ORIGINAL RESEARCH

Genetic and Phenotypic Characterization of Community Hospital Patients With QT Prolongation

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***Background*—**Congenital long-QT syndrome (LQTS) is a genetic disorder characterized by prolongation of the corrected QT interval (QTc) on an ECG. The aim of the present study was to estimate the prevalence of pathogenic and likely pathogenic sequence variants in patients who had at least 1 ECG with a QTc ≥500 ms.

***Methods and Results*—**Telemark Hospital Trust is a community hospital within the Norwegian national health system, serving ≈173 000 inhabitants. We searched the ECG database at Telemark Hospital Trust, Norway, from January 2004 to December 2014, and identified 1531 patients with at least 1 ECG with a QTc ≥500 ms. At the time of inclusion in this study (2015), 766 patients were alive. A total of 733 patients were invited to participate, and 475 accepted. The 17 genes that have been reported to cause monogenic LQTS were sequenced among the patients. Pro-QTc score was calculated for each patient. A molecular genetic cause of LQTS was detected in 31 (6.5%) of 475 patients. These patients had a lower pro-QTc score than those without pathogenic or likely pathogenic variants (1.7±1.0 versus 2.8±1.6; $P<0.001$).

***Conclusions*—**Compared with the general population, hospitalized patients with a QTc ≥500 ms in at least 1 ECG recording had an increased likelihood for pathogenic and likely pathogenic variants in LQTS genes. We recommend increased awareness of the possibility of LQTS in patients with at least 1 ECG with a QTc ≥500 ms. **(*J Am Heart Assoc*. 2018;7:e009706. DOI: 10.1161/JAHA.118.009706.)**

Key Words: genetic testing • inherited arrhythmia • long-QT syndrome

Congenital long-QT syndrome (LQTS) is a genetic disorder characterized by prolongation of the corrected QT interval (QTc) on an ECG. It is associated with increased risk of torsade de pointes ventricular tachycardia and sudden cardiac death. The estimated prevalence of LQTS is 1:2000 live births.[1]

QTc ≥500 ms is considered to be highly abnormal and associated with increased risk of torsade de pointes ventricular tachycardia,[2] but also carriers of LQTS mutations with a QTc ≤440 ms have an increased risk of life-threatening cardiac events.[3] Treatment with β-blocker medication significantly reduces the risk of adverse outcomes.[4]

More than 1500 sequence variants in 17 genes have previously been reported to be pathogenic or likely pathogenic for LQTS, although the evidence for some of these is limited.[5] Autosomal dominant is the most common inheritance pattern, and *KCNQ1* (LQT1) harbors most genetic defects, followed by *KCNH2* (LQT2) and *SCN5A* (LQT3).[6,7] Penetrance is incomplete, and expression is variable within families; these factors complicate both interpretation of pathogenicity of sequence variants in LQTS genes and genetic counseling.[8]

Most molecular genetic studies of LQTS have been conducted in cohorts clinically diagnosed with LQTS or ascertained through cascade screening of relatives for a specific causative variant, and 80% of families meeting clinical diagnostic criteria have detectable pathogenic or likely pathogenic sequence variants in the 17 known LQTS genes.[9]

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Accompanying Data S1, Tables S1 through S6, and Figure S1 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.009706

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Clinical Perspective

What Is New?

- A molecular genetic cause of long-QT syndrome was detected in 6.5% of patients with at least 1 ECG with a corrected QT interval ≥500 ms.
- In most patients with a pathogenic or likely pathogenic variant, the prolonged corrected QT interval had not been acknowledged in the medical records.

What Are the Clinical Implications?

- We recommend increased awareness of the possibility of congenital long-QT syndrome in patients with at least 1 ECG with a corrected QT interval ≥500 ms.

Current guidelines recommend genetic testing of asymptomatic patients if repetitive ECGs show a QTc ≥480 ms (European Society of Cardiology guidelines[2]) or a QTc >500 ms (Heart Rhythm Society/European Heart Rhythm Association Expert Consensus Statement[7]) in the absence of secondary causes that may prolong the QT interval. An LQTS risk score (Schwartz) >3 is also considered diagnostic.[2,7,10] In accordance with current clinical guidelines for LQTS, genetic analyses have often been limited to the 3 main LQTS genes. The prevalence and spectrum of pathogenic and likely pathogenic sequence variants in the remaining LQTS genes are less well documented.

The aim of the present study was to estimate the prevalence of pathogenic and likely pathogenic sequence variants in patients who had at least 1 ECG with a QTc ≥500 ms admitted to a community hospital. Targeted sequencing was used to analyze all 17 known LQTS genes. We further wanted to determine if these patients fulfilled the clinical criteria for LQTS, according to current guidelines.

Methods

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Study Population and Selection of ECGs

The patients in this study have previously been described (Figure 1).[11] Briefly, the ECG database at Telemark Hospital Trust contained 225 117 ECGs from 63 286 unique patients collected throughout a period of 11 years (January 2004–December 2014). The ECG database was searched with the following criteria: QTc (Bazett's formula) ≥500 ms, QRS width ≤120 ms, age ≥15 years, heart rate (beats per minute) >30 and ≤100 (because of the limitations of Bazett's formula), no acute ST-segment–elevation infarction, and no atrial fibrillation or atrial flutter. All ECGs with a QTc ≥500 ms were manually reviewed, and a total of 1531 patients with at least 1 ECG with a QTc ≥500 ms were included in the previous study. These patients' first ECG with a QTc ≥500 ms is further referred to as "ECG 1."

The QT interval was measured manually in the lead showing the longest QT interval as the mean of 3 consecutive beats. We determined the end of the T wave by the tangent method, and U waves were not included if distinct from the T wave.[11,12] The average heart rate over the entire recording was used if the rhythm was regular. ECGs with frequent premature ventricular beats or short runs of supraventricular tachycardia were excluded.[11] The QTc was calculated according to Bazett's formula.

At the time of inclusion in the present study (2015), 766 patients were alive, but 33 of these were excluded because of severe health problems, such as advanced dementia. A total of 733 patients were invited to participate in the study, and those who consented delivered blood for genetic testing and had a new ECG. This ECG is referred to as "ECG 2."

Clinical Data

Clinical data, including whether the QT prolongation were acknowledged by the clinician, were obtained from the medical records at time of ECG 1, and pro-QTc risk score was calculated for each patient.[12] QT-prolonging conditions included in the pro-QTc score are listed in Table 1. QT-prolonging medication was defined as any medication on the Arizona CredibleMeds QTdrugs lists.[13] Hypomagnesemia was defined as serum magnesium ≤0.71 mmol/L, and hypokalemia was defined as serum potassium ≤3.6 mmol/L. Hypocalcemia was defined as corrected serum calcium ≤2.17 mmol/L or ionized serum calcium ≤1.18 mmol/L.

QT Interval and LQTS Risk Score (Schwartz)

It was noted whether the patients with a pathogenic or likely pathogenic variant and the patients with variants of uncertain significance (VUS) met the criteria for a clinical diagnosis of LQTS on the basis of current guidelines.[2,7] The LQTS risk score (Schwartz) was based on QTc from ECG 2 because it was least affected by environmental factors.[2,10] The difference in QTc between ECG 1 and ECG 2 was also calculated.

