HUMAN GENETICS · ORIGINAL PAPER

Identification novel LQT syndrome-associated variants in Polish population and genotype-phenotype correlations in eight families

Małgorzata Szperl[1] · Urszula Kozicka[2] · Agnieszka Kosiec[3] · Piotr Kukla[4] · Marta Roszczynko[3] ·
Elżbieta Katarzyna Biernacka[2]

Abstract

Congenital long QT syndrome (LQTS) is a primary cardiac channelopathy. Genetic testing has not only diagnostic but also prognostic and therapeutic implications. At present, 15 genes have been associated with the disease, with most mutations located in 3 major LQTS-susceptibility genes. During a routine genetic screening for *KCNQ1*, *KCNH2* and *SCN5A* genes in index cases with LQTS, seven novel variants in *KCNH2* and *SCN5A* genes were found. Genotype-phenotype correlations were analysed in these patients and their families. An open reading frame and splice site analysis of the exons was conducted using next-generation sequencing. In novel variants, phenotypes of carriers and their affected relatives were analysed. In 39 unrelated patients, 40 pathogenic/putative pathogenic mutations were found. Thirty-three of them, predominantly missense, were reported previously: 11 were in the KCNQ, 17 in the KCNH2 and 5 in the SCN5A gene. Seven novel missense variants were found in eight families. Among them, four variants were in typical for LQTS location. Two variants in the *KCNH2* gene (p.D803Y and p.D46F) and one in the *SCN5A* gene (G1391R) were in amino acid (AA) position which up to present has not been reported in LQTS. Phenotype analysis showed the life-threatening course of the disease in index cases with a history of sudden cardiac death in six families. Mutation carriers presented with ECG abnormalities and some of them received beta-blocker therapy. We report three novel variants (*KCNQ1* p.46, *KCNH2* p.D803Y, *SCN5A* p.G1391R) which have never been reported for this AA location in LQTS; the phenotype-genotype correlation suggests their pathogenicity.

Keywords Long QT syndrome · Genetic screening · Novel variants

---

What's new?

Novel variants which have never been reported before in patients with LQT syndrome

Communicated by: Michal Witt

✉ Małgorzata Szperl
mszperl@yahoo.com

Urszula Kozicka
pocztaurszula@wp.pl

Agnieszka Kosiec
akosiec@ikard.pl

Piotr Kukla
kukla_piotr@poczta.onet.pl

Marta Roszczynko
mroszczynko@ikard.pl

Elżbieta Katarzyna Biernacka
k.biernacka@ikard.pl

[1] Department of Epidemiology, Cardiovascular Disease Prevention and Health Promotion, Institute of Cardiology, Ul. Niemodlińska 33, 40-635 Warsaw, Poland

[2] Department of Congenital Cardiac Defects, Institute of Cardiology, Warsaw, Poland

[3] Department of Molecular Biology, Institute of Cardiology, Warsaw, Poland

[4] Department of Cardiology and Internal Medicine, Specialistic Hospital, Gorlice, Poland

Introduction

Hereditary Long QT syndromes (LQTS) belong to the channelopathies, which results from mutations that disrupt the assembly and function of ion channel proteins which in turn causes delayed ventricular repolarization. Clinically, LQTS is characterised by prolonged heart rate-corrected QT (QTc) interval, T-wave abnormalities on electrocardiogram (ECG) and cardiac events including syncope, cardiac arrest and sudden cardiac death (Crotti et al. 2008). The prevalence of symptomatic LQT syndrome is estimated to be about 1:5000, whilst prevalence of asymptomatic carriers of pathogenic mutations is estimated about 1:2000–1:2500. These discrepancies indicate the low penetrations and variable expression of the syndromes (Priori et al. 1999). Some of genotyped patients can carry two mutations causing LQTS (Goldenberg and Moss 2008); there are also reports about some mosaicism germline cells cases (Miller et al. 2004). Most of families have private mutation, and therefore diagnostics is problematic in those cases. To date, 15 genes have been involved in LQTS. Those genes code proteins responsible for correct repolarization of the cardiac action potential. Three "major" LQTS genes (*KCNQ1*, *KCNH2* and *SCN5A*) that encode ion channel α subunits critical for a generation of an action potential account for approximately 75% of all LQTS cases and 95% of genotype-positive cases. The 12 "minor" genes, encoding additional ion channel α subunits (*CACNA1C*, *KCNJ5*), cardiac potassium (*AKAP9*, *KCNE1*, *KCNE2*, *KCNJ2*) and sodium (*CAV3*, *SCN4B*, *SNTA1*, *ANK2*) channel interacting proteins and calcium-binding messenger proteins (*CALM1*, *CALM2*), account for less than 5% of LQTS cases.

