

Functional phenotype variations of two novel $K_V7.1$ mutations identified in patients with Long QT syndrome

Sofia Hammami Bomholtz PhD^{1,2} | Marwan Refaat MD^{3,4}  | Annette Buur Steffensen PhD^{1,2} | Jens-Peter David PhD^{1,2} | Karin Espinosa MD^{1,2} | Robert Nussbaum MD⁵ | Julianne Wojciak MS⁵ | Bo Hjorth Bentzen PhD^{1,2} | Melvin Scheinman MD⁵ | Nicole Schmitt PhD^{1,2} 

¹Danish National Research Foundation Centre for Cardiac Arrhythmia, University of Copenhagen, Copenhagen, Denmark

²Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

³Department of Internal Medicine, Division of Cardiology, American University of Beirut Medical Center, Beirut, Lebanon

⁴Department of Biochemistry and Molecular Genetics, American University of Beirut, Beirut, Lebanon

⁵Department of Medicine, University of California, San Francisco, San Francisco, California

Correspondence

Nicole Schmitt, Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen, Denmark.
Email: nschmitt@sund.ku.dk

Present address: Annette Buur Steffensen, Department of Neuroscience, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen, Denmark

Present address: Jens-Peter David, Sanofi A/S, Lyngbyvej 2, 2100 Copenhagen, Denmark

Funding information

Danmarks Grundforskningsfond

Abstract

Background: The slow delayed rectifier potassium current I_{K_S} is crucial for the repolarization of the cardiac action potential. It is conducted by the voltage-gated channel $K_V7.1$ encoded by *KCNQ1*, together with its β -subunit *KCNE1*. Loss-of-function (LOF) mutations in *KCNQ1* have been associated with heritable cardiac arrhythmias such as Long QT syndrome (LQTS). This disease is characterized by prolonged ventricular repolarization and propensity to ventricular tachyarrhythmia that may lead to syncope, cardiac arrest, and sudden death. We aimed to functionally characterize two $K_V7.1$ mutations (p.A150T and p.L374H) identified in two independent LQTS patients with different severity of disease phenotype, family history, and co-segregation of LQTS.

Methods: We performed whole-cell patch clamp recordings in CHO-K1 cells, and confocal imaging in Madin-Darby Canine Kidney (MDCK) cells.

Results: I_{K_S} -A150T showed significantly decreased current amplitudes from above +20 mV (approximately 52% decrease at +40 mV), but demonstrated cell membrane localization similar to wild-type (WT). I_{K_S} -L374H, however, exhibited a complete LOF compared to WT channels. Confocal imaging showed endoplasmic reticulum retention of the channel in MDCK cells. Mimicking the heterozygous state of the patients by co-expressing WT and mutant subunits resulted in an approximately 22% decrease in current at +40 mV for A150T. The L374H mutation showed a more pronounced effect (62% reduction at +40 mV compared to WT channel).

Conclusion: Both mutations, $K_V7.1$ A150T and L374H, led to loss of channel function. The degree of LOF may mirror the disease phenotype observed in the patients.

KEYWORDS

cardiac electrophysiology, I_{K_S} current, risk stratification

1 | INTRODUCTION

The hereditary Long QT syndrome (LQTS) is a cardiac arrhythmia diagnosed, among other factors, by a prolongation of the heart rate-corrected QT interval (QT_C) on the surface electrocardiogram (ECG).^{1,2} QT_C is a measure of ventricular repolarization, and prolonged repolarization of the ventricles may lead to tachycardia of the *torsades de pointes* type. Ultimately, it may degenerate into sudden

cardiac death.² LQTS is a heterogeneous disease, currently associated with mutations in 15 genes subdividing the syndrome in LQT1-15.² Most of the identified mutations reside in genes encoding ion channel subunits or proteins modulating ion channel function. LQT1 is the most common form of LQTS with more than 250 different mutations identified so far.² The underlying molecular entity is the voltage-gated potassium channel $K_V7.1$ encoded by *KCNQ1*.^{3,4} $K_V7.1$ is a pore-forming α -subunit that assembles with the β -subunit *KCNE1* to form

the I_{Ks} channel complex.^{5,6} This slowly activating potassium current is crucial for the repolarization of the cardiac action potential.⁴

Mutations in *KCNQ1* have not only been linked to LQT1, but also to Short QT syndrome (SQTS) and atrial fibrillation (AF) (reviewed in refs. 7 and 8) with the overall concept that loss-of-function (LOF) mutations lead to LQTS, whereas gain-of-function mutations are associated with SQTS or AF (reviewed in ref. 9). Yet, increasing evidence suggests that ventricular and atrial phenotypes may overlap (reviewed in ref. 4). Furthermore, reduced penetrance and variable expressivity hamper the risk stratification of patients and their family members.¹⁰ Considerable efforts have been made to establish rules for genotype-phenotype correlations and subsequent clinical management, leading to algorithms that aid the interpretation of mutational findings.¹¹ It is, however, debated to which extent knowledge of mutations can be used for prophylactic treatment of patients.¹²

In this study, we investigated the functional effect of two $K_{V7.1}$ mutations, A150T and L374H, identified in LQTS patients with varying severity of disease phenotype to address a possible correlation between degrees of functional effects with the clinical phenotypes of the patients.

