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Genetic Study in Argentinian Patients with Clinical Long QT Syndrome Diagnosis

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Abstract

Long QT Syndrome (LQTS) is a genetic cardiac condition in which varying degrees of severity and treatment response. Three are primarily affected by mutations that cause cardiac ion channel dysfunction. In Argentina, most of the LQTS diagnoses are made by clinical examination and ECG analysis. In this study, we evaluated a group of individuals to correlate their clinical diagnosis of LQTS with genetic variations.

Using gDNA isolation, PCR, and exome sequencing, we screened the coding sequences of the *KCNQ1*, *KCNH2*, and *SCN5A* genes. We identified several changes in these genes, most of them previously described in the literature, but also a novel variation. We found an alteration in the sequence of *KCNQ1* exon 16 which did not allow us to amplify it. This is the first analysis of genetic variations in LQTS in Argentina conducted by a national research laboratory. The combination of the detected variations may explain the prolongation of the QT interval observed in the ECG of some of the individuals and may help to improve the treatment making it more rational as well as provide genetic counselling to first-degree relatives.

Keywords: Long QT syndrome; Genetic screening; Argentina; Exon sequencing

Abbreviations: LQTS: long QT syndrome; ECG: Electrocardiogram; QTc: Corrected QT interval; gDNA: Genomic DNA; PVT: Polymorphic ventricular tachycardia

Introduction

Long QT syndrome (LQTS) is a genetic disorder that can cause lethal cardiac arrhythmia and sudden cardiac death [1]. Patients with LQTS display a prolonged corrected QT interval (QTc) on resting ECG with a world prevalence of 1:2,000 persons [2]. About 17 genes encoding ion channels or associated proteins have been implicated in this disease [3,4], and a pathogenic or likely pathogenic variant is considered a diagnostic criterion of LQTS regardless of clinical and/or ECG findings. The genes most frequently involved in LQTS are *KCNQ1*, *KCNH2,* and *SCN5A* [5]. The *KCNQ1* gene encodes the KCNQ1 potassium channel subunit responsible for slow rectifying K^+ current (I_{Ks}) [6,7]. Mutations in the *KCNQ1* gene reduce I_{Ks} by preventing subunit assembly, interruption of membrane trafficking, or by impairment of channel opening. The *KCNH2* gene encodes the hERG channel (Kv11.1), responsible for the rapid rectifying K^+ current (I_{Kr}) [8]. The majority of mutations reported in this

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gene interfere with proper folding, assembly, or trafficking to the membrane [9]. The *SCN5A* gene encodes the sodium channel Nav1.5. Gain-of-function mutations in this channel lead to increased sodium entry into the heart cells, prolonging the QT interval and loss-of-function mutations, result in lower expression levels or translation into aberrant proteins [10]. Approximately 80% of genotype-positive patients have mutations in one of these 3 genes: *KCNQ1* (40-45%), *KCNH2* (30-35%), and *SCN5A* (10%) [2] allowing the disease to be classified into three major subtypes: LQT1, LQT2, and LQT3, respectively. In addition, between 5-10% of the patients with LQTS carry several alterations in more than one of these genes simultaneously. These patients generally have a more severe phenotype and earlier onset [11].

QT interval prolongation is a pathognomonic sign of the disease, but up to one-third of mutation carriers may have normal QT intervals on resting ECGs [5]. Therefore genetic testing for mutations that lead to cardiac channelopathies is essential for an accurate diagnostic evaluation and familial screening. Molecular identification of the causes of this disease contributes to better diagnosis, risk stratification, genetic counseling, and treatment of affected individuals. We aimed to correlate clinical LQTS diagnosis with genetic variants by analyzing LQTS cases in Argentinian subjects and their relatives. We examined the three most common LQTS genes (*KCNQ1*, *KCNH2*, and *SCN5A*) using exon amplification and direct DNA sequencing.

Methods

Ethics statement

Written informed consent for genetic explorations and authorization for publication of the results of this study were obtained from patients and family members. This study was conducted under the approval of the ethics committee of the *Hospital Municipal de Agudos L. Lucero* de Bahía Blanca, (Argentina) (expedient Nº 43-30154-2017) following the Declaration of Helsinki.

Table 1: Clinical features of individuals with clinical LQTS diagnosis.

Case	Sex	Age	Presentation	Family history	Arrhythmia	QTc (ms)	Therapy
1	F	0.3	asymptomatic	None	None	<450	None
2	M	27	asymptomatic	None	None	510	BB
3	F	33	Presyncope and PVT	None	Yes	460	BB + CA
4	M	45	palpitations	None	Yes	495	BB
5	M	8	asymptomatic	Yes	None	500	BB
6	F	62	bradycardia	None	None	480	BB

M: male, F: female, BB: beta-adrenergic receptor blocker, PVT: polymorphic ventricular tachycardia, CA: catheter ablation, ICD: Implantable cardioverter-defibrillator

Table 2: List of primers used to amplify KCNQ1 coding exons.