Recently, the technology of NGS offers new possibilities of screening the genes. As a result of using, this cost-effective methodology of identifying known and novel variants can be used on a larger scale. Here, we present 8 index cases with LQTS, presenting life-threatening arrhythmias, where novel autosomal variants were identified in the *KCNQ*1, *KCHN2* and *SCN5A* genes.

Patients and methods

The study group consisted of 8 index cases (6 women) with LQTS related to novel variants, which were identified among 39 index cases with genetically determined LQTS and their relatives, who agreed to undergo genetic study and clinical evaluation. NGS revealed 33 known mutations (11 in KCNQ1, 17 in KCNH2, 5 in SCN5A genes) and seven novel variants. A phenotype-genotype correlation was analysed in each family.

The clinical evaluation consisted of detailed history included family and cardiological exam. Parameters the repolarization process were assessed manually by one cardiologist.

Corrected QT was calculated using Bazett's formula to adjust the intervals to heart rate (Bazett 1920). Electrocardiographic measurements of T-wave and U-wave were made in leads with the highest amplitude using the tangent technique (Goldenberg et al. 2006; Postema et al. 2008).

The study was approved by the local Bioethics Committee of Institute of Cardiology. The written consent from each individual patient was obtained.

Genetic study Ten millilitres of blood was collected on EDTA. The genomic DNA was isolated from peripheral blood by phenol or by standard salting out method. Screening for mutation 3 major gene causing LQTS: *KCNQ1*, *KCNH2* and *SCN5A* was performed by next-generation sequencing (NGS) using exon-flanking intronic primers designed by our laboratory. Amplicons were analysed by GS Junior System (Roche, France) according to the Amplicon Library Preparation Method Manual. All libraries were sequenced with minimum depth of 20 reads. Data analysis was done by using GS Amplicon Variant Analyser (AVA) software (Roche, France). Each novel variant was validated using the Sanger sequencing method in two individual samples from each index case. For this purpose, DNA was amplified using site-specific primers and amplicons were subjected to bidirectional capillary-based sequencing using 3130XL Genetic Analyser (Applied Biosystem, Hitachi, Japanese). Data were analysed by using DNA Sequencing Analysis Software version 5.3.1 Applied Biosysytem. Obtained sequences were compared to the consensus sequences for the *KCNQ1* (GeneBank Accession Number NM_000218), *KCNH2* (GeneBank Accession Number NM_000238) and *SCN5A* (GeneBank Accession Number NM_001099404) genes. Mutation was considered as a novel when absent from HGMD, Phase 3 of 1000 Genomes (http://www.1000genomes.org/) and Version 0.3 of ExAC (http://exac.broadinstitute.org/). Specific primers for each novel variant are available on request. Having the opportunity, we conducted co-segregation within the family. Prediction analysis of novel variants was made by using SIFT and PolyPhen-2 algorithms. To localise the variants, we used the Swissprot database (http://ca.expasy.org/uniprot/).