DNA Sequencing

The 17 genes in which mutations are known to cause monogenic LQTS were sequenced: *AKAP9* (NM_005751.4), *ANK2* (NM_1148.4), *CACNA1C* (NM_000719.6), *CALM1* (NM_006888.4), *CALM2* (NM_001743.4), *CALM3* (NM_005184.2), *CAV3* (NM_033337.2), *KCNE1* (NM_000219.5), *KCNE2* (NM_172201.1), *KCNH2* (NM_000238.3), *KCNJ2*

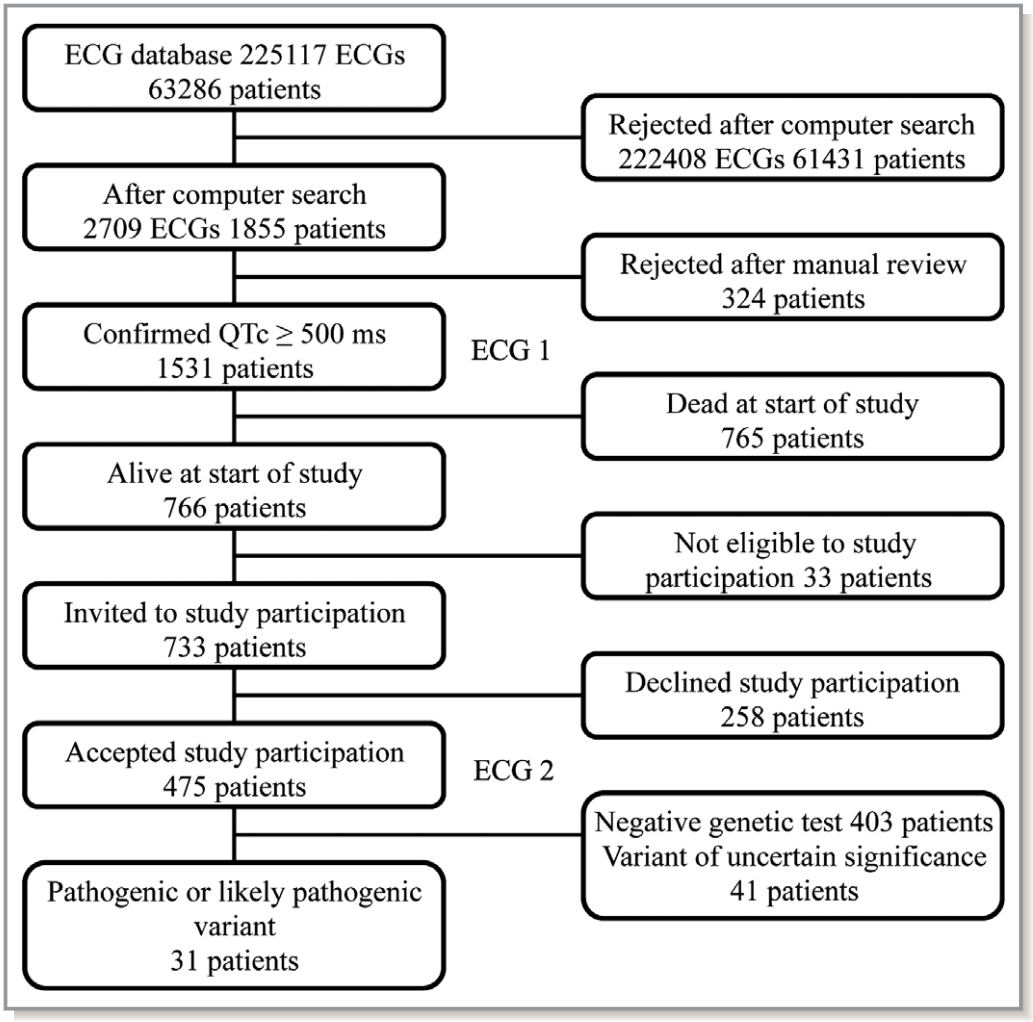

Figure 1. Flow chart of the inclusion process. QTc indicates corrected QT interval.

(NM_000891.2), *KCNJ5* (NM_000890.3), *KCNQ1* (NM_000218.2), *SCN4B* (NM_174934.3), *SCN5A* (NM_198056.2), *SNTA1* (NM_003098.2), and *TRDN* (NM_006073.3). The patients were not referred to testing in a clinical setting, but were included in a research project based solely on prolonged QTc.[11] Further details on DNA sequencing, bioinformatics, and RNA analyses are found in Data S1.

Variant Interpretation

An alternative allele frequency cutoff of 0.1% from the Exome Aggregation Consortium database (populations: all or non-Finnish Europeans) was used for filtering variants (Figure 2). Additional databases of allele frequencies, such as gnomAD,[14] SweGen,[15] 2000 Danes,[16] and the in-house Telemark database with ≈1000 exomes, were consulted when manually reviewing the variants that remained after filtering. Sequence variants that were synonymous (predicting no change in amino acids), intronic (outside splice sites), or in untranslated regions were discarded, unless they had previously been reported as pathogenic or likely pathogenic. Genes and variants that remained after filtering were manually reviewed in the light of available clinical and biological data to evaluate causality. The key tools used for this were Alamut Visual decision-support software (Interactive Biosoftware), Human Gene Mutation Database,[5] and ClinVar.[17]

Sequence variants were divided into 5 classes: class 5, pathogenic; class 4, likely pathogenic; class 3, uncertain significance; class 2, likely benign; and class 1, benign. Classification was based on guidelines from the Association for Clinical Genetic Science and the American College of Medical Genetics and Genomics.[18] Previously reported pathogenic and likely pathogenic variants were not accepted as pathogenic without scrutinizing newly available evidence, such as allele frequencies and in vitro studies.

Statistical Analysis

Continuous data were described by mean±SD or median (range) and compared using the unpaired Student *t* test or

Table 1. Medical Conditions and Factors Known to Prolong QTc at the Time of ECG 1 and ECG 2 for 31 Patients With Pathogenic or Likely Pathogenic Variants and 41 Patients With VUS

QT-Prolonging Medical Conditions	Pathogenic or Likely Pathogenic Variants (n=31)		VUS (n=41)	
	ECG 1	ECG 2	ECG 1	ECG 2
Acute coronary syndrome within 7 d	1 (3)	0 (0)	9 (22)	0 (0)
Anorexia or starvation	0 (0)	0 (0)	0 (0)	0 (0)
Heart rate <45 bpm	0 (0)	0 (0)	0 (0)	0 (0)
Diabetes mellitus 1 and 2	4 (13)	4 (13)	8 (20)	8 (20)
Ejection fraction <40%	0 (0)	0 (0)	7 (17)	1 (2)
Female sex	20 (65)	20 (65)	21 (51)	21 (51)
Hypertrophic cardiomyopathy	0 (0)	0 (0)	0 (0)	0 (0)
Hypoglycemia (in the absence of diabetes mellitus)	0 (0)	0 (0)	0 (0)	0 (0)
Intoxication with QT-prolonging drugs	0 (0)	0 (0)	2 (5)	0 (0)
Known genetic LQTS	1 (3)	1 (3)	0 (0)	0 (0)
Pheochromocytoma	0 (0)	0 (0)	0 (0)	0 (0)
Renal dialysis	0 (0)	0 (0)	1 (2)	1 (2)
Status <7 d after AF conversion	1 (3)	0 (0)	1 (2)	0 (0)
Status <24 h after cardiac arrest	0 (0)	0 (0)	1 (2)	0 (0)
Status <24 h after syncope or seizure	1 (3)	0 (0)	4 (10)	0 (0)
Status <7 d after stroke, subarachnoid hemorrhage, or head trauma	1 (3)	0 (0)	1 (2)	0 (0)
Serum electrolyte disturbances	3 (10)	1 (3)	17 (41)	0 (0)
Drugs with known risk of TdP*	4 (13)	3 (10)	9 (22)	9 (22)
Drugs with possible risk of TdP*	2 (6)	1 (3)	4 (10)	8 (20)
Drugs with conditional risk of TdP*	11 (35)	10 (32)	18 (44)	19 (46)
Drugs with special risk for patients with LQTS*	2 (6)	1 (3)	6 (15)	2 (5)

Data are given as number (percentage) of patients. AF indicates atrial fibrillation; bpm, beats per minute; LQTS, long-QT syndrome; QTc, corrected QT interval; TdP, torsade de pointes ventricular tachycardia; VUS, variants of uncertain significance.
*The QT-prolonging drug categories are according to AZCERT, Inc.[13]

independent-samples Mann-Whitney *U* test, as appropriate. Categorical data were described as proportions and analyzed by the χ^2 test (SPSS, version 23.0; IBM, Armonk, NY). A 2-sided $P<0.05$ was considered statistically significant.