2 | METHODS

2.1 | Study subjects

The probands have been referred to our clinic and diagnosed with LQTS in accordance with the Schwartz criteria.¹ The study was carried out in accordance with the principles outlined in the Declaration of Helsinki. The Institutional Review Board of the University of California, San Francisco approved the study and the included patients gave written informed consent.

2.2 | Genetic screening

Genetic testing was performed at GeneDx (Gaithersburg, MD, USA) (Proband 1) and Familion (New Haven, CT, USA) (Proband 2) and included analysis of the target exons and flanking intronic regions of known LQTS genes *AKAP9*, *CACNA1C*, *CAV3*, *KCNE1*, *KCNE2*, *KCNH2*, *KCNJ2*, *KCNQ1*, *SCN4B*, *SCN5A*, and *SNTA1*. Laboratory control groups consisted of 350-1300 healthy subjects of Caucasian and African American ancestry depending time of testing. For details, see Supporting Information.

2.3 | Molecular biology

The point mutations p.A150T (c.448G>A) and p.L374H (c.1120 T>A) in $K_{V7.1}$ were introduced using standard techniques. For details, see Supporting Information.

2.4 | Antibodies

Primary antibodies are as follows: goat polyclonal anti- $K_{V7.1}$ (1:100, C-20, Santa Cruz Biotechnology, Heidelberg, Germany), mouse anti-

c-myc-tag antibody (1:100, clone 9E10), and rabbit polyclonal anti-calnexin (1:2,000, Stressgen, San Diego, CA, USA). See Supporting Information for secondary antibodies.

2.5 | Cell culture and transfections

For electrophysiological experiments, CHO-K1 (Chinese Hamster Ovary) cells were used. CHO-K1 cells were transfected with 1.8 μ g DNA (WT or MUT) using SiLentFect Lipid Reagent (Bio-Rad Laboratories, Hercules, CA, USA). For imaging, Madin-Darby Canine kidney (MDCK) (strain II) cells were transfected with 3 μ g plasmid DNA using Lipofectamine and Plus Reagent (Invitrogen, Nærum, Denmark) according to manufacturer's protocol. For experimental details, see Supporting Information.

2.6 | Patch-clamp recordings and data analysis

Currents were recorded using a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA). See Supporting Information for details. Data were sampled with PClamp software and analyzed with Clampfit software (Molecular Devices) and GraphPad Prism 5 (GraphPad Software, CA, USA). Current-voltage relations were obtained from the step protocol by plotting the outward current at the end of the test pulse as a function of the test potential. Data are represented as mean \pm SEM. Unpaired *t*-test or two-way ANOVA followed by Bonferroni test was used as appropriate to compare the wild-type (WT) and mutated I_{Ks} channel complex. $P < .05$ was considered statistically significant.

2.7 | Immunofluorescence and imaging

Images were acquired using Zeiss LSM710 laser scanning confocal microscopy system with a 63 \times /numerical aperture (NA) = 1.40 oil objective and the pinhole diameter was set between 0.9 and 1.0 μ m. Line averaging and sequential scanning were applied to reduce noise and allow separation of the individual channels, respectively. All images were obtained with a pixel format of 1024 \times 1024 and treated using ZEN 2010 Edition (Zeiss, Oberkochen, Germany) and Illustrator CS5 (Adobe, San José, CA, USA). Quantification was performed as described earlier.¹³ For details, see Supporting Information.

3 | RESULTS

3.1 | LQTS patients 1 and 2 are differently affected by the disease

Proband 1 is a woman who presented at age 50 with a history of syncope and palpitations. Medical records show that paramedics were called as an ECG showed AF; however, normal sinus rhythm was demonstrated on ECGs in the emergency room. The QT_C was prolonged (multiple ECG showing $QT_C = 483-519$ ms). She had an implantable cardioverter defibrillator (ICD) placed and is taking beta

