beta-blocker therapy for 1 year. This controlled the syncope, but myopia manifested after beginning therapy and deteriorated to -4 D. Therapy was therefore suspended. The patient is currently stable, without syncope.

In family 3, the proband bears two mutations in genes encoding potassium channel α and β-subunits (KCNNE1 and KCNH2, respectively); mutations in these genes are associated with reduced outgoing K+ current in phase 3 of the action potential and are expected to express a severe phenotype. The effect of this combination was shown to cause a dominant-negative effect that reduced IK, by Nof, et al and Yoshikane, et al, who however considered the p.(Asp85Asn) variation in KCNNE1 combined with different KCNH2 mutations. This situation could explain the proband’s LQTS phenotype but fails to explain the overt disease in his brother.

A third mutation in the ANK2 gene was found in the proband, his affected brother, and the healthy father. Mutations in this gene have been proposed as modulators of human arrhythmias, but p.(Ile3285Thr) is described as “likely benign” in dbSNP and therefore not associated with the condition. Accordingly, results from this family seem to reject any possible association of this variant with the disease.

Variable expression was also found in symptom-free family members in whom 12-lead ECG and Holter monitoring showed a subclinical phenotype.

Although it is described as having a role as LQTS modifier, we showed a perfect segregation in our family tree for the p.(Asp85Asn) variation in the KCNNE1 gene.

Nevertheless, we cannot exclude the presence of important variations in other genes not evaluated in this study.

Wolff-Parkinson-White syndrome (syncope + ECG with long QT-interval) was suspected in the proband. As the first line treatment for this syndrome, radiofrequency catheter ablation was performed but syncope and the ECG-pattern persisted. A defibrillator was implanted with good clinical effect. Other members of the family with LQT respond well to beta-blockers.

The proband of family 4 showed two new variations of uncertain significance (VUS), namely p.(Leu342Pro) and p. (Ile337Met) in the KCNQ1 gene and the “benign” p.(His47Tyr) variant in the AKAP9 gene. Mutations in KCNQ1 are associated with the common LQTS type 1. Both missense variants were predicted as potentially pathogenic variants by 3/3 pathogenicity prediction tools (Table II). Both missense variants were predicted as potentially pathogenic variants by 3/3 pathogenicity prediction tools (Table II). However, both VUS were also found in the proband’s mother, who did not have any clinical sign of the disease. This situation cannot be explained by the concomitant presence of the p.(His47Tyr) variant in the AKAP9 gene, since the protein is known to possess the N-terminus binding sites for KCNQ1 located on its residues 29-46; residue 47 is not conserved among species; thus confirming that it is not important for the protein’s function. The mutation p.(Leu342Phe) in the same codon has already been described in LQTS patients and in a case of syncope before 10 years of age. The importance of this new VUS is suggested by the fact that neighbour codon 341 in the same protein region, i.e., transmembrane segment S6, is commonly mutated in LQTS 1 patients and mutant patients have a high risk of cardiac events; in a study of 244 patients with KCNQ1-A341V genotype, about 70% experienced at least one cardiac event before...
40 years of age, 30% of whom had a fatal or near-fatal event, the other 30% were asymptomatic in the first 4 decades, like the proband’s mother who is only 27. In her case, we cannot exclude a first cardiac event before the age of 40 years. The proband is on beta-blocker therapy which controls LQT and is well tolerated.

Conclusions: Several studies associate LQTS with genetics but these associations are mainly demonstrated in a population-dependent fashion; to our knowledge this is the first study reporting clinical and genetic evaluation of LQTS patients from Russian families. It is also one of the few reports considering all known genes associated with the disease until now. Despite this, the results remain controversial and further studies are therefore required to fully understand the underlying causative mechanisms.

Interestingly, in 3 out of 4 families, probands were found positive to at least two mutations in different genes. We cannot say anything about the links between digenic mutations and more severe phenotypes because of our small statistical sample, but some reports in the literature highlight this possibility.

Approximately 70–75% of LQTS patients have mutations in one of the 3 major LQTS-causing genes (KCNQ1, KCNH2 and SCNSA), the result of which is that a genetic diagnosis cannot be established in 25–30% of patients. In our study, the mutation detection rate was 44% (4/9 probands) even with application of NGS for 14 LQTS-related genes; this is unlikely to reflect the real genetic variant distribution in Russian patients because of the small number of subjects.

In conclusion, our study confirms the genetic heterogeneity of this disease, while the presence of 5 patients negative to genetic testing suggests that other unknown genes are involved.

This next generation sequencing approach confirmed to be of invaluable help to study diseases characterized by marked genetic heterogeneity as LQTS, though the pathogenicity of mutations could be difficult to demonstrate in large pedigrees also.

**References**


Supplemental Files

Supplemental Figure
Please see supplemental files:
https://www.jstage.jst.co.jp/article/ihj/58/1/58_16-133/_article/supplement