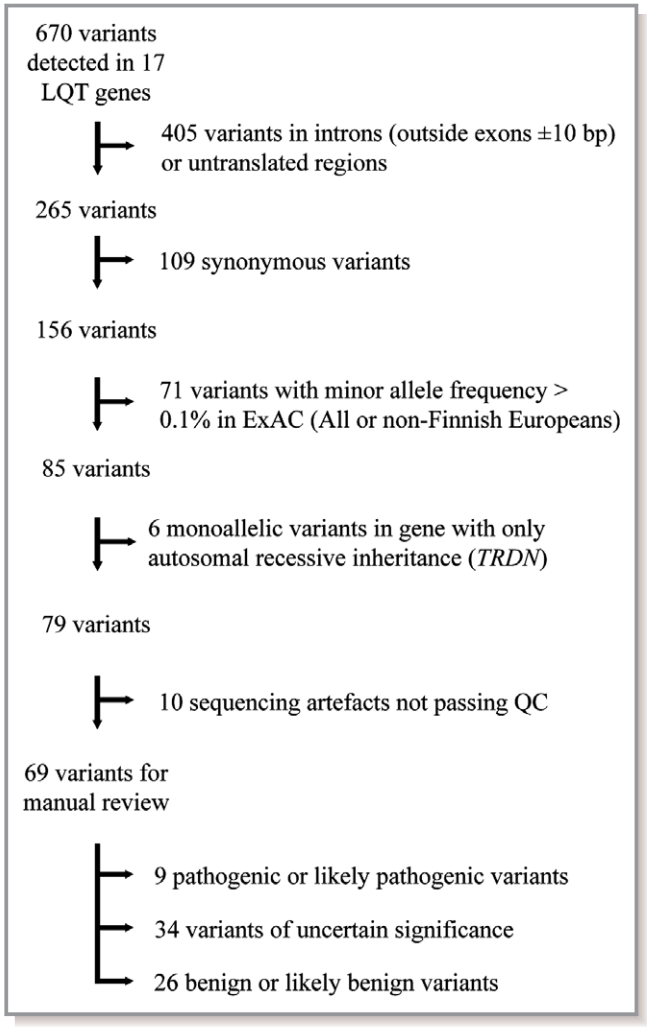

Figure 2. Flow chart of filtering and evaluation of sequence variants. Bp indicates base pair; ExAC, Exome Aggregation Consortium; LQT, long QT; QC, quality control.

Ethics

The study complies with the Declaration of Helsinki. The Norwegian Regional Committee for Medical and Health Research Ethics has approved the study (2013/1090), and informed consent was obtained from all patients. Genetic counseling was offered to all patients with pathogenic or likely pathogenic mutations. Patients with a pathogenic or likely pathogenic variant were assigned an LQTS diagnosis in an inherited disease clinic.

Results

Characteristics of Study Population at Time of First ECG With QTc ≥500 ms (ECG 1)

Demographics for the 475 (65%) of 733 patients who participated in the study are shown in Table 2. They underwent genetic testing and were on average 3 years

Table 2. Demographics of 733 Invited Patients at the Time of the First ECG With QTc ≥500 ms ("ECG 1")

Demographics	Total (n=733)	Participants (n=475)	Nonparticipants (n=258)	*P* Value
Age, y	64±15	63±14	66±16	0.01
Female sex	448 (61)	294 (62)	154 (60)	0.58
Heart rate, bpm	78 (39–100)	77 (39–100)	81 (48–100)	<0.01
QRS duration, ms	94±12	93±12	94±12	0.32
QTc, ms	512 (500–669)	512 (500–669)	515 (500–634)	0.17
Hypokalemia	200/683 (29)	124/441 (30)	76/242 (31)	0.60
No. of QT-prolonging drugs	1 (0–5)	1 (0–5)	1 (0–5)	0.34
No. of QT-prolonging conditions*	1 (0–4)	1 (0–4)	1 (0–3)	0.29
Pro-QTc score	2.7±1.5	2.7±1.6	2.7±1.5	0.95

Data are given as mean±SD, number/total (percentage), or median (range). Bpm indicates beats per minute; QTc, corrected QT interval.
*Female sex, electrolyte disturbances, and medication not included.

younger than those who declined (63±14 versus 66±16 years; *P*=0.01). There were no significant differences in QTc, sex, hypokalemia, number of QT-prolonging conditions, number of QT-prolonging drugs, or pro-QTc score between study participants and nonparticipants at the time of ECG 1 (Table 2).

A pathogenic or likely pathogenic genetic variant was identified in 31 (6.5%) of 475 patients (Table 3). The criteria applied for classification of genetic variants are shown in Table 4. These patients had a lower pro-QTc score than those without pathogenic or likely pathogenic variants (1.7±1.0 versus 2.8±1.6; *P*<0.001) (Table 5). Of the 31 patients with a pathogenic or likely pathogenic variant, 12 (39%) had an additional nongenetic explanation for QTc prolongation at the time of ECG 1 (Table 1). The median serum potassium level was 3.5 mmol/L (range, 3.4–3.6 mmol/L) among those with hypokalemia. The sole acute coronary syndrome was minor, without any other ECG changes than the QTc prolongation. Three patients with pathogenic or likely pathogenic variants used potent QT-prolonging antiarrhythmic drugs (Table S1).

Among the patients without pathogenic or likely pathogenic variants, 75 (74%) of the 101 patients with acute coronary syndrome had ischemic ECG changes. The median serum potassium level was 3.4 mmol/L (range, 1.9–3.6 mmol/L) among those with hypokalemia.

A genetic VUS was identified in 41 (8.6%) of 475 patients (Table S2). There were no significant differences in QTc, sex, hypokalemia, number of QT-prolonging conditions, number of QT-prolonging drugs, or pro-QTc score between these patients and patients with negative genetic test results (Table S3). All of the patients carrying a VUS had a plausible nongenetic explanation for QTc prolongation at the time of ECG 1 (Table 1, Table S4).

Awareness of QT Prolongation by Healthcare Provider

QTc prolongation was acknowledged in the medical records in 7 (23%) of the 31 patients with a pathogenic or likely pathogenic variant at the time of ECG 1. Only 1 of those had been offered a follow-up visit and was found to have a QTc of 472 ms after discontinuation of QT-prolonging medication. QT prolongation was acknowledged in the medical records in 59 (13%) of the 444 patients without a pathogenic or likely pathogenic variant.

QT Interval and LQTS Risk Score (Schwartz)

Among the patients with a pathogenic or likely pathogenic variant, the median QTc in ECG 1 was 511 ms (range, 501–577 ms) and the median QTc in ECG 2 was 479 ms (range, 413–565 ms) (Table 3). One patient missed ECG 2. The median difference between the QTc in ECG 1 and ECG 2 was −33 ms (range, −96 to 52 ms) (Figure 3). Of 30 patients, 4 (13%) had a QTc ≥500 ms and 15 (50%) had a QTc <480 ms on ECG 2 (Figure 4). Only 1 of the patients with a pathogenic or likely pathogenic variant had a longer QTc at the time of ECG 2 compared with ECG 1.

Of the 31 patients with a pathogenic or likely pathogenic variant, 6 (19%) fulfilled the criteria for a clinical diagnosis of LQTS on the basis of LQTS risk score (Schwartz). One patient had a family history of sudden death in a first-degree relative with congenital deafness who died at the age of 6 years. Another patient had a known family history of LQTS. Furthermore, 1 patient had a previously diagnosed LQTS. From the age of 6 years, 1 patient had experienced fainting spells, previously thought to be of epileptic origin, but now