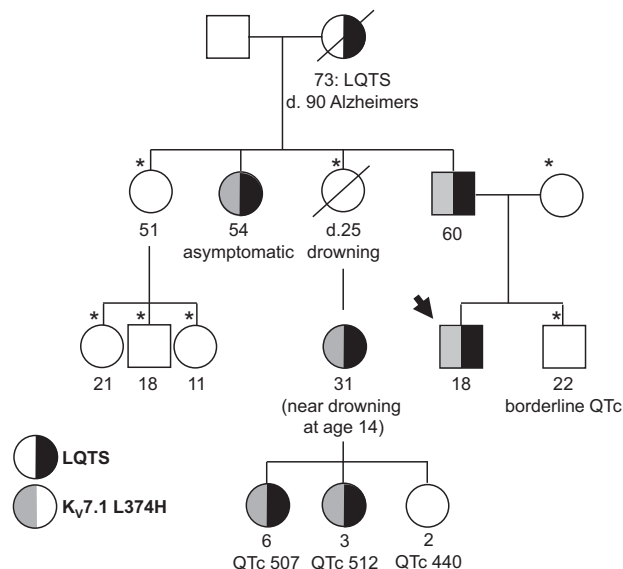


FIGURE 1 Pedigree of proband carrying $K_{V7.1}$ -L374H (circles, females; squares, males) with phenotypic and genotypic information where available

Note. Proband 2 is indicated by an arrow. Age at diagnosis/inclusion and QTc, when available. d, diseased; * not genetically tested

blockers. She had a documented episode of ventricular fibrillation where she had an appropriate shock in April 2007. On follow-up in 2012, she was found to have paroxysmal AF with an average of 2% burden on device interrogation. The proband has no reported family history of LQTS (Figure S1). Her paternal uncle died suddenly in his teens in China, yet the cause of death is unknown. Her two children and three siblings all had normal ECGs in 2007 and have not been evaluated since.

Proband 2 is male and was diagnosed with LQTS at 1 year of age. He has been treated with β -blockers since age 2 years. He had one episode of possible loss of consciousness in 2009. He was then febrile, stressed, and dehydrated. He has had no further episodes. He has been managed with the nonselective β -blocker nadolol and exercise testing shows adequate β -blockage. His past ECGs clearly showed LQTS with QTc intervals as high as 560 ms. The ECG after treatment showed sinus bradycardia with a heart rate of 43 beats per minute and corrected QT interval of 485 ms. The proband has an extensive family history illustrated in Figure 1. The father, age 60, reportedly had a syncopal episode at age 42. He was diagnosed with LQTS on exercise test after his niece's diagnosis about 17 years ago. The brother, age 22, reportedly has a borderline prolonged QTc interval. A paternal aunt drowned at age 25. She had a history of syncope occurring with exercise or stress. Her daughter, age 31, had a near drowning event at age 14. Evaluation led to a diagnosis of LQTS. She was treated with beta-blockers and ICD, which has discharged twice in the past 15 years. Her daughter, age 6, was diagnosed with LQTS via an exercise test. Another paternal aunt, age 54, was reportedly diagnosed with LQTS but the family does not know how the diagnosis was made. She is asymptomatic. The paternal grandmother had a history of syncope triggered by startle in her 20s. She was

diagnosed with LQTS at age 73 after others in the family were diagnosed. She died at age 90 from complications of Alzheimer's disease.

3.2 | Genetic screening reveals two mutations

Genetic screening revealed two nonsynonymous mutations in *KCNQ1* in evolutionary conserved amino acids (Figure S2); a schematic overview of the positions is depicted in Figure S2. The patients were heterozygous (HET) for the mutations. Proband 1 carries p.A150T that was absent in controls, and ClinVar,¹⁴ Gnomad, and ExAc databases.¹⁵ The residue resides in transmembrane segment S2 of the channel protein. Proband 2 carries p.L374H residing in the C-terminus. *In silico* phenotype prediction tool SIFT¹⁶ and Polyphen-2¹⁷ predicted A150T to be tolerated and L374H damaging (SIFT) or probably damaging (Polyphen-2). L374H is present in large population databases, albeit at very low frequency (1/246 038 alleles in Gnomad). This variant has previously been reported in two unrelated individuals tested for LQTS, with no published segregation data.^{18,19} Three labs classify this variant as a variant of uncertain significance in ClinVar. GeneDx has seen it in four unrelated families in which a proband underwent LQTS panel testing. Family saw it in seven unrelated LQTS patients. Variants in nearby residues (A372D, S373P, W379G, and W379S) have also been reported in the Human Gene Mutation Database in association with LQTS.²⁰

Genetic testing of family members revealed that the proband's father, aunt, and cousin are obligate carriers. Two of that cousin's daughters are also positive and both have LQTS based on QTc intervals of >500 ms. Hence, this variant co-segregates with LQTS in six members of this patient's family.