Results

We showed the presence of seven novel variants in Polish population. One of the variants was identified in *KCNQ1* (T587R) gene and five were identified in *KCNH2* (V822L, 2D46Y, D803Y, F617V, F656L) gene, where two of them (D46Y, LF56L) were identified in two unrelated index cases. One variant was identified in *SCN5A* gene (p.R1391R). These variants were neither found in ExAC nor 1000 Genomes and have not been reported in the dbSNP and HGMD genomic database (Table 1). Genetic alterations were characterised by

Table 1 Results of the analysis of the novel variants

Gen/Genbank transcript ID	Substitution bp	Position of altered AA	Prediction, SIFT	Prediction, PolyPhen-2	Another variant in same AA residue reported in arrhythmias
KCNQ1 NM_000218	ACG>AGG	T587R	Damaging, score − 0	Damaging, score − 1	T587M rs120074189
KCNH2 NM_000238	GAC>TAC	D46Y	Damaging, score− 0	Damaging, score − 1	No
KCNH2 NM_000238	TTC>GTC	F617V	Damaging, score − 0	Damaging, score − 1	F617L rs796052195
KCNH2 NM_000238	TTC>TTA	F656L	Damaging, score − 0	Damaging, score − 1	F656C rs199472977
KCNH2 NM_000238	GAC>TAT	D803Y	Damaging, score − 0	Damaging, score − 1	No
KCNH2 NM_000238	GTG>TTG	V822L	Damaging score − 0	Damaging score − 1	V822M V822L rs121912506
SCN5A NM_001099404	GGA>AGA	G1391R	Damaging score − 0	Damaging score − 1	No

type and location of novel variants. Three of new variants (*KCNQ1*:T587R, QT2:F617V, F656) are located in the transmembrane region. The other two variants were located in C-terminal (*KCNH2*: V822L, D803Y) and one in N-terminal region (*KCNH2*: D46Y). Variant p.G391R was located in DIII-S4 region of *SCN5A* gene. To determine penetration, we examined relatives and pedigree analysis was done if it was possible. Penetrance defined as the ratio between patients with the clinical phenotype LQT (symptoms and/or incorrect ECG) and number of carriers mutation.
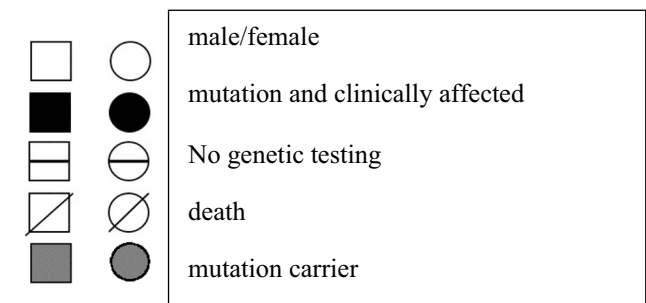
Family number 1

Heterozygous missense mutation in *KCNQ1* gene causing a protein change p.T587R (Fig. 1(B)) was identified in a 28-year-old man (LQT1) with a history of multiple syncopal episodes during emotional stress, 12-lead ECG showed QTc max 580 msec, biphasic T-wave in V1 and broad-based T-wave in V2-V4 (Fig. 1(C)) was successfully treated with metoprolol controlled-release tablets 100 mg (metoprolol CR). His brother died at the age of 22 years during swimming due to sudden cardiac death (SCD). This novel variant has been also identified in his asymptomatic mother who presented only borderline QTc (QTc max 460 msec) (Fig. 1(A)).
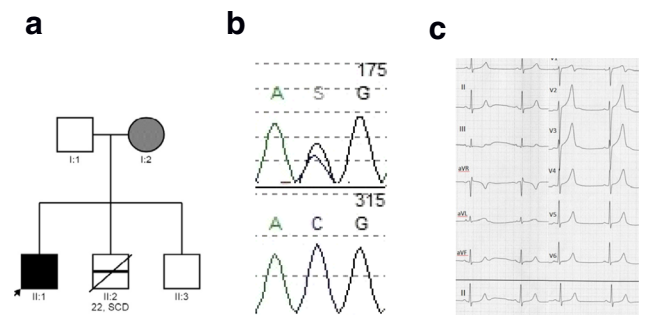
Family number 2