Table 3. Patients With a Pathogenic or Likely Pathogenic Variant

No.	Gene	cDNA Change	Protein Change	Age at ECG 1, y	Sex	QTc at ECG 1, ms	QTc at ECG 2, ms	Family History	Syncope
1	*KCNH2*	c.157G>A	p.(Gly53Ser)	56	Male	535	496	No	Yes
2	*KCNH2*	c.2257G>T	p.(Ala753Ser)	15	Male	520	450	No	Yes
3	*KCNH2*	c.2682_2685dup	p.(Asp896Hisfs*25)	53	Male	513	565	No	No
4	*KCNH2*	c.2775dup	p.(Pro926Alafs*14)	58	Female	517	511	No	No
5	*KCNQ1*	c.573_577del	p.(Arg192Cysfs*91)	62	Male	504	492	No	No
6	*KCNQ1*	c.1588C>T	p.(Gln530*)	39	Female	508	508	No	Yes
7	*KCNQ1*	c.1588C>T	p.(Gln530*)	39	Male	509	413	No	No
8	*KCNQ1*	c.1588C>T	p.(Gln530*)	43	Female	501	481	No	No
9	*KCNQ1*	c.1588C>T	p.(Gln530*)	45	Female	520	458	No	Yes
10	*KCNQ1*	c.1588C>T	p.(Gln530*)	51	Male	551	477	No	No
11	*KCNQ1*	c.1588C>T	p.(Gln530*)	57	Female	504	473	No	No
12	*KCNQ1*	c.1588C>T	p.(Gln530*)	60	Female	504	469	No	No
13	*KCNQ1*	c.1588C>T	p.(Gln530*)	62	Female	510	467	Yes	Yes
14	*KCNQ1*	c.1588C>T	p.(Gln530*)	62	Female	543	459	No	No
15	*KCNQ1*	c.1588C>T	p.(Gln530*)	64	Female	502	470	No	No
16	*KCNQ1*	c.1588C>T	p.(Gln530*)	66	Male	501	486	No	No
17	*KCNQ1*	c.1588C>T	p.(Gln530*)	68	Female	538	482	No	No
18	*KCNQ1*	c.1588C>T	p.(Gln530*)	70	Male	508	495	No	No
19	*KCNQ1*	c.1588C>T	p.(Gln530*)	70	Male	503	457	No	No
20	*KCNQ1*	c.1588C>T	p.(Gln530*)	73	Male	577	NA	No	No
21	*KCNQ1*	c.1588C>T	p.(Gln530*)	77	Female	520	484	No	No
22	*KCNQ1*	c.1588C>T	p.(Gln530*)	79	Female	506	474	No	No
23	*KCNQ1*	c.1588C>T	p.(Gln530*)	81	Female	506	499	No	No
24	*KCNQ1*	c.1588C>T	p.(Gln530*)	83	Female	515	459	No	No
25	*KCNQ1*	c.1588C>T	p.(Gln530*)	86	Female	516	475	No	No
26	*KCNQ1*	c.1588C>T	p.(Gln530*)	86	Female	508	474	No	No
27	*KCNQ1*	c.1588C>T	p.(Gln530*)	87	Female	532	520	No	Yes
28	*KCNQ1*	c.1588C>T	p.(Gln530*)	88	Female	511	462	Yes	No
29	*KCNQ1*	c.1591-1G>A	p.?	56	Female	502	496	No	No
30	*KCNQ1*	c.1760C>T	p.(Thr587Met)	38	Female	504	493	Yes	No
31	*SCN5A*	c.4931G>A	p.(Arg1644His)	58	Male	513	481	No	Yes

NA indicates not available; QTc, corrected QT interval.

considered symptoms of LQTS. No patients had any documented episodes of torsade de pointes ventricular tachycardia or cardiac arrest, but 6 patients had previously had a syncope that could have been related to LQTS.

For the patients with a VUS, the median QTc in ECG 1 was 510 ms (range, 500–639 ms) and the median QTc in ECG 2 was 449 ms (range, 377–498 ms). One patient missed ECG 2. The median difference between the QTc in ECG 1 and ECG 2 was −73 ms (range, −5 to −228 ms) (Figure 3). None of the patients carrying a VUS had a QTc ≥500 ms, and 35 (88%) of 40 patients had a QTc of <480 ms on ECG 2 (Figure 4). None of the patients with VUS had an LQTS risk score (Schwartz) >3. None had a family history of sudden death in a first-degree relative. One patient with a VUS (c.5962A>C p.[Met1988Leu] in ANK2) had a syncope that could have been related to LQTS, had documented polymorphic ventricular tachycardia, and had an implantable cardioverter-defibrillator implanted.

Patients with a pathogenic or likely pathogenic variant had a higher median QTc in ECG 2 than the patients with VUS (479 versus 449 ms; $P<0.001$).

Table 4. ACMG Criteria Used for Classification of Pathogenic and Likely Pathogenic Variants

Gene	cDNA Change	Protein Change	ACMG/AMP Criteria	Classification
KCNH2	c.2775dup	p.(Pro926Alafs*14)	PVS1, PS3, PM2	Pathogenic
KCNH2	c.2682_2685dup	p.(Asp896Hisfs*25)	PVS1, PM2, PP5	Pathogenic
KCNH2	c.2257G>T	p.(Ala753Ser)	PM1, PM2, PP2, PP3, PP5	Likely pathogenic
KCNH2	c.157G>A	p.(Gly53Ser)	PM1, PM2, PM5-S*, PP2, PP5	Pathogenic
KCNQ1	c.573_577del	p.(Arg192Cysfs*91)	PVS1, PP1, PP5	Pathogenic
KCNQ1	c.1588C>T	p.(Gln530*)	PVS1, PS4, PP1	Pathogenic
KCNQ1	c.1591-1G>A	p.?	PVS1, PS3, PM2	Pathogenic
KCNQ1	c.1760C>T	p.(Thr587Met)	PS1, PS3, PM1, PM2, PP5	Pathogenic
SCN5A	c.4931G>A	p.(Arg1644His)	PS1, PS3, PM2, PP3	Pathogenic

ACMG indicates American College of Medical Genetics and Genomics; AMP, Association for Molecular Pathology; PM, pathogenic moderate; PP, pathogenic supporting; PS, pathogenic strong; PVS, pathogenic very strong.
*PM5 assigned strong evidence of pathogenicity because both Gly53Asp and Gly53Arg previously have been reported as pathogenic and both were trafficking deficient.

Genetic Analyses

A total of 670 sequence variants were detected in the 17 known LQTS genes. Of these, 69 were coding or splice site variants with a minor allele frequency <0.1% in the Exome Aggregation Consortium database (Figure 2). On average, the patients harbored 0.26 such rare coding variants. All pathogenic or likely pathogenic variants were heterozygous. The previously reported variant *KCNQ1* c.1588C>T p.(Gln530*) was present in 23 patients. The other 8 variants were all detected in single cases. Patients heterozygous for p.(Gln530*) were older than the other patients with a pathogenic or likely pathogenic variant (67±16 versus 50±16 years; P=0.01) and more likely to be women (74% versus 38%; P=0.08) (Table S5). By excluding patients with the Gln530* variant, the 8 patients with pathogenic or likely pathogenic variants other than *KCNQ1* c.1588C>T p.(Gln530*) were younger than the patients with negative genetic test results (50±16 versus 63±14 years; P<0.01).

Two novel pathogenic null variants were detected. *KCNH2* c.2682_2685dup p.(Asp896Hisfs*25) is located in the 3′ end of exon 11 and within a few base pairs reach of 3 other frameshift variants previously reported in patients with LQTS.[19–21] *KCNQ1* c.1591-1G>A p.(?) disrupts normal pre-mRNA splicing (Figure S1).

Discussion

In the present study, a genetic diagnosis was obtained in 31 (6.5%) of 475 patients with at least 1 ECG with a QTc ≥500 ms. This illustrates that a QTc ≥500 ms in at least 1 ECG recording may greatly increase the likelihood of pathogenic or likely pathogenic variants in LQTS genes in hospitalized patients compared with the general population. This is a 130-fold increase relative to the estimated population prevalence of 1:2000.[1]

Table 5. Demographics of 475 Genetically Tested Patients at the Time of the First ECG With QTc ≥500 ms ("ECG 1")

Demographics	Total (n=475)	Pathogenic or Likely Pathogenic Variant (n=31)	VUS and Negative Genetic Test Result (n=444)	*P* Value
Age, y	63±14	63±17	63±14	0.86
Female sex	294 (62)	20 (65)	274 (62)	0.85
Heart rate, bpm	77 (39–100)	66 (41–97)	77 (39–100)	<0.01
QRS duration, ms	93±12	88±10	94±12	<0.01
QTc, ms	512 (500–669)	511 (501–577)	512 (500–669)	0.50
Hypokalemia	124/441 (28)	3/22 (14)	121/419 (30)	<0.001
No. of QT-prolonging drugs	1 (0–5)	0 (0–2)	1 (0–5)	0.071
No. of QT-prolonging conditions*	1 (0–4)	0 (0–1)	1 (0–4)	0.001
Pro-QTc score	2.7±1.6	1.7±1.0	2.8±1.6	<0.001

Data are given as mean±SD, number/total (percentage), or median (range). Bpm indicates beats per minute; QTc, corrected QT interval; VUS, variants of uncertain significance.
*Female sex, electrolyte disturbances, and medication not included.