3.3 | Mutant $K_{V7.1}$ -A150T channels show an ambiguous phenotype

To investigate whether the mutations found in $K_{V7.1}$ could explain the LQTS phenotype observed in the probands, we expressed WT or mutant (MUT) channels in a mammalian expression system and performed whole cell patch clamp experiments. Expression of $K_{V7.1}$ -A150T in the absence of the β -subunit KCNE1 did not reveal any difference between WT and MUT channels (data not shown). To mimic the native I_{K5} current, we co-expressed $K_{V7.1}$ WT or MUT with KCNE1 (Figure 2). As expected,^{5,6} WT complexes gave rise to slowly activating and deactivating noninactivating potassium current when submitted to a voltage-step protocol (10-mV increments, 2-s pulses) ranging from -100 to $+40$ mV. In contrast, I_{K5} -A150T induced a significant LOF, observed as a decrease in current amplitude from $+20$ mV potential and greater compared to the WT complex ($P < .05$; Figure 2A-B). At $+40$ mV, the current amplitude was 587.1 ± 46.6 pA/pF ($n = 8$) for WT and 365.7 ± 82.8 pA/pF ($n = 7$) for the MUT channel. When mimicking the HET state of the patient by co-expressing WT and $K_{V7.1}$ -A150T together with KCNE1, this loss of function was partially rescued, although still significantly different at $+40$ mV when compared to WT (HET: 454.9 ± 64.4 pA/pF, $n = 10$; $P < .05$). Analysis of

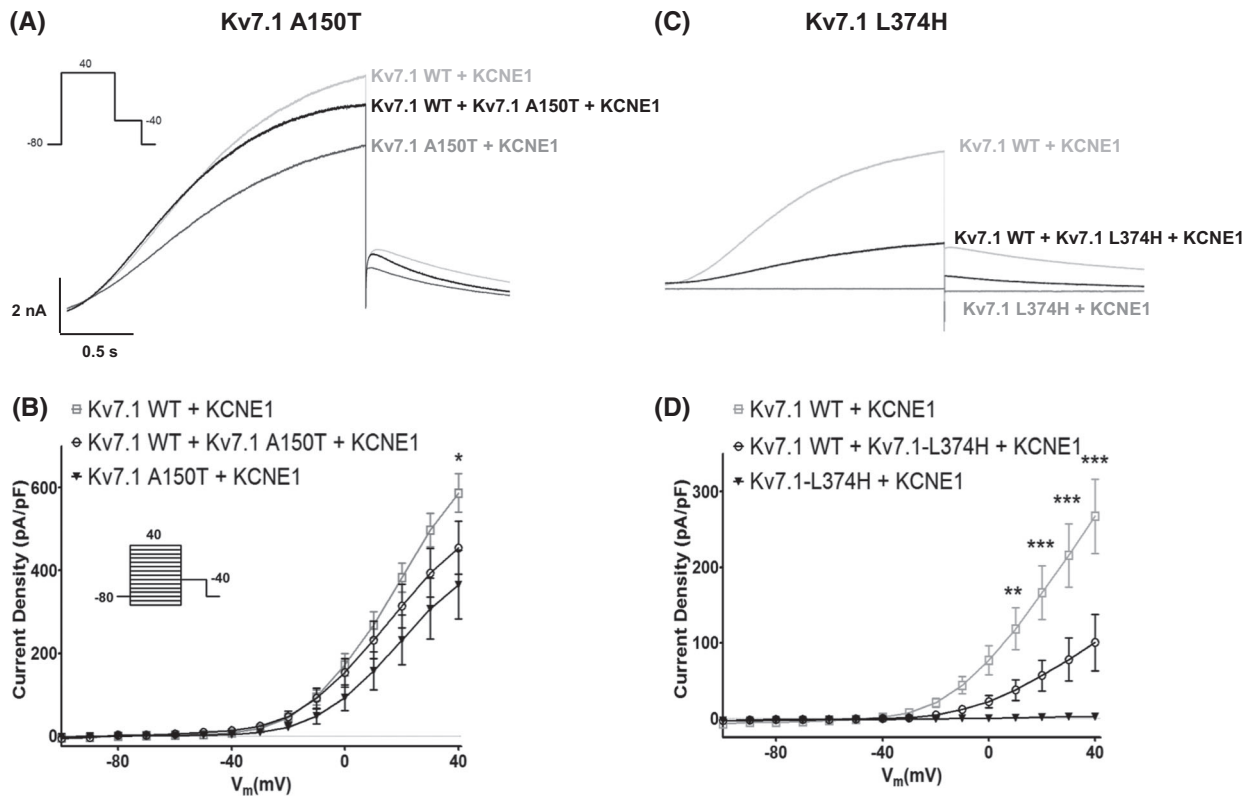


FIGURE 2 Comparison of $K_v7.1$ -WT/KCNE1 with $K_v7.1$ -A150T/KCNE1 and $K_v7.1$ -L374H/KCNE1 whole cell currents measured in CHO-K1 cells. A and C, Representative current traces when clamped at +40 mV. Both $K_v7.1$ and $K_v7.1$ -A150T produce I_{K_S} -like currents when co-expressed with KCNE1. B and D, Currents measured at the end of each voltage step were used to construct the current-voltage (I/V) relationship ($n = 7$ -11 from three to four independent experiments). The current step protocol is shown as inset