Point heterozygous missense mutation in *KCNH2* gene caused a protein change p.V822L (Fig. 2(B)). This variant has been identified in a 35-year-old woman, and 12-lead ECG showed QTc max 490 msec, and broad-based T-waves, notched in V2 (Fig. 2(C)), after two out-of-hospital episodes of sudden cardiac arrest, with a number of episodes of syncope

associated with high emotions, with family history of SCD during sleep (two women: her sister at the age of 23 years and father's cousin at the age of 24 years). A dual-chamber implantable cardioverter defibrillator (ICD) was placed for secondary prevention. Medical therapy with metoprolol CR 50 mg was implemented. In 3-year follow-up, she has not received any therapy from the ICD. Family genetic screening for long QT gene mutations revealed three asymptomatic

male/female

mutation and clinically affected
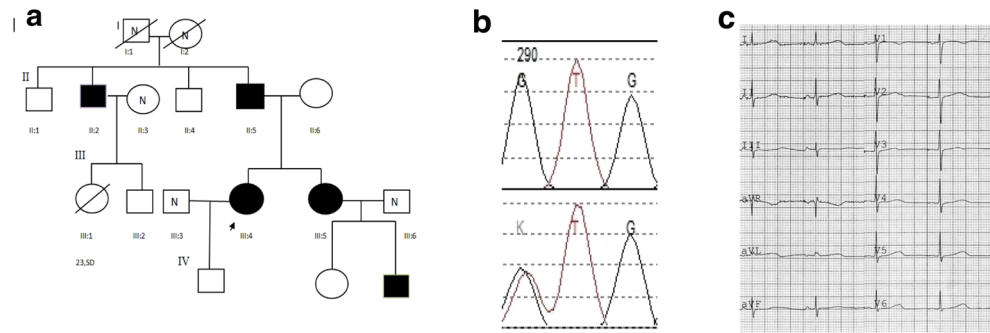
No genetic testing

death

mutation carrier

↗ - arrow indicates index case

Fig. 1 Family number 1. (A) Pedigree of family. (B) Variant ACG>AGG (p.T587R) in *KCNQ1* gene (C) Index case ECG (paper speed 25 mm/s)

Fig. 2 Family number 2. (A) Pedigree of family. (B) Variant GTG>TTG (p.V822L) in *KCNH2* gene. (C) Index case ECG (paper speed 25 mm/s)

carriers of the mutation: father (QTc max 490 msec), paternal uncle (QTc max 480 msec) and son of younger sister (max 590 msec, at night sinus bradycardia). The last patient received subcutaneous ICD in primary prevention (Fig. 2(A)).

Family number 3 and family number 4

In two unrelated index cases, we identified the same heterozygous missense mutation in *KCNH2* gene causing a protein change p.D46Y (Fig. 3(B)).

Family number 3

The D46Y variant in the gene *KCNH2* was identified in a 47-year-old woman (QTc max 530 msec, low amplitude T-waves—Fig. 3(C)) with recurrent episodes of syncope provoked by sudden loud noises (alarm clock) and emotions. Her father died suddenly without known cause in middle age (Fig. 3(A)). Metoprolol CR 50 mg is efficient in 3-year observation.

Family number 4

The second patient carrying this variant is a 47-year-old woman (QTc max 510 msec, Fig. 3(C′)) with negative family history of SD who experienced cardiac arrest due to torsade de pointes (TdP) during sleep 5 months after delivery. An implantable cardioverter defibrillator was placed and treatment of beta-blocker therapy (metoprolol CR 100 mg) was

implemented. One appropriate ICD therapy was observed during 3-year follow-up. This variant has been discovered in her 73-year-old father (QTc max 492 msec), treated with a single-chamber ventricular pacemaker and beta-blocker because of atrial fibrillation and tachy-brady syndrome (Fig. 3(A′)).