Target	Forward (5`-3`)	Reverse (5`-3`)	Product (bp)
Exon 1	GGCTAAGCAGGTGGGCTCG	CAGAGCTCCCCCACACCAG	853
Exon 2	TCGAAGCACTGTCTGTTCCT	GTTCCCCTCAGTCCTTGGTC	433
Exon 3	GCATGGCTGGGTTCAAACAGG	TGCTGAGGGCTGCCAATGC	380
Exon 4	AGACGAGAGCAGGGTGTATG	GCATCTGAGCAAGGTGGATGG	261
Exon 5	CCTGTCGGGATGGACATATAC	CTTGGGCTTGCTCTGAGTC	418
Exon 6	TTAGGCGTCTGCACAGGAG	CAAGCACAGGTTTGTGGACAG	361
Exon 7	ATCAGAGTGGTGGGTTTGG	CTCTGGAGTATAGCACCTTC	340
Exon 8	TTCCAGCACTGACCATACC	CAATGATGGTTCTGACAGGT	304
Exon 9	CATGTCAAGCCTGTGACTCTG	GGACATTGGGATGGCAGGAAC	459
Exon 10	TGTGTGAAGACACTGGAGCTGG	GAAGGCACCTGGAAGGTTTAC	473
Exon 11	ATTGTCAGGGCTGGAGCTTC	GCACTAGGCGAGTAGATAGCAC	479
Exon 12	TGAACACTCTCCTTGTTTCTGG	CCTTGCAACCCTCCACTATG	306
Exon 13	AACCAGGCTTATGCCATCAC	GGTTGAGAGGCAAGAACTCAG	359
Exon 14	GAGGAAGTCTGAGAGGCAGC	TTTCCACACCTAGAGCCTAACC	565
Exon 15	TTTGACTCTCAGCTACCTCC	CAGGAGCTTCACGTTCACAC	266
Exon 16A	TGCACTTGCAGAGACGGTTG	GAAGAGGTGGCCTTGCTGAG	548
Exon 16B	TAGTGGTGTCCCCGCTAGG	CCTGTCCTGTGTAGGAACCG	1820

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Cases selection

The Cardiac Electrophysiology Service of the *Hospital Privado del Sur de Bahía Blanca,* (Argentina) selected the cases with a clinical diagnosis of LQTS to perform the molecular analysis, according with the Schwartz criteria [12]. We investigated 6 Argentinian individuals (men and women) with an age of 0-62 years. 5 out of the 6 individuals, showed a prolonged QTc interval (i.e. >460 ms) while one of them (case #1 in Table 1) was a first-degree relative presenting a normal QTc interval (<450 ms). For case #5, we added 2 first-degree relatives to evaluate the same variation. As a control population, we studied 5 healthy unrelated individuals, with same age range.

PCR amplification

Genomic DNA (gDNA) was extracted from whole blood cells using a commercial kit (Inbio Highway, Argentina). gDNA was used for PCR amplification of coding exons of *KCNQ1*, *KCNH2,* and *SCN5A.* The *KCNQ1* gene has 16 coding exons, the *KCNH2* gene has 15 and the *SCN5A* gene has 28. Specific primers were designed to amplify each exon (Tables 2, 3, and 4). Each primer pair was intended to hybridize ~100 bp before and after the coding region. For the KCNH2 gene, exon 1 codifies for 25 aminoacids however few polymorphisms were reported in it, so it was not analyzed. For the *SCN5A* gene, exon 1 is not a codifying region, so it was also not tested either. PCR products were separated by agarose gel electrophoresis and purified using a commercial kit (PB-L, Argentina).

DNA sequencing and analysis

PCR products were sequenced in both strands (5`→3`and 3`→5`) using an external service (Macrogen Inc., Korea). DNA alignments and polymorphism detection were carried out online using SnapGene (V5.2.4) and Benchling software. The sequences of the complete human *KCNQ1* (NG_008935.1), *KCNH2* (NG_008916.1), and *SCN5A* (NG_008934.1) genes described in GenBank were used as reference.

Structural analysis of human KCNQ1, hERG, and $Na_v1.5$

Homology modeling was performed using the SWISS-MODEL server (https://swissmodel.expasy.org) [13]. The 3D-protein models were generated employing as target protein the FASTA amino acid sequences for human KCNQ1 (NP_000209.2), hERG (NP_000229.1), and $Na_v1.5$ (NP_932173.1).

Results

Clinical features

Out of the six individuals, five exhibited a prolonged QTc interval on their ECG while the remaining one was a first-degree relative presenting a normal QTc interval (case #2, Table 1). One case had a history of sudden cardiac death before the age of 40, while another experienced presyncope and documented polymorphic ventricular tachycardia (Table 1 and Figure 1A). Most subjects are currently under pharmacological treatment with β-adrenergic receptor blockers, while one of them has undergone catheter ablation and has an implantable defibrillator (Table 1).

KCNQ1 sequencing analysis

The results of the molecular screening of the *KCNQ1* gene are presented in Table 5 and Figure 2. Exons 2 to 16 of this gene were analyzed in all six individuals and the control subjects. Exon 1, on the other hand, was not possible to be amplified in any sample. For this gene, we detected the synonymous NM_000218.3(KCNQ1):c.1638G>A (p.Ser546=) variant in exon 13 of cases #2 and #6 (Table 5 and Figure 2), located at the intracellular domain near the C-terminal (Figure 3). In both cases, this variant was found in heterozygosis. Additionally, in our experimental conditions, we were unable to amplify exon 16 of case #5 but on the contrary, we were able to do it in all other five cases and the five control samples. To verify this result, two different samples from the patient were used. To exclude the possibility that the primers would not anneal correctly on the sequence, we designed a second pair located far before and after in the sequence (Table 1). Also, in this case, we were unable to amplify this exon in this individual. To verify the same variant in 2 first-degree relatives (father and sister), we performed the same PCR reaction for exon 16 in both, resulting positive for them. The modeling shown in Figure 3 does not include this area due to resolution limitations.