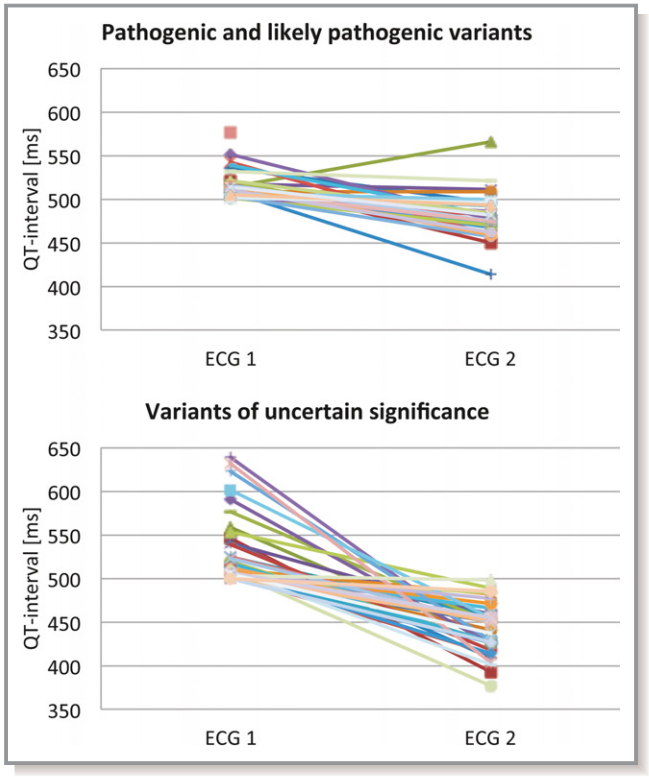

Figure 3. Corrected QT interval at the time of ECG 1 and ECG 2 among 31 patients with pathogenic or likely pathogenic variants and 41 patients with variants of uncertain significance.

Current guidelines from the European Society of Cardiology recommend clinical diagnosis and genetic testing in an asymptomatic individual if repetitive ECGs show a QTc ≥480 ms. An LQTS risk score (Schwartz) >3 is also clinically diagnostic.[2] Genetic testing is usually reserved for patients with persistent QTc prolongation after withdrawal or resolution of the QT-prolonging factors. We, therefore, included ECG 2 after QT-prolonging factors had been recognized and modified when appropriate and the patients with an acute illness had recovered. If European Society of Cardiology guidelines had been followed, 47% of the patients with a pathogenic or likely pathogenic variant in the present study would probably not have been considered for the diagnosis. The Heart Rhythm Society/European Heart Rhythm Association Expert Consensus Statement recommends cutoff on repetitive ECGs to QTc >500 ms, which would have failed to diagnose 73% of the patients with a pathogenic or likely pathogenic variant in the present study.[7]

The awareness of QT prolongation and the possibility of LQTS was low. At Telemark Hospital Trust, the physician ordering the ECG is responsible for the interpretation. At the time of the present study, the QTc duration was indicated on the ECG, but without automated description or flagging of the QT prolongation. QT alert systems have been described previously and could increase awareness of QT prolongation.[12]

The previously reported *KCNQ1* c.1588C>T p.(Gln530*) was identified in 23 of 31 patients with a pathogenic or likely pathogenic variant. Thus, this founder mutation inflates the prevalence of LQTS in Telemark. The Gln530* carriers were significantly older than the other patients with pathogenic or likely pathogenic variants, which could question this variant's effect on QTc interval and mortality. Several other variants previously reported to cause LQTS have recently been proved to have no effect on QTc, syncope, or overall mortality.[22] This illustrates the need for reevaluation before accepting previous

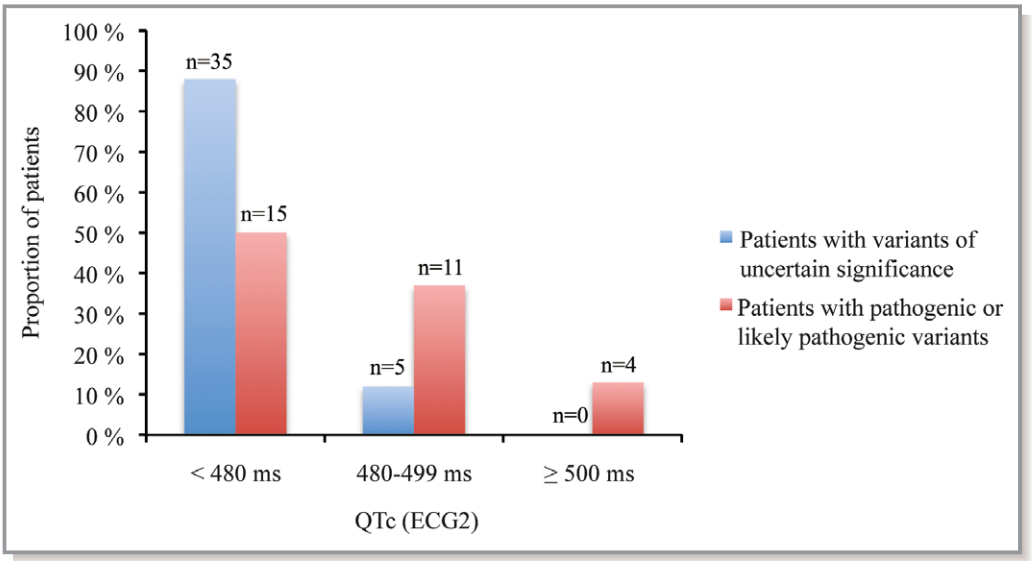

Figure 4. Bar chart showing the corrected QT interval (QTc) distribution at the time of ECG 2 among 30 patients with pathogenic or likely pathogenic variants (red) and 40 patients with variants of uncertain significance (blue).

claims of pathogenicity. Gln530* has previously been reported as the most prevalent pathogenic KCNQ1 variant in Norway,[6] and it is most common in Telemark's neighbor district, Agder. It has consistently been reported as pathogenic in several populations.[17] In the gnomAD database, Gln530* is present in 7 of 55 818 non-Finnish European individuals, but absent from all other populations. The variant is also absent from SweGen, 2000 Danes, 1000 genomes Norway, and the Telemark database, which together contain 8000 alleles.[15,16,23] This supports that the variant is associated with a significant increase in QTc and with an increased risk of cardiac events, although this risk is reported to be lower than for *KCNQ1* missense variants.[24]

Most studies on the risk of life-threatening outcomes in LQTS have been performed in children and young adults, in contrast to the population in our study. Risk of adverse outcome has been assumed to be low in patients >40 years of age. However, in a previous study from the International Long QT Syndrome Registry, the risk of aborted cardiac arrest, sudden cardiac death, and implantable cardioverter-defibrillator therapy was maintained until the age of 60 years.[25] The likelihood of serious cardiac events is increased in carriers of a pathogenic variant, even when the QTc is within the normal range.[3] Thus, for the patients with a pathogenic or likely pathogenic variant in our population, lifestyle modifications, evaluation of drug use, and in some cases prophylactic medical treatment are indicated.