Family number 5

Heterozygous missense variant in *KCNH2* gene causing a protein change p.D803Y (Fig. 4(B)) was identified in a 27-year-old woman (QTc max 470 msec, T-wave abnormalities, Fig. 4(C)). The patient had a history of several episodes of syncope. A single-chamber atrial pacemaker was inserted to enable beta-blocker therapy (metoprolol CR 100 mg was given) with good result. Her mother (age 38, during puerperium, after alarm clock) and her sister (age 21, during sleep) died suddenly. The mutation was found in her older asymptomatic brother (QTc max 458 msec, T-wave abnormalities). Both index case and her brother carried another new variant—*SCN5A* gene p.G1391 (Fig. 4(A)).
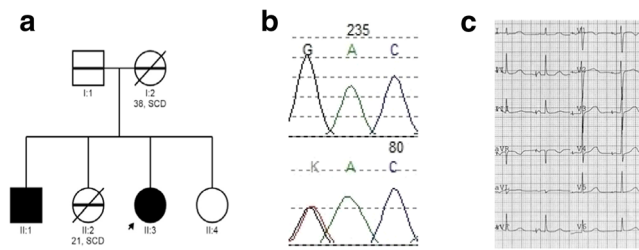
Family number 6

Genetic screening identified a new heterozygous missense mutation in *KCNQ1* gene causing a protein change p.F617V (TTC>GTC) index case (Biernacka et al. 2015). The mutation has been identified in a 50-year-old woman with QTc max 540 msec (Fig. 5(C)), several episodes of syncope induced by psychological stress (Torsades de Pointes, TdP) and a
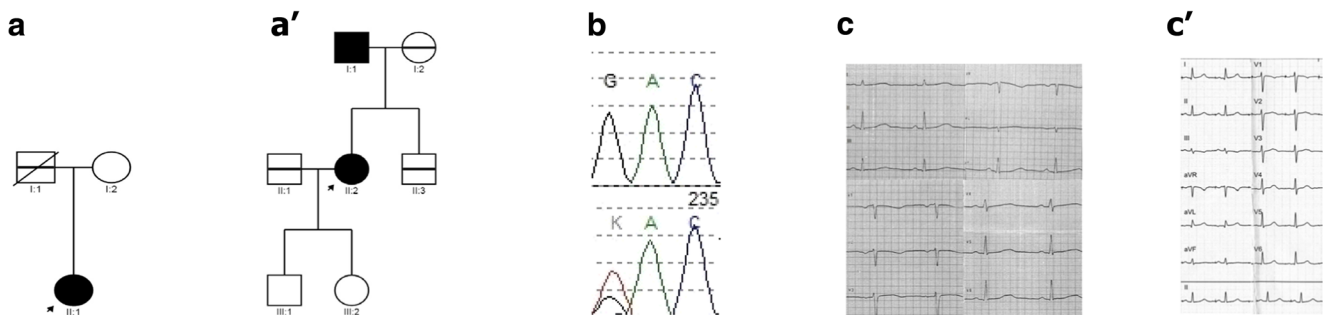
Fig. 3 (A) Pedigrees of family number 3 and family number 4 (A′). (B) Variant GAC>TAC (D46YF) *in KCNH2* gene. (C) Index case number 3 and index case number 4 (C′) ECG (paper speed 50 mm/s and 25 mm/s, respectively)

Fig. 4 Family number 5. (A) Pedigree of family. (B) Variant p.D803Y (GAC>TAC) in *KCNH2* gene. (C) Index case ECG (paper speed 25 mm/s)

family history of SCD among the first-degree relatives during sleep: mother (at the age of 42) and sister (at the age of 20). Beta-blocker therapy (propranolol 120 mg per day given in three divided doses) and a single -chamber atrial pacemaker was implanted due to drug-related bradycardia. Neither arrhythmia nor syncope has been observed in 10-year follow-up. Mutation has been discovered in the youngest asymptomatic but with ECG abnormality son (560 msec and significant ST-T changes) (Neyroud et al. 1999).