KCNH2 sequencing analysis

The results of the molecular screening of the *KCNH2* gene are presented in Table 5. We identified the missense NM_000238.4(*KCNH2*):c.982C>T (p.Arg328Cys) variant in exon 5 of case #3, which is located at the proximal domain (Figure 3 and 4); the synonymous NM_000238.4(*KCNH2*):c.1692A>G (p.Leu564=) variant in exon 7 of case #1, located at the transmembrane domain (TM) 5 (Figure 3); the missense NM_000238.4(*KCNH2*):c.2690A>C (p.Lys897Thr) variant in exon 11 of case #2 and #6, located at the intracellular region near the C-terminal (Figure 3 and 4); and the synonymous NM_000238.4(*KCNH2*):c.1956T>C (p.Tyr6520) variant, located at the TM 6 of the channel, in all cases except case #3 (Table 5 and Figure 3).

SCN5A sequencing analysis

The results of the molecular evaluation of the *SCN5A* gene are presented in Table 5. We were able to identify 7 polymorphisms of the *SCN5A* gene in our population (Table 2): the synonymous NM_000335.5(*SCN5A*):c.87A>G (p.Ala29=) in exon 2 of cases #2, #4, #5 and #6, and the missense NM_000335.5(*SCN5A*):c.100C>T (p.Arg34Cys) in

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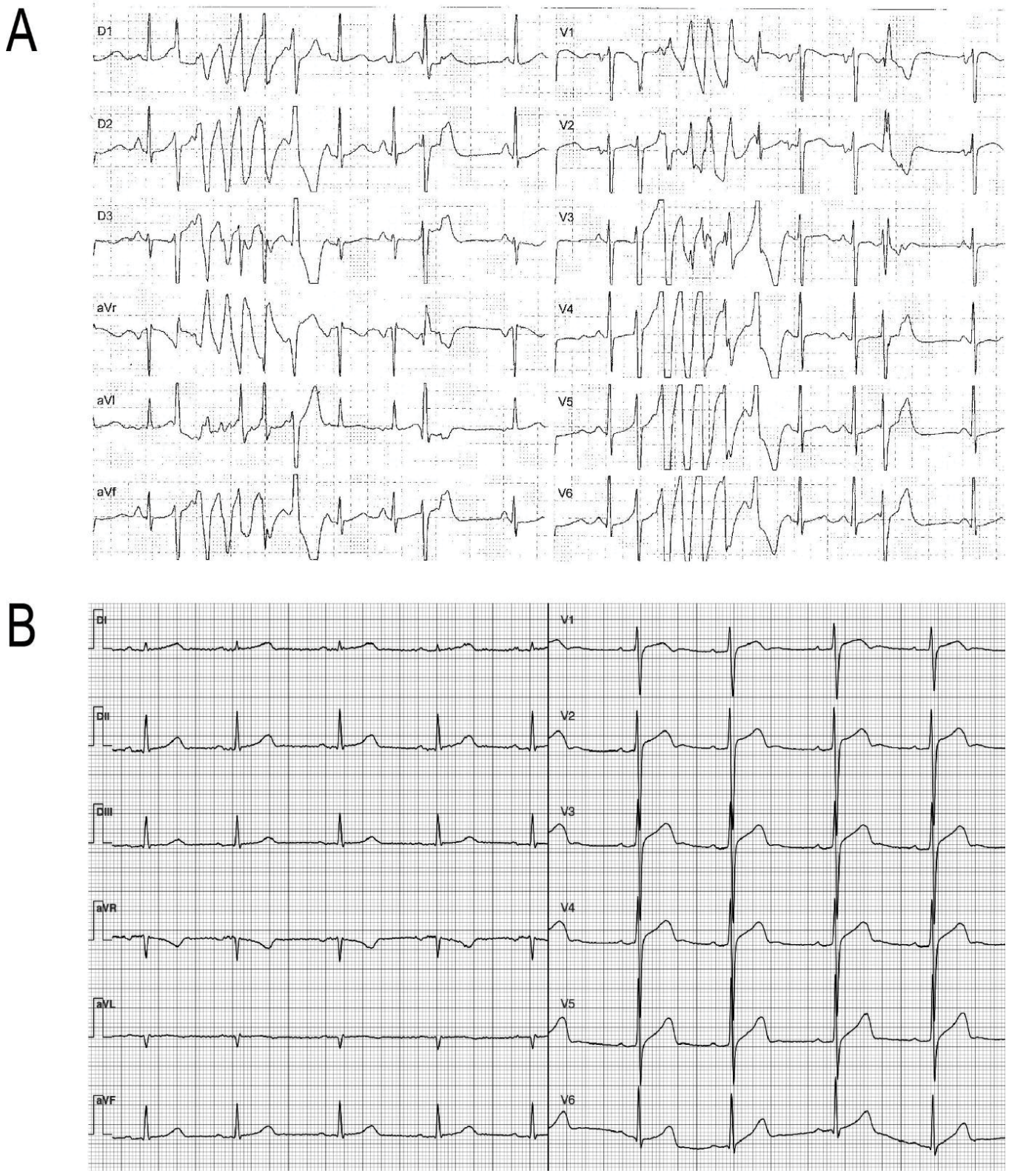

Figure 1: Example of ECG recordings from some cases.