Genetic cascade testing for detected LQTS mutations is warranted by the ability to identify relatives at risk. Many at-risk individuals are likely to be unaware of their status in light of the disorder's incomplete penetrance, variable expressivity, and unpredictable course.[6,26] The risk of life-threatening cardiac events is highest in young adolescence, and identification of a pathogenic variant in an LQTS gene has the potential to prevent life-threatening cardiac events in offspring or siblings.[7,27]

More patients with a pathogenic or likely pathogenic variant had a diagnostic Schwartz score, no clear QT-prolonging factor, and persistent QT prolongation on ECG 2 compared with the patients with a VUS. Furthermore, many of the patients with a pathogenic or likely pathogenic variant had QT prolongation exceeding the values that one may expect from a specific QT-prolonging factor, favoring these patients when considering patients for genetic testing. A role for common variants on QT interval and arrhythmia has been demonstrated, but they still have limited clinical utility and their contribution in this cohort has not been analyzed.[28,29]

Of the 17 genes tested, pathogenic or likely pathogenic variants were detected only in the 3 major genes *KCNQ1*, *KCNH2*, and *SCN5A*. In contrast, 7 genes contained at least 1 VUS, and only 16 of 47 VUS were identified in the 3 major genes. Five patients carried 2 VUS each. One of these patients was asymptomatic but had consistently QTc >480 ms in >10 ECGs, and another patient had QTc ≥497 ms in 4 ECGs. It could be speculated that the coexistence of >1 VUS could increase QTc, because additive effects have been suggested by others.[30,31] However, the clinical characteristics of the 41 patients with VUS are comparable to the mutation-negative group. This supports the notion that VUS are of limited clinical importance. Whether a VUS represents a benign variant, a proarrhythmic variant requiring secondary provocation, or a truly pathogenic variant remains to be determined. Until then, they should not be used for clinical decisions.

Study Limitations

All patients were recruited from Telemark County in Norway, which has 173 000 inhabitants who are relatively homogeneous from a genetic perspective. The mutation spectrum would thus likely be different in other populations. The Gln530* variant inflates the prevalence of LQTS in Telemark, and the prevalence of LQTS may be different in other populations.

Only patients still alive at the time of inclusion were included. The mutation spectrum and prevalence of pathogenic or likely pathogenic variants may be different in the deceased individuals. It is possible that patients with a history of cardiac disease (themselves or relatives) are more prone to accept genetic testing.

Only the 17 known LQTS genes have been sequenced, and not all genetic defects in these genes will be detected by next-generation sequencing. Most intronic variants and deletions/duplications larger than ≈40 base pairs escape detection. Interpretation of the pathogenicity of genetic variation is based on current genetic and medical knowledge and is likely to change over time.

The pro-QTc score was developed to predict mortality in hospitalized patients with QT prolongation. In the present study, the pro-QTc score was used to assess the number of QT-prolonging factors. The CredibleMeds QTdrugs lists were accessed in 2015, and the pro-QTc score, therefore, does not include the most recent drugs added to the lists. Automated T-wave morphological analyses were not performed in the present study. Future studies should assess T-wave morphological characteristics in patients with a prolonged QT interval.

Bazett's formula overestimates QTc at high heart rates, but there were no significant differences between the groups at the time of ECG 1 using QTc Fredericia or QTc Framingham (Table S6).

Conclusion

We detected a molecular genetic diagnosis of LQTS in 31 (6.5%) of 475 patients with at least 1 ECG with a QTc ≥500 ms in a community hospital. Compared with the general

population, hospitalized patients with a QTc ≥500 ms in at least 1 ECG recording had an increased likelihood for pathogenic and likely pathogenic variants in LQTS genes. If European Society of Cardiology guidelines had been followed, 47% of the patients carrying pathogenic and likely pathogenic variants in the present study would not have been diagnosed. If the Heart Rhythm Society/European Heart Rhythm Association Expert Consensus Statement had been adhered to, 73% of the patients would not have been diagnosed. We recommend increased awareness of the possibility of LQTS in patients with at least 1 ECG with a QTc ≥500 ms.

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Disclosures

None.

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Supplemental Material

Data S1.

DNA sequencing

Genomic DNA was extracted from EDTA blood using Agencourt Genfind v2 (Beckman Coulter). A panel targeting the 17 genes *AKAP9* (NM_005751.4), *ANK2* (NM_1148.4), *CACNA1C* (NM_000719.6), *CALM1* (NM_006888.4), *CALM2* (NM_001743.4), *CALM3* (NM_005184.2), *CAV3* (NM_033337.2), *KCNE1* (NM_000219.5), *KCNE2* (NM_172201.1), *KCNH2* (NM_000238.3), *KCNJ2* (NM_000891.2), *KCNJ5* (NM_000890.3), *KCNQ1* (NM_000218.2), *SCN4B* (NM_174934.3), *SCN5A* (NM_198056.2), *SNTA1* (NM_003098.2) and *TRDN* (NM_006073.3) was designed using DesignStudio (Illumina). NGS sample preparation and enrichment was performed using Illumina's Nextera Rapid Capture Custom Enrichment kit according to the manufacturer's recommendation. Samples were sequenced 2x150 bp on a NextSeq 500 (Illumina).

All pathogenic and likely pathogenic variants were confirmed with Sanger sequencing on an ABI3130, using a validated and accredited method in accordance with ISO15189.

Bioinformatics pipeline

The reads were mapped to the reference sequence (GRCh37/hg19) by BWA (Burrows-Wheeler Aligner) [21]. GATK (Genome Analysis Toolkit) was used for base quality score recalibration, indel realignment, duplicate removal and SNP and INDEL discovery [22-24]. Mean target coverage was 876X, and on average 97 % of all targeted bases had coverage greater than 20X. Variants were annotated by Annovar [25]. Filtus software was used for bioinformatic filtering [26].

RNA-analyses

PAXgene Blood RNA tubes were used for collection of blood samples for RNA analyses. Total RNA was extracted with PAXgene Blood RNA Kit (Qiagen). RT-PCR was performed

with OneStep RT-PCR Kit (Qiagen). Amplicons were run on 1.5 % agarose gels, DNA was extracted from individual bands using QIAquick Gel Extraction Kit (Qiagen) and Sanger sequenced on an ABI3130 (Thermo Fisher Scientific).

Table S1. Number of drugs from CredibleMeds QTdrugs lists at time of ECG 1 and ECG 2 for 31 patients with a pathogenic or likely pathogenic variant.

	ECG1	ECG2
Drugs with known risk TdP	Sotalol (n=1) Amiodarone (n=1) Flecainide (n=1) Citalopram (n=1)	Amiodarone (n=1) Flecainide (n=1) Citalopram (n=1)
Drugs with possible risk TdP	Tamoxifen (n=1) Lithium (n=1)	Lithium (n=1)
Drugs with conditional risk TdP	Hydrochlorothiazide (n=4) Furosemide (n=3) Solifenacin (n=1) Sertraline (n=1) Pantoprazole (n=2)	Hydrochlorothiazide (n=4) Furosemide (n=1) Pantoprazole (n=3) Solifenacin (n=1) Sertraline (n=1) Paroxetine (n=1)
Drugs with special risk for LQTS patients	Salbutamol (n=1) Salmeterol (n=1) Formeterol (n=1)	Salmeterol (n=1) Formeterol (n=1)

ECG=Electrocardiogram; TdP=torsades de pointes; VUS= variants of uncertain significance

Table S2. Variants of uncertain significance.