Family number 7 and family number 8

We identified the same heterozygous missense variant in *KCNH2* gene causing a protein change p.F656L (Fig. 5(B)) in two unrelated index cases.

Index case number 7

A 35-year-old man had an episode of syncope and cardiac arrest at home as first manifestation of the disease—CPR made efficiently by his wife. QTc was not prolonged (413 ms) (Fig. 5(C)). Epinephrine test unmasked LQTS, with QTc prolongation to 670 msec associated with TdP induction. After TdP recurrence when patient was treated with beta-blocker (propranolol 60 mg/day), dual-chamber ICD was implanted. Mutation has been discovered in asymptomatic but with ECG abnormality mother. There was no family history of SCD (Fig. 5(A)).
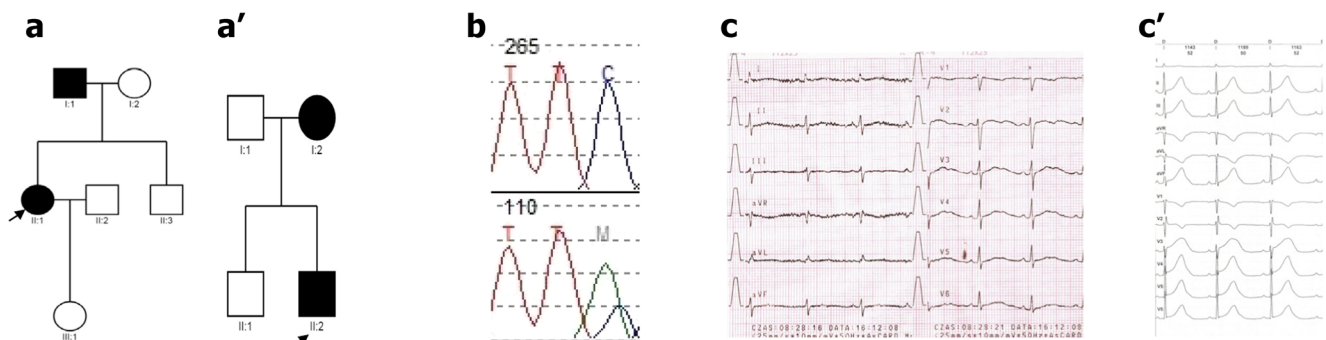
Index case number 8

A 22-year-old woman had an episode of loss of consciousness 6 months after delivery. Holter monitoring showed episodes of QTc prolongation max. QTc was 618 ms and medium QTc was 538 ms with different morphology types of T-wave (Fig. 5(C′)). Beta-blocker (BB) therapy (metoprolol was initiated) titrated up to 200 mg daily. The patient was out of symptoms. After 2 years during the second pregnancy, she reduced BB to 50 mg/day. In the postpartum period, 3 months after delivery, she had syncopal episodes. Holter monitoring showed polymorphic ventricular tachycardia. The cardioverter defibrillator was implanted. Mutation has been discovered in asymptomatic but with ECG abnormality father (Fig. 5(A′)).

Discussion

Genetic research showed a presence of seven novel variants in Polish population. In silico analysis predicted all seven novel variants are probably damaging protein. One of the variants was identified in *KCNQ1* (T587R) gene, the others were identified in *KCNH2* (V822L, 2D46Y, D803Y, F617V, F656L) gene, where two of them (D46Y, LF56L) were identified in two unrelated index cases. One variant was identified in *SCN5A* gene (p.R1391R). These variants were neither found in ExAC nor 1000G and have not been reported in the dbSNP and HGMD genomic database. We will discuss each variant separately.

Variant p.T587R in *KCNQ1* (family no. 1) is located in the pore region. Another variant has been reported at the same AA position in patient with Jervel and Lange-Nielsen syndrome (p.T587M, rs120074189, HGMD CM981131) (Neyroud et al. 1999). Family segregation shows uncompleted penetration (Fig. 1(A)). Based on phenotype-genotype correlation and the pore localisation of the *KCNQ1*, p.T587R variant strongly suggests its causative role (Napolitano et al. 2005) but only men are symptomatic.