A. Case #3: resting 12-lead ECG. Polymorphic ventricular tachycardia. B. Case #5: resting 12-lead ECG showing bradycardia (54 bpm) and a mild QTc prolongation (ECG after BB treatment).

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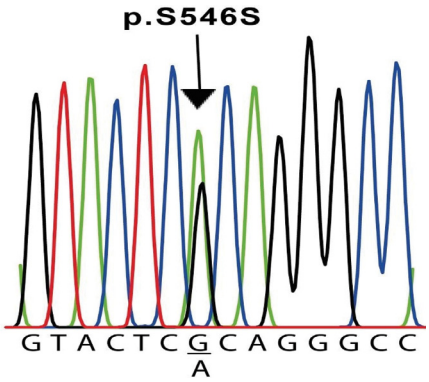

Figure 2: Nucleotide sequence of the KCNQ1 variant found in cases #2 and #6.

Both patients underwent a c.1638G>A exchange in exon 13 in a heterozygous configuration, resulting in a silent mutation (p.S564S). The sequences shown correspond to the sense strand of patient #2 gDNA.

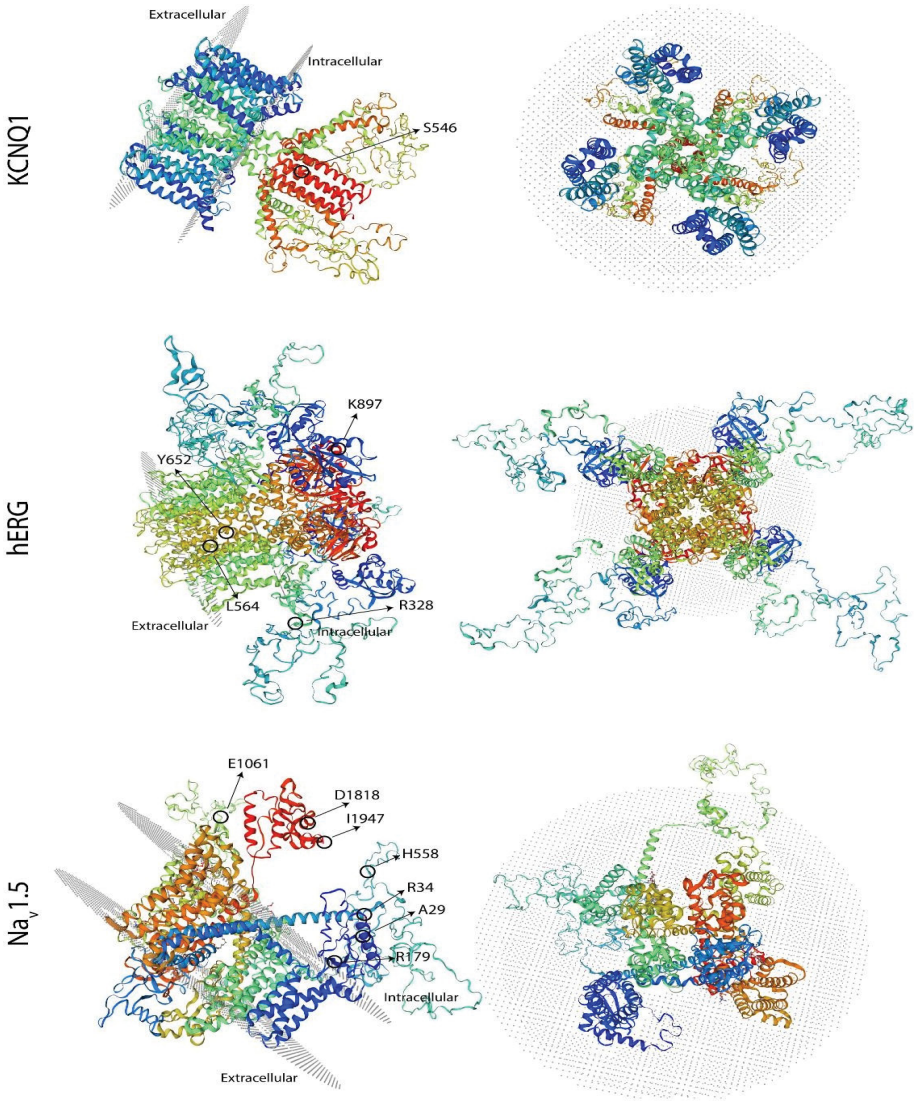

Figure 3: 3D protein model with the mutations found in each channel.

Left panel: side view, and right panel: extracellular view. The structures are oriented in space in such a way that the location of all the changes found can be observed.