activation and deactivation kinetics did not reveal any significant changes (Figure S3).

3.4 | $K_v7.1$ -L374H shows complete LOF

When applying the same set of experiments for $K_v7.1$ -L374H, we found a complete LOF for the MUT channel. Representative current traces and current-voltage relationship are depicted in Figure 2C-D. Figure 2D illustrates that mimicking the HET state of the patient by co-expressing MUT with WT resulted in significantly decreased steady-state current amplitude at potentials of +10 mV and above with a 62.4% reduction at +40 mV indicating a mild dominant-negative effect (WT: 267.4 ± 49.0 pA/pF, $n = 9$, HET: 100.5 ± 37.4 pA/pF, $n = 7$; $P < .001$). Tail current analysis showed no significant alteration of the voltage-dependent activation; however, a tendency to a more depolarized half maximal activation voltage indicated a LOF (Figure S4). Time constants of activation and deactivation kinetics were not affected (Figure S4).

3.5 | $K_v7.1$ mutations display different subcellular localization

We further examined whether the observed LOF phenotypes were due to changes in the subcellular localization caused by the mutations. We employed the well-established MDCK cell system^{13,21} that is

well suited to analyze trafficking and targeting behavior of the MUT channel proteins. WT and MUT proteins were transiently expressed in the MDCK cell line together with c-myc-tagged KCNE1. Laser confocal microscopy imaging was performed using specific antibodies against $K_v7.1$ and c-myc-tagged KCNE1 (Figure 3). Fluorescent-conjugated Phalloidin (Pha) that stains the F-actin located beneath the plasma membrane in MDCK cells was used as a cell membrane marker. As shown in the merged pictures, $K_v7.1$ -WT is located at the cell membrane in polarized MDCK cells in line with previous reports.¹³ $K_v7.1$ -A150T behaved similarly to WT both in the absence (data not shown) and presence of KCNE1. In contrast, we observed $K_v7.1$ -L374H primarily intracellularly where it appeared to be retained in the endoplasmic reticulum (ER). Quantification of surface expression showed significant reduction for $K_v7.1$ -L374H (73% reduction of WT level, $n = 22$ -25 cells from three independent experiments). Yet, in experiments co-expressing $K_v7.1$ -L374H with WT, the channel seemed to be expressed at the cell membrane comparable to WT, which is in contrast to our observations in patch-clamp experiments.

4 | DISCUSSION

In this study, we addressed a possible correlation of functional effects of two LQTS mutations with the disease phenotype seen in the patients. The patient carrying $K_v7.1$ -A150T was diagnosed in adult age

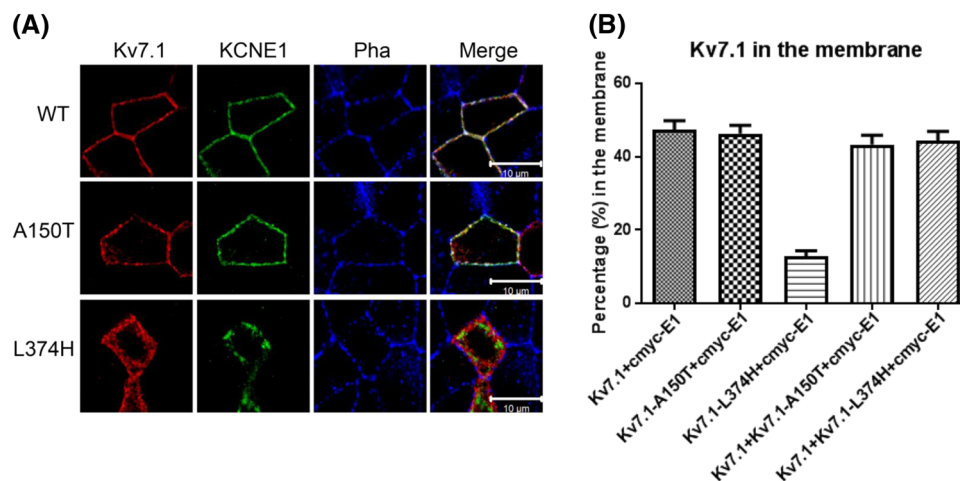


FIGURE 3 Subcellular localization of $Kv7.1$ mutants. A, Confocal images of polarized MDCK cells transiently expressing $Kv7.1$ -WT or MUT together with c-myc-KCNE1 as indicated and labeled with antibodies against $Kv7.1$ and c-myc. Fluorescent-conjugated Phalloidin (Pha) stains the F-actin located beneath the plasma membrane. Merged pictures are shown in right panels. B, Quantification of $Kv7.1$ surface membrane expression ($n = 22$ -25 from three independent experiments) [Color figure can be viewed at wileyonlinelibrary.com]