Fig. 5 (A) Pedigree family number 7 and family number 8 (A′). (B) Variant p.F656L (TTC>TTA) in KCNH2 gen. (C) Index case number 7 and index case number 8 (C′) ECG (paper speed 25 mm/s)

Variant p.V822L (GTG>TTG) in *KCNH2* gene (family no. 2)
Variant resulting in the same AA substitution but with another allele has been reported in LQTS patients in dbSNP (rs121912506). Also, in the same AA residue, variant p.Val822Met has been reported as pathogenic (rs121912506) (Satler et al. 1996). Although, there is a full penetration in this family, but only women are clinical symptomatic. Men in this family are clinical asymptomatic with abnormal ECG.

Variant p.D46Y (family no. 3 and family no. 4) Other variants in the same AA position have been submitted by AVA_EXAC (rs752503743, p.D46E) and GeneDX Laboratory (rs 794728408, p.D46N) (Lek et al. 2016; GeneDx n.d.). Those variants were validated only by frequency and have not been reported in patients with LQT syndrome. Novel variant is located in the N-terminal region. According to pedigree of family nos. 3 and 4, all carriers are symptomatic (Fig. 3(B, B′)), so in this case occurs a full penetration including symptoms and ECG.

Variant p.D803Y (GAC>TAC) in *KCNH2* gene (family no. 5) We reported for the first time missense variant in this AA position in LQT syndrome. This variant is located in the C-terminal region. In silico analysis shown that this variant is probably protein damaging. Both index case and her brother carried another new variant: *SCN5A* gene p.G1391, rs780405533 (GeneDx Variant Classification 06012015). Variant D803Y was not observed in approximately 6500 individuals in the NHLBI Exom Sequencing Project (NHLBI 2015). Wattanasirichaigoon showed that mutation in this region of *SCN5A* gene interferes with the attachment of DIII-DIV linker to the pore and result in reduced fast activation (Wattanasirichaigoon et al. 1999). Unfortunately, women who died suddenly were not genotyped and we do not know if women carrying those two variants are particularly susceptible, whilst men carrying those two variants are asymptomatic with ECG abnormality (border QTc and T-wave abnormality). A protective effect of the presence of G1391R variant in SCN5A gene cannot be excluded.

According to pedigree analysis, it seems that women carrying those two variants are particularly susceptible, whilst men carrying those two variants are asymptomatic with ECG abnormality (border QTc and T-wave abnormality).

Variant p.F617V (TTC>GTC) in *KCNH2* (family no. 6) Another mutation in this AA position (Table 1) has been reported as likely pathogenic (Shigemizu et al. 2015). The revealed variant is located in the pore region. A history of sudden death in the first-degree relative, TdP and syncope in index case suggested that this variant is potentially life-threatening in young women (Biernacka et al. 2015). The youngest son (carrying mutation) is clinically asymptomatic but he has abnormal ECG.

Variant p.F656L (TTC>TTA) in *KCNH2* gen (family no. 7 and 8)
This mutation is present in two separate families. The novel variant is located in the pore region. Given segregation in family number 7 and family number 8, this variant shows full co-segregation with disease (symptoms and/or ECG), but affected individuals who harbour the same variant display variable expression of the disease (Fig. 5(A, A′)). Napolitano reported another variant in the same AA position F656C, rs199472977 in LQT syndrome (Napolitano et al. 2005).

Apart from 4 cases affecting known AA residue, we report for the first time three variants in AA residue which have never been reported in patients with LQT syndrome: two in *KCNH2* gene (D803Y and D46) and one in *SCN5A* gene (G1391R). In silico analysis predicts all new variants are probably damaging to the protein structure or function. Among *KCNH2* p.V822L, *KCNH2* p.F617V and *KCNH2* p.D803Y + *SCN5A* p.G1391R variants, carriers' occurrence of disease and risk of cardiac events is higher for females than males. Based on phenotype-genotype correlation and the pore localisation of the *KCNQ1*, p.T587R variant strongly suggests its causative role but only men in this small family are clinically symptomatic.