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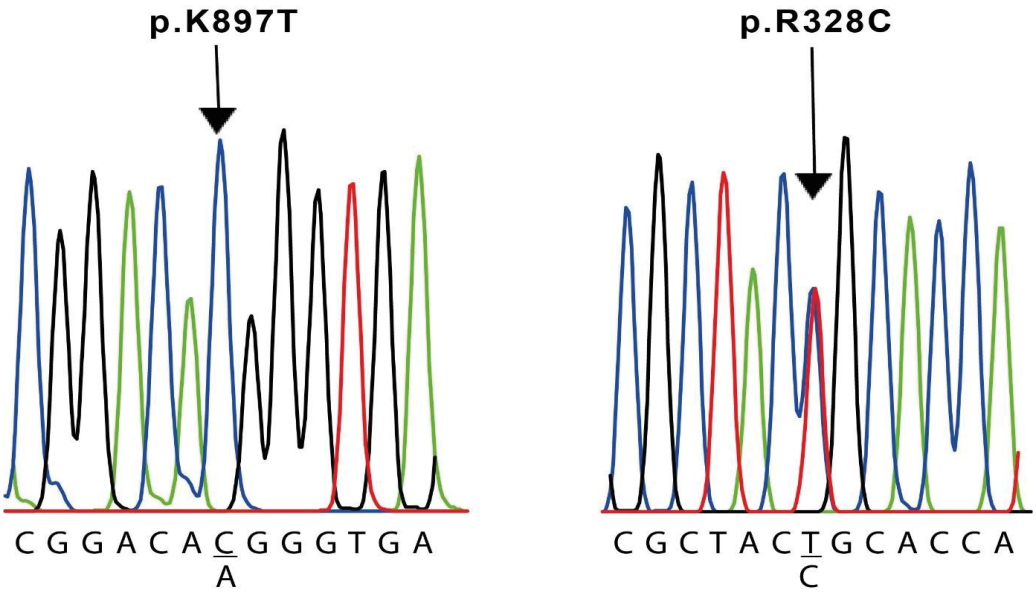

Figure 4: Nucleotide sequence of KCNH2 variants.

R238C was founded in case #3 (left) and K897T was found in cases #2 and #6 (right). The sequences shown correspond to the sense strand of gDNA.

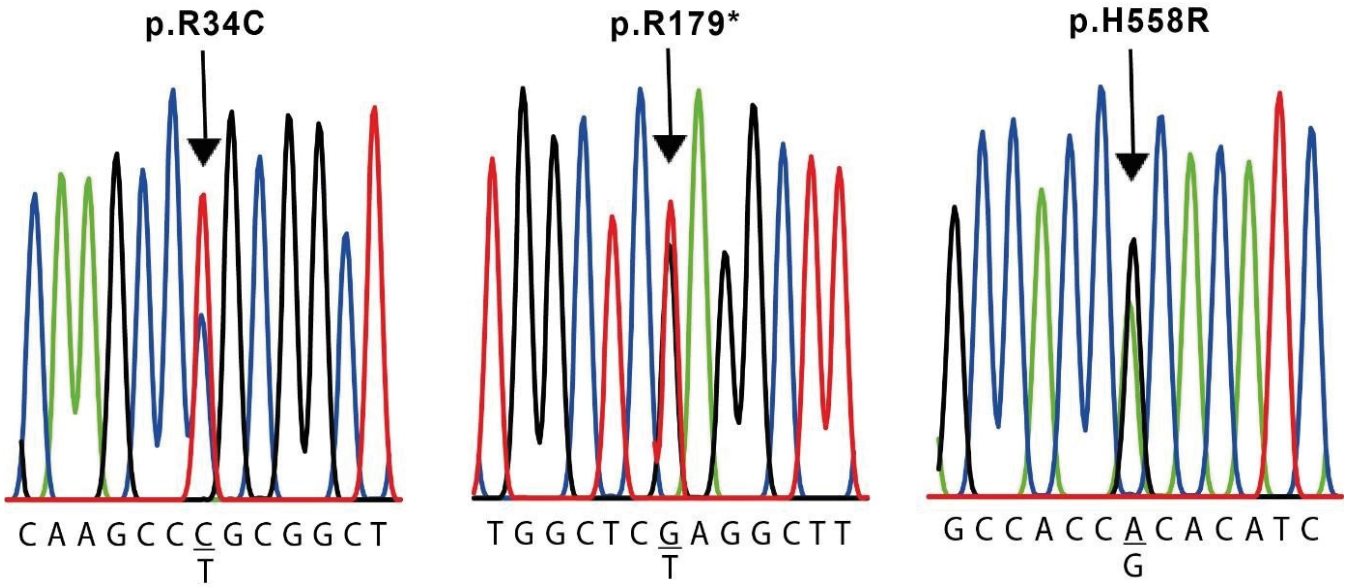

Figure 5: Nucleotide sequence of SCN5A variants in cases #4 and #6.

The sequences shown correspond to the sense strands of gDNA.

exon 2 of case #4, both located in the N-terminal region (Figure 3 and 5). The nonsense NM_000335.5(*SCN5A*):c.535C>T (p.Arg179Ter) in exon 5 of case #6, located in the TM2 of the domain I ((Figure 3 and 5), the missense NM_000335.5(*SCN5A*):c.1673A>G (p.His558Arg) in exon 12 of cases #3 and #4, located in the interdomain I-II (Figure 3 and 5); the synonymous NM_000335.5(*SCN5A*):c.5454T>C (p.Asp1818=) in exon 28 of case #2 and #4 and the synonymous NM_000335.5(*SCN5A*):c.5841C>T (p.Ile1947=) in exon 28 of case #4, both located after domain IV (Figure 3). The synonymous NM_000335.5 (*SCN5A*):c.3183A>G (p.Glu1061=) in exon 17 was found in all cases except case #4, in the interdomain III-IV (Figure 3). All of these changes are located in the intracellular side of the channel (Figure 3).