and had no family history of LQTS, whereas the patient carrying $Kv7.1$ -L374H had severely prolonged QT intervals, symptoms since the age of one, and an extensive family history of LQTS with several incidents of cardiac arrest while swimming. We found this variant to co-segregate with LQTS in six members of this patient's family, which strongly suggests that this variant is responsible for the patient's LQTS.

Both mutations induced LOF observed as a decrease (p.A150T) or absence (p.L374H) of I_{Ks} current in patch-clamp experiments. Further supportive of a complete LOF is the ER retention of $Kv7.1$ -L374H channels as shown in Figure 3. $Kv7.1$ -A150T displayed only partial current reduction and no difference in trafficking/membrane expression compared to WT channels. The LOF is compatible with a prolongation of the cardiac action potential and severity of the LQTS phenotype observed in the patients. Mimicking the HET state of the patients showed that the MUT phenotype could be partially rescued, leaving the possibility that the mutations are not causative of the LQTS diagnosed in the patients. However, as the functional data indicate that these mutations have the potential to result in severe LOF, the clinical manifestation of the MUTs might be affected by additional factors such as allele-specific expressivity or differences in autonomic responses in the patients.^{12,22,23}

By analyzing the clinical phenotypes of 387 LQT1 patients and correlating those to biophysical properties of the corresponding $Kv7.1$ MUTs determined by electrophysiological experiments, Jons and colleagues have proposed that the biophysical phenotype analysis may be more useful for risk stratification than clinical parameters. Specifically, they found that in patients with moderate QT prolongation (defined as $QT_C < 500$ ms), slower activation was an independent predictor for cardiac events.²⁴ Nielsen and colleagues have recently reported an association between QT_C interval duration and risk of AF. The authors investigated more than 280 000 digital ECGs and found that short (≤ 372 ms) and long (≥ 458 ms) QT_C intervals increased the hazard ratio for AF.²⁵ Curiously, proband 1 in our study

had QT_C values around 500 ms and activation kinetics tended to be faster, which could be indicative of a gain-of-function phenotype. In line with this, she was admitted to the hospital with symptoms and ECG recording compatible with AF, although no AF episodes have been recorded afterward. The mutation resides in transmembrane segment S2. There is emerging evidence for the participation of the transmembrane segments S1 and S2 and the S1/S2-linker in gating processes of voltage-gated potassium channels. Here, residues in S1 or S2 facing the extracellular side of the membrane or the extracellular S1/S2-linker come in close proximity to the voltage sensor S4 as shown for *Shaker* channels,²⁶ $KvAP$ channels,²⁷ or $Kv7.1$ channels.²⁸ Of note, the S1/S2-linker of the $Kv7.1$ channel harbors a cluster of mutations associated with either AF or SQTS (S140G, V141M, and T144A),²⁹⁻³² and Q147R associated with an overlapping phenotype of AF and QT_C prolongation.³³ Intriguingly, Q147R did only exhibit alterations in presence of KCNE1 subunits³³ and has recently been implicated in the $Kv7.1$ /KCNE1 interaction interface.³⁴ In our study, A150T also showed only alterations in the presence of KCNE1. $Kv7.1$ -L374H resides in helix A of the intracellular C-terminus of the channel protein involved in calmodulin binding of $Kv7.1$,³⁵ thereby affecting channel gating, folding, and membrane trafficking.^{36,37}

A number of studies have proposed an association between the position of the mutation and the severity of the LQTS clinical phenotype.³⁸⁻⁴⁰ They show a higher risk of cardiac events for patients having a mutation in the transmembrane segments or loops compared to mutations located in the N- and C-termini. However, in our study, L374H located in the proximal C-terminus of the $Kv7.1$ channel protein displayed a much more severe phenotype of the disease compared to the A150T mutation located in the S2 segment. This implies that even the establishment of rules for positional genotype-phenotype correlations is not sufficient for risk assessment and management of patients with LQTS. However, a combination of clinical and mutation location data, together with the analysis of the effects of Long QT

mutations on both channel function and trafficking, is indicated to accurately determine the mechanisms underlying the disease, which might improve proper patient management and counseling.