Conclusions

1. This study reveals new variants whose pathogenicity is difficult to assess. Genotype-phenotype correlations are not very strong and require confirmation in larger families. Although genotype-phenotype correlations are not very strong and require confirmation in larger families, our data can be helpful in the diagnosis of LQT syndrome.
2. Risk stratification is not as simply as additionally genetic variants may modify clinical severity. Affected individuals who harbour the same putative causative mutation display variable expression of the disease.

Limitations

1. Small families do not allow to study a penetration of reported new variants.
2. Silico tools are helpful for the initial assessment but not sufficient to ultimately assess the pathogenicity of novel variants.

Compliance with ethical standards

References

Bazett H (1920) An analysis of the time relationship of electrocardiograms. Heart 7:353–370

Biernacka E, Szperl M, Kosiec A, Roszczynko M, Hoffman P (2015) A novel life-threatening mutation in long QT2 syndrome. Kardiol Pol 73(11):1097–1100. https://doi.org/10.5603/KP.a2015.0096

Crotti L, Celano G, Degradi F, Schwartz PJ (2008) Congenital long QT syndrome. Orphanet J Rare Dis 7:3–18

GeneDx, (n.d.)207 Perry Parkway, Gaithersburg Maryland, United States – 20877. http://www.genedx.com/ Organization ID: 26957

Goldenberg I, Moss AJ (2008) Long QT syndrome. J Am Coll Cardiol 51(24):2291–2300

Goldenberg I, Moss AJ, Zareba W (2006) QT interval: how to measure it and what is "normal". J Cardiovasc Electrophysiol 17:333–336

Lek M, Karczewski KJ, Minikl EV, Samocha KE, Banks E, Fennell T et al (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536:285–291. https://doi.org/10.1038/nature19057

Miller TE, Estrella E, Myerburg RJ, Garcia de Viera J, Moreno N et al (2004) Recurrent third-trimester fetal loss and maternal mosaicism for long-QT syndrome. Circulation 109:3029–3034

Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J et al (2005) Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. JAMA 294(23):2975–2980

Neyroud N, Richard P, Vignier N, Donger C, Denjoy I, Demay L et al (1999) Genomic organization of the KCNQ1 K+ channel gene and identification of C-terminal mutations in the long-QT syndrome. Circ Res 84(3):290–297

NHLBI (2015) Exome Sequencing Project (ESP) . Exome Variant Server Available at http://evs.gs.washington.edu (accessed 1/15/2015)

Postema PG, De Jong JS, Van der Bilt IA et al (2008) Accurate electrocardiographic assessment of the QT interval: teach the tangent. Heart Rhythm 5:1015–1018

Priori SG, Napolitano C, Schwartz PJ (1999) Low penetrance in the long-QT syndrome. Clinical Impact Circulation 99:529–533

Satler C, Walsh E, Vesely M, Plummer M, Ginsburg G, Jacob H (1996) Novel missense mutation in the cyclic nucleotide-binding domain of HERG causes long QT syndrome. Am J Med Genet 65:27–35

Shigemizu D, Aiba T, Nakagawa H, Ozaki K, Miya F, Satake W et al (2015) Exome analyses of long QT syndrome reveal candidate pathogenic mutations in calmodulin-interacting genes. PLoS One 10(7): e0130329. https://doi.org/10.1371/journal.pone.0130329 eCollection 2015

Wattanasirichaigoon D, Vesely MR, Duggal P, Levine JC, Blume ED, Wolff GS et al (1999) Sodium channel abnormalities are infrequent in patients with long QT syndrome: identification of two novel SCN5A mutations. Am J Med Genet 86(5):470–476