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Discussion

Cases #1 and #2

Case #1 is a first-degree relative of case #2 (proband). We only found silent variants in it. We detected the L564L variant in exon 7, and the Y652Y variant located in exon 8, both of the *KCNH2* gene classified as benign (Table 5) [14-16]. For the *SCN5A* gene we detected the benign silent variant E1061E, in exon 17. Case #1 shows a normal ECG, so it seems that those detected changes do not affect the QT interval.

Case #2

Case #2 is a young male with a considerably prolonged QT interval but asymptomatic and under treatment with BB. We found the infrequent benign S546S variant in exon 13 of the *KCNQ1* gene (Table 5). It has been associated with LQT syndrome in European individuals [16,17] and with alterations in plasmatic lipid levels [18] in the Chinese population. The other silent variants were Y652Y, for *KCNH2* gene, and A29A, E1061E, and D1818D, for *SCN5A,* all classified as benign [14,19-21]. Despite all of them are benign, some authors reported that synonymous mutations can affect the thermodynamic stability of mRNA secondary structures, having consequences in protein functional expression [22].

We also detected the K897T variant in exon 11 of *KCNH2* gene (Figure 3), classified as benign but also associated with channel-kinetic changes and reduced hERG expression [23-25]. This variant acquires a pathogenic profile when it is associated with *KCNQ1* polymorphisms [3,23]. hERG channels are regulated by thyroid hormone through PI3K signaling. This change, creates a phosphorylation site for Akt which reverses hormonal regulation through the PI3K signaling and inhibits channel activity, prolonging the QT Interval [26].

Considering that some of the silent variants are shared with Case 1 (normal QT interval), possibly, the combination of the silent variants with the K897T variant might be responsible for the prolongation of the QT interval observed in this case. In particular, silent variant in *KCNQ1* gene that is not shared with Case #1.

Case #3

This is a young adult female with a medical history of presyncope and PVT, exhibiting a mild prolongation of QT interval (Table 1 and Figure 1). We identified two changes in the protein sequence: the R328C in *KCNH2* gene and the H558R in the *SCN5A* gene, along with a silent variant E1031E. Despite they are not associated with a pathogenic status, these variants are reported in individuals with cardiac alterations [23,27]. Although the R328C variant does not reduce the I_{Kr} or alters channel function [28], it was reported in a case of Torsades de Pointes with respiratory distress, left ventricular dysfunction, and a prolonged QT interval [29]. Additionally, it has been associated with changes in conductance when is co-expressed with the *KCNQ1* mutation R591H [30].On the

Table 3: List of primers used to amplify KCNH2 coding exons.

Target	Forward (5`-3`)	Reverse (5`-3`)	Product (bp)
Exon 2	CACGCACAGCTGCGTTCG	GCCCTCCTGAAAACCATCTC	626
Exon 3	ATTGAGGGGAGCCATAAGGG	CAAGCCACATCCTCAGGGTA	524
Exon 4	TGGCGGTTTATGATGACTGGA	GCACCCAGGACGTAGTGAAAA	746
Exon 5	GTTTCTTGTGACTCCCCTGG	AGCCCTTTACCAGACCTCTC	584
Exon 6	GGGTGGGCATTCTGATGGAA	GAGCATAGGTTTGCTGGGGT	756
Exon 7	CAGTGTGGGCTTCACCTCTT	CCCCCAAACCATGTCACGAT	678
Exon 8	TGGAGCGCAGATGTACAAGG	CATGGGCAAAAAGGGGCAAC	488
Exon 9	CAGGCCTGGAGGTTGAGATTT	AAGGAGAATGTGGGAACCCC	586
Exon 10	ATTGCTTCCCCGGTTGTGTG	TCCCTGCCCCCAATGTGATT	538
Exon 11	TAGAGCAGCCTACAAAAGTCCC	GGAAGGGATGGGAAGGTCTGA	402
Exon 12	ATAGAACAAAGGAGGGCCAGG	AGCTGAAAATGTTGGACACTCC	682
Exon 13	CGAGAAGAGCAGCGACACTTG	GAATGGAAGAAGGGGATCCAGC	437
Exon 14	TCCCCTTCTTCCATTCCTAGCC	GCAGGAACAAGGTTCAGGGAG	466
Exon 15	TACTTCCCACCTTGGTGCCT	GCTGTGCTTTCGAGTTCCTC	485

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other hand, it has been demonstrated that residues 326–345 located in the proximal domain (Figure 3) might be required for hormonal modulation of hERG [31,32]. This case also presents the H558R variant in exon 12 of *SCN5A* gene. It has been associated with alterations in intracellular trafficking [23] and reported as a pathogenic variant [33,34] (Table 3). R558-containing sodium channels are wild-type–like except when co- expressed with alternatively spliced transcripts [35].This change is located at the linker between DI and DII of the $Na_V1.5$ channel (Figure 3), which was reported to be a hot-spot for Arginine methylation, affecting channel properties [36]. The combination of all these changes may act synergistically contributing to the severe clinical presentation observed in this individual.

Table 4: List of primers used to amplify SCN5A exons.