No.	Gene	cDNA change	Protein change	gnomAD	References	SampleID	Comments
1	*AKAP9*	c.815C>G	p.(Thr272Ser)	-	-	LQT0232	c.814A>G p.(Thr272Ala) has one allele in gnomAD (1/245900)
2	*AKAP9*	c.974del	p.(Gln325Argfs*10)	-	-	LQT0082	Loss-of-function in *AKAP9* has not previously been reported to cause LQT.
3	*AKAP9*	c.4604C>G	p.(Pro1535Arg)	4/107712 NFE	-	LQT0376	-
4	*AKAP9*	c.5084T>C	p.(Val1695Ala)	1/17362 AFR	-	LQT0175	Macaque has alanine in this position. Predicted benign.
5	*AKAP9*	c.10748C>T	p.(Ser3583Leu)	2/112130 NFE	-	LQT0246	1/492 NOR alleles
6	*ANK2*	c.158G>C	p.(Gly53Ala)	2/126490 NFE	-	LQT0171*	-
7	*ANK2*	c.2890A>G	p.(Ile964Val)	4/125206 NFE	-	LQT0130	Predicted benign
8	*ANK2*	c.3584G>A	p.(Gly1195Asp)	-	-	LQT0037	-
9	*ANK2*	c.4147G>C	p.(Val1383Leu)	-	-	LQT0371	2/1600 alleles in Telemark inhouse database. c.4147G>A p.(Val1383Ile) and c.4147G>T p.(Val1383Phe) each has 1/245990 alleles in gnomAD
10	*ANK2*	c.4952C>A	p.(Pro1651His)	2/245854 ALL	-	LQT0009	Predicted benign
11	*ANK2*	c.5962A>C	p.(Met1988Leu)	-	-	LQT0442	-
12	*CACNA1C*	c.2078C>T	p.(Pro693Leu)	-	-	LQT0078 LQT0482	-
13	*CACNA1C*	c.2437G>A	p.(Gly813Arg)	4/71678 NFE	-	LQT0118 LQT0143	Highly conserved glycine. Likely benign in ClinVar
14	*CACNA1C*	c.2444C>A	p.(Ser815Tyr)	-	-	LQT0120	-
15	*CACNA1C*	c.2654C>G	p.(Ser885Cys)	-	-	LQT0327	Predicted benign
16	*CACNA1C*	c.2954G>T	p.(Gly985Val)	-	-	LQT0105	-
17	*CACNA1C*	c.3080G>T	p.(Arg1027Leu)	-	-	LQT0132*	Predicted likely pathogenic. c.3079C>T p.(Arg1027Trp) has 2/246242 alleles in gnomAD
18	*CACNA1C*	c.4043C>T	p.(Thr1348Met)	1/17240 EA	-	LQT0302	-
19	*CACNA1C*	c.4819C>T	p.(Pro1607Ser)	3/70458 NFE	1	LQT0235 LQT0329 LQT0430*	Predicted likely pathogenic. Published by Lieve et al. as pathogenic mutation in one patient [1].
20	*CACNA1C*	c.5657G>T	p.(Gly1886Val)	-	-	LQT0471	7/31786 alleles in CentoMD (unclassified). 1/3930 DAN. G>A

							p.(Gly1886Asp) in 2/30778 SA, G>C p.(Gly1886Ala) in 1/111406 NFE
21	*CACNA1C*	c.6235G>A	p.(Asp2079Asn)	7/98762 NFE	-	LQT0228 LQT0263	-
22	*CALM1*	c.14T>C	p.(Leu5Pro)	-	-	LQT0233	Reported pathogenic mutations in *CALM1* are C-terminal whereas p.(Leu5Pro) is N-terminal.
23	*KCNH2*	c.889C>T	p.(Pro297Ser)	4/14522 NFE	2-4	LQT0161 LQT0199*	Found in one LQT case by Kapa et al.[2] and in one young sudden unexplained death case by Winkel et al.[3]. Reported as potential false positive by Giudicessi et al. [4]. This is a poorly covered region in exomes.
24	*KCNH2*	c.2653C>T	p.(Arg885Cys)	10/25116 FIN	5-7	LQT0162	2/15875 alleles in CentoMD (unclassified). Reported as pathogenic by Berge et al.[5], and as VUS by Amendola et al. [6] and Dorschner et al.[7] Predicted likely pathogenic.
25	*KCNH2*	c.3118A>G	p.(Ser1040Gly)	-	8	LQT0312 LQT0334 LQT0344 LQT0430*	1/492 NOR alleles. Found in one sudden infant death syndrome case by Arnestad et al.[8].
26	*SCN4B*	c.34G>A	p.(Ala12Thr)	3/108260 NFE	-	LQT0171*	VUS in ClinVar. Predicted benign. Localized to exon 1, whereas all reported pathogenic variants in *SCN4B* are in exon 4 and 5.
27	*SCN5A*	c.3707A>C	p.(Lys1236Thr)	-	-	LQT0064	Predicted likely deleterious. Other variants affecting the same codon, p.(Lys1236Arg) and p.(Lys1236Asn) have each been reported in one patient with Brugada syndrome [9,10].
28	*SCN5A*	c.3430_3434del	p.(Met1144Glnfs*4)	-	-	LQT0273	Most, but not all, loss-of-function variants in *SCN5A* result in Brugada syndrome.
29	*SCN5A*	c.4501C>G	p.(Leu1501Val)	5/112248 NFE	11-14	LQT0217* LQT0359	Likely pathogenic in ClinVar. Pathogenic in CentoMD. Predicted to introduce a cryptic splice site, but mRNA analyses show only partially

							disrupted splicing (Figure S1). Reported previously in both LQT- and Brugada syndrome patients.
30	*SCN5A*	c.5579G>A	p.(Arg1860Lys)	-	-	LQT0123 LQT0199* LQT0360	Another variant affecting the same codon, p.(Arg1860Ser), is reported in an LQT case who also harbored the known pathogenic *KCNQ1* (p.Arg243Cys) [15].
31	*SNTA1*	c.205G>A	p.(Glu69Lys)	0/	-	LQT0148	VUS in ClinVar
32	*SNTA1*	c.759T>A	p.(Asp253Glu)	18/126524 NFE	-	LQT0132*	VUS in ClinVar. 3/31714 alleles in CentoMD (unclassified)
33	*KCNH2*	c.1475A>G	p.(His492Arg)	-	-	LQT0203	Predicted likely pathogenic. Other variant affecting the same codon, p.(His492Tyr) has been reported to cause a milder LQT phenotype [16].
34	*KCNH2*	c.2738C>T	p.(Ala913Val)	22/9116 FIN (1 hom)	1, 17-20	LQT0217*	Uncertain and likely pathogenic in ClinVar.4/15875*2 alleles in CentoMD (uncertain). Conflicting results from *in vitro* functional testing. Predicted to introduce a cryptic splice site. It is our opinion that this variant is too common to be a highly penetrant pathogenic mutation, but it could be a risk factor.

* These patients harbored two VUS each

Predicted benign: Consistently predicted benign by SIFT, MutationTaster, PolyPhen-2 and Revel (accessed 24.01.2018)

Predicted likely pathogenic: Consistently predicted likely pathogenic/deleterious or pathogenic/deleterious by SIFT, MutationTaster, PolyPhen-2 and Revel (accessed 24.01.2018)

NOR The Norwegian 1000 genomes project http://kreftgenomikk.no/en/1000genomes/ (accessed 04.01.2016)

DAN 2,000 Danes WES (Diabetes Type 2 Study accessed through AlaMut software (accessed 24.01.2018)

gnomAD categories FIN=Finnish population, NFE=Non-Finnish European population, EA=East Asian population, AFR=African population

gnomAD=Genome Aggregation Database, VUS= Variants of uncertain significance

Table S3. Demographics of 41 patients with variants of uncertain significance (VUS) at time of first ECG with QTc ≥ 500 ms ("ECG 1").

	Total (n=444)	VUS (n=41)	Negative genetic test (n=403)	P- value
Age (years)	63 ± 14	62 ± 15	63 ± 14	0.73
Female sex	274 (62 %)	21 (51 %)	253 (63 %)	0.18
Heart rate (beats/min)	77 (39-100)	76 (48-98)	78 (39-100)	0.81
QRS duration (ms)	94 ± 12	95 ± 11	93 ± 12	0.56
QTc (ms)	512 (500-669)	510 (500-639)	512 (500-669)	0.39
Hypokalemia	121/419 (30 %)	17/39 (44 %)	104/380 (27 %)	0.09
Number of QT-prolonging drugs	1 (0-5)	1 (0-5)	1 (0-5)	0.94
Number of QT-prolonging conditions *	1 (0-4)	1 (0-3)	1 (0-4)	0.55
Pro-QTc score	2.8 ± 1.6	2.9 ± 1.6	2.8 ± 1.6	0.68

*Female sex, electrolyte disturbances and medication not included.

ECG=Electrocardiogram; QTc=corrected QT interval; VUS=Variants of uncertain significance

Table S4. Number of drugs from CredibleMeds QTdrugs lists at time of ECG 1 and ECG 2 for 41 patients with VUS.