4.1 | Limitations

Although we employed well-established heterologous expression systems, these do not completely mirror the conditions in human cardiomyocytes. Patients were only screened in the known LQTS genes, which preclude detection of genetic modifiers elsewhere in the genome. There was no family history for proband 1; hence, we are not able to conclude unambiguously on the co-segregation of the mutation with the disease phenotype.

5 | CONCLUSIONS

In two independent LQTS patients with different severity of disease phenotype and family history of LQTS, two mutations were identified. Both, $K_V7.1$ A150T and L374H, led to loss of channel function observed as a decrease or absence of current where the latter was due to impaired trafficking. The results add to our understanding of the molecular basis of I_{Ks} current and may contribute to development of better drugs. Although the degree of LOF matched the disease phenotype observed in the patients, careful clinical follow-up is warranted for stratification of family members at risk.

ACKNOWLEDGMENT

This work was supported by the Danish National Research Foundation (ABS, BHB, JPD, KE, SHB, and NS).

AUTHOR CONTRIBUTIONS

Concept/design: SHB, MM, NS; Data collection/analysis/statistics/interpretation: SHB, MR, ABS, JPD, KE, JW, BHB, NS; Drafting article: SHB, ABS, MR, NS; Critical revision of article: all authors, Approval of article: all authors.

ORCID

Marwan Refaat MD  <https://orcid.org/0000-0002-3210-1987>

Nicole Schmitt PhD  <https://orcid.org/0000-0001-9482-9749>

REFERENCES

- Schwartz P, Crotti L. QTc behavior during exercise and genetic testing for the long-QT syndrome. *Circulation*. 2011;124:2181-2184.
- Bohnen MS, Peng G, Robey SH, et al. Molecular pathophysiology of congenital long QT syndrome. *Physiol Rev*. 2017;97:89-134.
- Wang Q, Curran ME, Splawski I, et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet*. 1996;12:17-23.
- Schmitt N, Grunnet M, Olesen S-P. Cardiac potassium channel subtypes: new roles in repolarization and arrhythmia. *Physiol Rev*. 2014;94:609-653.
- Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. K(V)LQT1 and Isk (minK) proteins associate to form the I(Ks) cardiac potassium current. *Nature*. 1996;384:78-80.
- Sanguinetti MC, Curran ME, Zou A, et al. Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. *Nature*. 1996;384:80-83.
- Hedley PL, Jørgensen P, Schlamowitz S, et al. The genetic basis of long QT and short QT syndromes: a mutation update. *Hum Mutat*. 2009;30:1486-1511.
- Mahida S, Lubitz SA, Rienstra M, Milan DJ, Ellinor PT. Monogenic atrial fibrillation as pathophysiological paradigms. *Cardiovasc Res*. 2011;89:692-700.
- Dvir M, Peretz A, Haitin Y, Attali B. Recent molecular insights from mutated IKS channels in cardiac arrhythmia. *Curr Opin Pharmacol*. 2014;15:74-82.
- Giudicessi JR, Ackerman MJ. Determinants of incomplete penetrance and variable expressivity in heritable cardiac arrhythmia syndromes. *Transl Res*. 2013;161:1-14.
- Giudicessi JR, Ackerman MJ. Genotype- and phenotype-guided management of congenital long QT syndrome. *Curr Probl Cardiol*. 2013;38:417-455.
- Amin AS, Pinto YM, Wilde AAM. Long QT syndrome: beyond the causal mutation. *J Physiol*. 2013;591:4125-4139.
- David J-P, Andersen MN, Olesen S-P, Rasmussen HB, Schmitt N. Trafficking of the I(Ks)-complex in MDCK cells: site of subunit assembly and determinants of polarized localization. *Traffic*. 2013;14:399-411.
- Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res*. 2018;46:D1062-D1067.
- Karczewski KJ, Weisburd B, Thomas B, et al. The ExAC browser: displaying reference data information from over 60 000 exomes. *Nucleic Acids Res*. 2017;45:D840-D845.
- Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc*. 2009;4:1073-1081.
- Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248-249.
- Tester DJ, Will ML, Haglund CM, Ackerman MJ. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. *Heart Rhythm*. 2005;2:507-517.
- Kapplinger JD, Tester DJ, Salisbury BA, et al. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. *Heart Rhythm*. 2009;6:1297-1303.
- Stenson PD, Mort M, Ball EV, Shaw K, Phillips A, Cooper DN. The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum Genet*. 2014;133:1-9.
- Jespersen T, Rasmussen HB, Grunnet M, et al. Basolateral localisation of KCNQ1 potassium channels in MDCK cells: molecular identification of an N-terminal targeting motif. *J Cell Sci*. 2004;117:4517-4526.
- Amin AS, Giudicessi JR, Tijssen AJ, et al. Variants in the 3' untranslated region of the KCNQ1-encoded Kv7.1 potassium channel modify disease severity in patients with type 1 long QT syndrome in an allele-specific manner. *Eur Heart J*. 2012;33:714-723.
- Diness TG, Hansen RS, Olesen SP, Grunnet M. Frequency-dependent modulation of KCNQ1 and HERG1 potassium channels. *Biochem Biophys Res Commun*. 2006;343:1224-1233.
- Jons C, O-Uchi J, Moss AJ, et al. Use of mutant-specific ion channel characteristics for risk stratification of long QT syndrome patients. *Sci Transl Med*. 2011;3:76ra28.
- Nielsen JB, Graff C, Pietersen A, et al. J-shaped association between QTc interval duration and the risk of atrial fibrillation: results from the Copenhagen ECG study. *J Am Coll Cardiol*. 2013;61:2557-2564.