Target	Forward (5`-3`)	Reverse (5`-3`)	Product (bp)
Exon 2	CTCTCTGCAAATGGTGTCCC	CACCAGTGACTCATTTCCCC	579
Exon 3	TGAGTCTACTGACCTGCCAA	GGAATCAGCGCTACTCTCAC	408
Exon 4	TCTCCTTGGAGACCCTGTTT	GGACTGGGAAAGGCAAAAGA	390
Exon 5	TTGATGGCCTCTGTTGAAGG	CTCTCCCCACCAGGATGAG	423
Exon 6	TAAGATGCCCAGGTTTGCCT	TCTGGTGACAGGCACATTCG	341
Exon 7	GTCTCAAAGCCCAGGAGAAG	TCTAGCCTGGGAAGTCACAA	572
Exon 8	CAGAGGAACAGAAGGAAGGC	CTCCAGAAGCTGTCTCCTCT	455
Exon 9	CTGTGGGGCATAAACTGGGT	TGCTGATCCCTTCTCCCTCA	407
Exon 10	TACCCTCCTCCCTAGGCTAT	TCAGCGATACCACATTCACA	539
Exon 11	TTGGGGTAGGTGTGCAAGTC	AGGCCATGGGAAACAGGAAG	435
Exon 12	GCCCTCAATGCTCTGAGAAG	TCTGTCTGTCCCCATTAGCA	667
Exon 13	AGGGAGTTGGGAACAGAGAA	AGGCCAGATGTGGGAGTATT	547
Exon 14	CACCTAGCAGCCCTGTCATC	GACCCTGAGATTCCCTCCCT	525
Exon 15	CCAAGCAAACCCCTACTGG	GGTGAAGGCATGACAGATCA	431
Exon 16	TAGTGGGTGCTCTGGGAGAA	TGGACGGATGGGTAGATGGA	620
Exon 17	ATAGCCAAACCTTCCACATT	CATGAGTGGTGGATAGCAG	883
Exon 18	CCTTGAGGGAGGAGTCTTCA	AGAATTTCCCATTGGCCCTG	527
Exon 19	TGACAGGCAAAAGTGGCTCT	TCTAAGGCAGGGTGTTGGTG	396
Exon 20	CACCCCCATCATCGTAGCTC	CCGTGGGGTTGAGAGTTTGT	447
Exon 21	GCAACAGAGCAAGACTGTCT	TACGTCCTCCTTCCTCTCTG	448
Exon 22	GCCAGGATACTCTTGGGCTT	ACGGCCATAGGACATCAGAA	455
Exon 23	CTCCCTTGAGTGTGGGATCT	GGCACTGTGATCCTCCTATG	557
Exon 24	CTCTGACCACCCAGGCATTT	ACGAGATCTTGCCCTTGTGG	335
Exon 25	TGGGCTAGTGACCTTCCTCT	TACATCCCTGGACACACCCT	452
Exon 26	GAGAAAGCCAGGAGGTGGTC	AGGCTGGGACCTCTCTTCAT	380
Exon 27	GGCTTTGGGCTCACTAGAGG	GAGAGGTGTGTGTGCGTGTA	562
Exon 28A	TGGCTCCTTGCCATATAGAGA	ATACGGAGTGGCTCAGACAG	777
Exon 28B	TGAGTGAGGACGACTTCGAT	GAACTCTGCCTGGTTGATCC	845

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Table 5: Detected variants for 6 cases of the studied cohort.

Case	Gene	Region	Nucleotide Change	Amino acid change	Zygosity	ClinVar
#1	*KCNQ1*	-	-			-
	KCNH2	Exon 7	c.1692A>G	p.L564L	Htz	B
		Exon 8	c.1956T>C	p.Y652Y	Htz	B
	SCN5A	Exon 17	c.3183A>G	p.E1061E	Hmz	B
#2	*KCNQ1*	Exon 13	c.1638G>A	p.S546S	Htz	B
	KCNH2	Exon 8	c.1956T>C	p.Y652Y	Htz	B
		Exon 11	c.2690A>C	p.K897T	Htz	B
	SCN5A	Exon 2	c.87A>G	p.A29A	Htz	B
		Exon 17	c.3183A>G	p.E1061E	Hmz	B
		Exon 28	c.5454C>T	p.D1818D	Htz	B/LB
#3	*KCNQ1*	-	-	-	-	-
	KCNH2	Exon 5	c.982C>T	p.R328C	Htz	B/LB/US
	SCN5A	Exon 12	c.1673A>G	p.H558R	Htz	B/LB
		Exon 17	c.3183A>G	p.E1061E	Hmz	B
#4	*KCNQ1*	-	-	-	-	-
	KCNH2	Exon 8	c.1956T>C	p.Y652Y	Hmz	B
	SCN5A	Exon 2	c.87A>G	p.A29A	Hmz	B
			c.100C>T	p.R34C	Htz	B
		Exon 12	c.1673A>G	p.H558R	Htz	B/LB
		Exon 28	c.5454T>C	p.D1818D	Htz	B
			c.5841C>T	p.I1947I	Htz	B
#5	*KCNQ1*	Exon 16	n. a.	-	-	n. r.
	KCNH2	Exon 8	c.1956T>C	p.Y652Y	Hmz	B
	SCN5A	Exon 2	c.87A>G	p.A29A	Htz	B
		Exon 17	c.3183A>G	p.E1061E	Hmz	B
#6	*KCNQ1*	Exon 13	c.1638G>A	p.S546S	Htz	B
	KCNH2	Exon 8	c.1956T>C	p.Y652Y	Hmz	B
		Exon 11	c.2690A>C	p.K897T	Hmz	B
	SCN5A	Exon 2	c.87A>G	p.A29A	Htz	B
		Exon 5	c.535C>T	p.R179*	Htz	P/LP
		Exon 17	c.3183A>G	p.E1061E	Hmz	B

n. a: not amplified, n. r.: not reported, Hmz: homozygous, Htz: heterozygous, ClinVar: ClinVar variant interpretation category, B: benign, LB: likely benign, P: pathogenic, LP likely pathogenic,US: uncertain significance.