	ECG1	ECG2
Drugs with known risk TdP	Amiodarone (n=3) Citalopram (n=4) Escitalopram (n=1) Ciprofloxacin (n=1) Propofol (n=1)	Citalopram (n=3) Amiodarone (n=2) Escitalopram (n=4)
Drugs with possible risk TdP	Venlafaxine (n=1) Mirtazapine (n=1) Lithium (n=1) Risperidone (n=1) Tacrolimus (n=1) Aripiprazole (n=1)	Venlafaxine (n=2) Promethazine (n=1) Mirtazapine (n=4) Tacrolimus (n=1) Aripiprazole (n=1) Lithium (n=1) Risperidone (n=1)
Drugs with conditional risk TdP	Furosemide (n=6) Pantoprazole (n=3) Hydrochlorothiazide (n=1) Metoclopramide (n=1) Metronidazole (n=2) Amitriptyline (n=1) Hydroxyzine (n=2) Paroxetine (n=1) Quetiapine (n=1) Amisulpride (n=1)	Quetiapine (n=2) Furosemide (n=4) Hydrochlorothiazide (n=5) Solifenacin (n=2) Pantoprazole (n=8) Metoclopramide (n= 1) Sertraline (n=1) Amisulpride (n=1)
Drugs with special risk for LQTS patients	Salbutamol (n=4) Formeterol (n=1) Noradrenaline (n=1)	Salmeterol (n=1) Formeterol (n=1)

ECG=Electrocardiogram; TdP=torsades de pointes; VUS= variants of uncertain significance

Table S5. Demographics of 23 patients with *KCNQ1* p.(Gln530*) compared to 8 patients with pathogenic or likely pathogenic variants at time of first ECG with QTc ≥ 500 ms ("ECG 1").

	Total (n=31)	*KCNQ1* p.(Gln530*) (n=23)	Pathogenic or likely pathogenic variant (n=8)	P- value
Age (years)	63 ± 17	67 ± 16	50 ± 16	0.01
Female sex	20 (65 %)	17 (74 %)	3 (38 %)	0.08
Heart rate (beats/min)	66 (41-97)	69 (41-97)	63 (49-86)	0.41
QRS duration (ms)	88 ± 10	88 ± 10	89 ± 10	0.74
QTc (ms)	511 (501-577)	510 (501-577)	513 (502-535)	0.95
Hypokalemia	3/22 (14 %)	3/17 (18 %)	0/5 (0 %)	0.72
Number of QT- prolonging drugs	0 (0-2)	1 (0-2)	0 (0-2)	0.24
Number of QT-prolonging conditions *	0 (0-1)	0 (0-1)	0 (0-1)	0.64
Pro-QTc score	1.7 ± 1.0	1.9 ± 0.9	1.1 ± 1.0	0.046

*Female sex, electrolyte disturbances and medication not included

ECG=Electrocardiogram; QTc=corrected QT interval

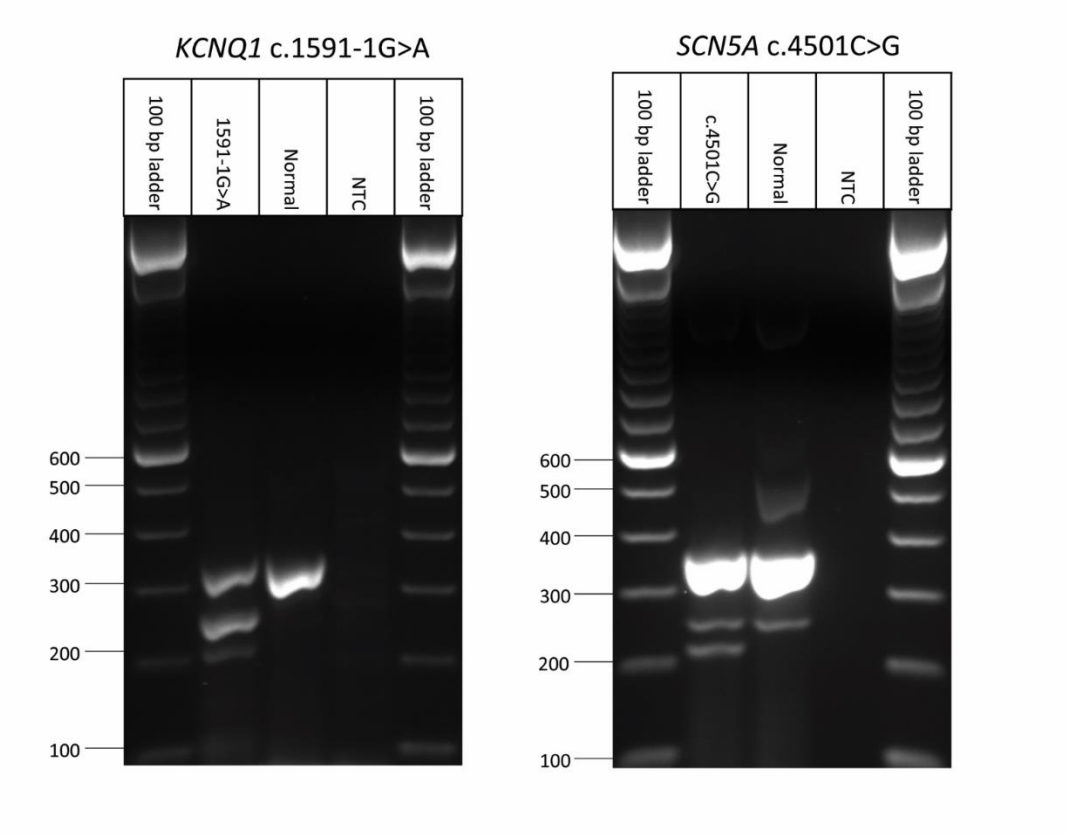
Table S6. Uncorrected QT duration, QTc Bazett, QTc Fredericia and QTc Framingham at time of first ECG with QTc ≥ 500 ms ("ECG 1").

Patients	# of patients	Heart rate (beats/min)	Uncorrected QT duration (ms)	QTc Bazett (ms)	QTc Fredericia (ms)	QTc Framingham (ms)
Participants	475	77 (39-100)	456 (392-698)	512 (500-669)	493 (462-631)	489 (452-631)
Non-participants	258	81 (48-100)	448 (392-730)	515 (500-634)	495 (462-786)	489 (452-761)
Pathogenic or likely pathogenic variants	31	66 (41-97)	488 (400-698)	511 (501-577)	500 (469-616)	498 (459-627)
VUS	41	76 (48-98)	464 (396-592)	510 (500-639)	497 (465-623)	494 (455-614)
Negative genetic test	403	78 (39-100)	456 (392-650)	512 (500-669)	493 (462-631)	488 (452-631)
VUS and negative genetic test	444	77 (39-100)	456 (392-650)	512 (500-669)	493 (462-631)	489 (452-631)
KCNQ1 p.(Gln530*)	23	69 (41-97)	480 (400-698)	510 (501-577)	500 (469-615)	496 (459-627)
Pathogenic or likely pathogenic variants other than *KCNQ1* p.(Gln530*)	8	63 (49-86)	493 (432-568)	513 (502-535)	500 (487-615)	500 (479-539)
P- value for participants vs non-participants		<0.01		0.17	0.56	0.26
P- value for pathogenic or likely pathogenic variants vs VUS and negative genetic test		<0.01		0.50	0.18	0.10

P- value for VUS vs negative genetic test		0.81		0.39	0.35	0.32
P- value for *KCNQ1* p.(Gln530*) vs pathogenic or likely pathogenic variants other than *KCNQ1* p.(Gln530*)		0.41		0.95	0.55	0.41

ECG=Electrocardiogram; QTc=corrected QT interval; VUS= variants of uncertain significance

Figure S1. RT-PCR of KCNQ1 c.1591-1G>A and SCN5A c.4501C>G.



KCNQ1 c.1591-1G>A affects the canonical acceptor splice site of exon 13 / intron 12. RT-PCR showed skipping of exon 13 from the mature mRNA. No production of normal mRNA from the mutated allele was observed by studying a marker variant (heterozygous c.1638G>A in exon 13). *SCN5A* c.4501C>G produced an aberrant transcript where 100 bp from the 3'-end of exon 25 and the first 4 bp of exon 26 had been excluded. The fainter band of the shorter, mutated transcript compared to normal, full-length transcript indicates that normal the mutated allele also produces some normal transcript. bp=base pairs; RT-PCR=Reverse transcription polymerase chain reaction.

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