26. Campos FV, Chanda B, Roux B, Bezanilla F. Two atomic constraints unambiguously position the S4 segment relative to S1 and S2 segments in the closed state of Shaker K channel. *Proc Natl Acad Sci USA*. 2007;104:7904-7909.
27. Cuello LG, Cortes DM, Perozo E. Molecular architecture of the KvAP voltage-dependent K⁺ channel in a lipid bilayer. *Science*. 2004;306:491-495.
28. Restier L, Cheng L, Sanguinetti MC. Mechanisms by which atrial fibrillation-associated mutations in the S1 domain of KCNQ1 slow deactivation of IKs channels. *J Physiol*. 2008;586:4179-4191.
29. Chen YH, Xu SJ, Bendahhou S, et al. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science*. 2003;299:251-254.
30. Hong K, Piper DR, Diaz-Valdecantos A, et al. De novo KCNQ1 mutation responsible for atrial fibrillation and short QT syndrome in utero. *Cardiovasc Res*. 2005;68:433-440.
31. Napolitano C, Priori SG, Schwartz PJ, et al. Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. *JAMA*. 2005;294:2975-2980.
32. Zareba W, Moss AJ, Sheu G, et al. Location of mutation in the KCNQ1 and phenotypic presentation of long QT syndrome. *J Cardiovasc Electrophysiol*. 2003;14:1149-1153.
33. Lundby A, Ravn LS, Svendsen JH, Olesen S-P, Schmitt N. KCNQ1 mutation Q147R is associated with atrial fibrillation and prolonged QT interval. *Heart Rhythm*. 2007;4:1532-1541.
34. Gofman Y, Shats S, Attali B, Haliloglu T, Ben-Tal N. How does KCNE1 regulate the Kv7.1 potassium channel? Model-structure, mutations, and dynamics of the Kv7.1-KCNE1 complex. *Structure*. 2012;20:1343-1352.
35. Sun J, MacKinnon R. Cryo-EM structure of a KCNQ1/CaM complex reveals insights into congenital long QT syndrome. *Cell*. 2017;169:1042-1050.e9.
36. Shamgar L, Ma L, Schmitt N, et al. Calmodulin is essential for cardiac IKs channel gating and assembly: impaired function in long-QT mutations. *Circ Res*. 2006;98:1055-1063.
37. Ghosh S, Nunziato DA, Pitt GS. KCNQ1 assembly and function is blocked by long-QT syndrome mutations that disrupt interaction with calmodulin. *Circ Res*. 2006;98:1048-1054.
38. Barsheshet A, Goldenberg I, O-Uchi J, et al. Mutations in cytoplasmic loops of the KCNQ1 channel and the risk of life-threatening events: implications for mutation-specific response to β -blocker therapy in type 1 long-QT syndrome. *Circulation*. 2012;125:1988-1996.
39. Costa J, Lopes CM, Barsheshet A, et al. Combined assessment of sex- and mutation-specific information for risk stratification in type 1 long QT syndrome. *Heart Rhythm*. 2012;9:892-898.
40. Shimizu W, Horie M, Ohno S, et al. Mutation site-specific differences in arrhythmic risk and sensitivity to sympathetic stimulation in the LQT1 form of congenital long QT syndrome: multicenter study in Japan. *J Am Coll Cardiol*. 2004;44:117-125.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Hammami Bomholtz S, Refaat M, Buur Steffensen A, et al. Functional phenotype variations of two novel K_v7.1 mutations identified in patients with Long QT syndrome. *Pacing Clin Electrophysiol*. 2020;43:210-216. <https://doi.org/10.1111/pace.13870>