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Case #4

This individual is a middle-aged male with arrhythmia, palpitations and a prolonged QT interval (Table 1). He presents two changes in the protein sequence coded by the *SCN5A* gene: R34C and H558R. R34C variant is a frequent polymorphism in Asian and black population, although it has also been detected among Hispanics [34]. It is located at the N-terminal (Figure 3) and is associated with a loss-of-function change. H558R, as mentioned before, is associated with cardiac electrical alterations. The co-expression of these two changes in the same individual has not been reported before. A synergistic negative effect of both changes combined, could be present in this individual. Moreover, he exhibits several silent mutations in *KCNH2* (Y652Y) and *SCN5A* genes (A29A, D1818D, and I1947I). Altogether these changes may contribute to the clinical manifestations that this individual shows.

Case #5

This is an asymptomatic pediatric individual with a family history of sudden death in a sports setting and exhibits a prolonged QTc interval (Figure 1). Gene analysis revealed several silent mutations in *KCNH2* (Y652Y) and *SCN5A* (A29A and E1061E) genes but also an unidentified change in exon 16 of *KCNQ1* gene. This change could result from a deletion or an insertion big enough to not be amplified by our PCR conditions. Either of these possibilities will affect the functional properties of the channel [37]. Young patients with deletions in exon 15 and 16 showed prolonged QTc interval [38,39]. Additionally, a small domain between residues 589 and 620 in the C-terminal region may function as an assembly domain for KCNQ1 subunits [37]. Without it, KCNQ1 would fail to assemble and functional channels will not be produced. Besides, this exon encodes the site of interaction between AKAP9 and KCNQ1, which is required for the functional channel regulation through phosphorylation [40]. Therefore, in this individual, the partial or complete lack of exon 16 may affect channel function through these mechanisms. A similar case was found in an adolescent patient who experienced QT lengthening following physical exercise [38]. In our case, a young individual with a family history of sudden death during sports activities, this exon 16 alteration could have similar implications.

Case #6

This case is a female who presents bradycardia and prolonged QT interval and shows several silent mutations: S546S in *KCNQ1*, Y652Y in *KCNH2* and A29A and E1061E in *SCN5A*. Moreover, she also shows two variants that change the protein sequence: K897T in *KCNH2* and the R179* in *SCN5A*. In this case, the K897T variant was found in homozygosity, so 100% of the channels will have the mutant subunit. More drastically, the R179* inserts a stop codon in exon 5 of *SCN5A* gene, generating a truncated protein that lacks all pore domains (Figure 3). Heterologous expression of the *SCN5A*-R179* alone results in non-functional channels [27]. This variant has been classified as pathogenic/likely pathogenic and also reported in individuals with Brugada Syndrome and LQTS [27,35,41]. As this mutation was found in heterozygosity, not all Nav1.5 channels are affected. Probably, the combination of both mutations leads to the development of an intermediate syndrome with characteristics of both, BS and LQTS.

Conclusion

This study conducted by a national research laboratory in Argentina represents the first public report of genetic variations in LQTS within this population. It marks the beginning of a systematic survey of the Argentinian population, which is known for its diverse genetic background.

The analysis of *KCNQ1*, *KCNH2*, and *SCN5A* genes in the studied individuals allowed the detection of both benign and pathological genetic variants. Among the cases, five individuals exhibited variations in these genes that may be associated with prolonged QT intervals and clinical symptoms.

Our study highlights the importance of detecting variants associated with alterations in the physiology and function of cardiac ion channels, since they can create a vulnerable substrate that, in the presence of specific triggers such as I_{Kr} blockers, can precipitate life-threatening ventricular arrhythmias.

Furthermore, the results demonstrate that the genetic classification of LQTS is not solely determined by a single pathogenic mutation in a gene. It is more likely that a combination of silent and protein-altering changes in different genes contributes to the phenotype observed in LQTS patients.

Consequently, genetic analysis of suspected LQTS cases, as well as their close relatives, can provide valuable information for accurate diagnosis, genetic counseling, and determining the most appropriate treatment strategies.

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Competing interests

The authors declare that they have no competing interests.

Ethics declarations

Ethics approval and consent to participate. This study was conducted under the approval of the ethics committee of the *Hospital Municipal de Agudos L. Lucero* de Bahía Blanca, (expedient Nº 43-30154-2017) following the Declaration of Helsinki.

Authors' contributions

LD, SS, EA, ER and LD, perform molecular biology experiments, analyzed, and interpreted the patient data. LO, FG and RK selected the patients for the study. LD, EA, GS and RK were a major contributors in writing the manuscript. All authors read and approved the final manuscript.

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