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Investigation of Ion Channel Gene Variants in Patients with Long QT Syndrome

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Abstract

Background: The long QT syndrome (LQTS) is an inherited arrhythmia syndrome with increased QT interval and risk of sudden death. Mutations in genes KCNQ1, KCNH2 and SCN5A account for 90% of cases with genotype determined, and genotyping is informative for genetic counseling and better disease management.

Objective: Molecular investigation and computational analysis of gene variants of KCNQ1, KCNH2 and SCN5A associated with LQTS, in families with the disease.

Methods: The coding regions of genes KCNQ1, KCNH2 and SCN5A in patients with LQTS and their family members were sequenced and analyzed using Geneious Pro™ software.

Results: Two families with clinical criteria for LQTS were investigated. The proband of Family A had QT_C = 562 ms, Schwartz Score = 5.5. The genotyping identified the G1714A mutation in the KCNH2 gene. QT_C = 521 ± 42 ms was observed in family members carrying the mutation against QT_C = 391 ± 21 ms for non-carriers. The proband of Family B had QT_C = 551 ms, Schwartz Score = 5.5. The genotyping identified the G1600T mutation, in the same gene. The analysis of family members revealed QT_C = 497 ± 42 ms in mutation carriers, compared with QT_C = 404 ± 29 ms in non-carriers.

Conclusion: Two gene variants previously associated with LQTS were found in two families clinically diagnosed with LQTS. The prolongation of the QT interval was observed in all family members carrying the mutations. A strategy was developed to identify variants of genes KCNQ1, KCNH2 and SCN5A, making it possible to train technical staff for future application to diagnosis routine. (Arq Bras Cardiol 2011;96(3):172-178)

Keywords: Long QT syndrome; ion channels; mutation; death sudden cardiac.

Introduction

The long QT syndrome (LQTS)[1,2] is a genetic disease with prevalence of 1:2,000[3], which involves risk of sudden death from ventricular arrhythmias in a structurally normal heart[4]. The diagnosis is made through the Schwartz score[5], which combines the clinical history and ECG criteria and the clinical history, rating the probability of LQTS as "low" (< 1) "intermediate" (2 and 3) and "high" (≥ 4). A Schwartz score ≥ 4 has specificity > 99% for the diagnosis of LQTS[6].

Different types of LQTS are caused by variations in ion channel genes[7,8] and in the ankyrin gene[9]. Currently, 12 types have been described[10], but types 1, 2 and 3 account for 90% of cases with identified genotype[11]. Although more than 700 variants have been described[12], the genetic basis was not identified in 29% of the cases[13]. Various types of LQTS differ with respect to risk of

sudden death[14], triggers for arrhythmia episodes[15,16] and response to drug treatment[17,18]. Furthermore, the severity depends on the affected region of the channel[19]. That is why the identification of the mutation is important for managing the disease.

Despite the large number of variants involved, the search for gene variants associated with LQTS (genotyping) is being increasingly used in clinical practice. To the best of our knowledge, no unit of the National Health System is currently conducting the genotyping of LQTS. For this reason, the gene variants associated with LQTS were investigated in two families clinically diagnosed with the disease. The conditions for the genotyping of genes KCNQ1, KCNH2 and SCN5A were established, and a computational analysis was used to facilitate the identification of mutations previously described and novel gene variants in the Brazilian population.

Methods

Patients

Patients referred to the National Institute of Cardiology with clinical suspicion of LQTS had their medical history

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taken and were physically examined by a cardiologist with expertise in LQTS (FESCFº). The electrocardiograms were independently analyzed by two researchers (EC and FESCFº), and the QT interval was measured by the tangent method[20]. None of the probands had electrocardiographic signs of cardiac hypertrophy, conduction disturbances or myocardial ischemia. Molecular analyses were carried out with probands with Schwartz score ≥ 4 and first-degree relatives, with a total of 29 individuals from two families.

DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was extracted from peripheral blood[21]. The exons corresponding to coding regions of the three genes investigated were amplified by PCR using the primers previously described[22,23]. Exons of genes KCNQ1 and KCNH2 were amplified using, respectively, the programs "B" and "C" previously published by Syrris et al[24] To amplify exons 1A of KCNQ1 and 4 of KCNH2, the PCRx Enhancer reagent (Invitrogen) was added to the reaction, according to manufacturer's instructions. The exons of the SCN5A gene were amplified using conditions that we established: 0.2 mM dXTP, 3 mM $MgCl_2$, 0.5 μM of primers (sense + antisense), 0.625 IU of recombinant Taq DNA polymerase (Invitrogen), 200 ng of DNA template, in a final volume of 50 ml. The program consisted in 94° C/10 min; 30 cycles of 94° C/1 min; 58° C/1 min; 72° C/2 min; final extension at 72° C/10 min.

Automated DNA sequencing

The sequencing of PCR products purified by Millipore's PCR Cleanup filters was performed using sense or antisense primers, or in a MegaBACE 1000 device (Amersham Biosciences) with DYEnamic ET Dye Terminator Kit reagents (GE Healthcare Life Systems), according to manufacturer's instructions.

Analysis of sequences obtained for identification of mutations

The Geneious Pro™[25] software was used to analyze the sequences obtained and to generate maps of mutations already described for the genes studied. In a nutshell, the sequences of the genomic and coding regions of genes KCNQ1 (access number NM_000218 and NG_008935.1), KCNH2 (access number NM_000238 and NG_008916) and SCN5A (access number NM_000335 and NG_008934.1) were imported from GenBank and known mutations (Inherited Arrhythmias Database[12]) were added to these in the software, with the respective references. Then, we marked the annealing sites for primers, intronic mostly, used in amplification reactions and the central portions of the introns were removed to facilitate the analysis. The direct comparison between mutation maps and the sequences obtained from patients allows viewing both the quality of sequencing and the mutations previously described.

Ethical considerations

This study was approved by the Research Ethics Committee of the National Institute of Cardiology (case number 0187/4.01.08). Patients and relatives were informed about the objectives and risks of the study and agreed to participate in it by signing the consent form.

Results

Research on gene variants of KCNQ1, KCNH2 and SCN5A was conducted in two families clinically diagnosed with LQTS, including the analysis of two probands and their families, totaling 11 individuals in family A and 17 in Family B. The analysis of the variants was optimized by creating mutation maps with Geneious Pro™ software (data not shown).

The proband of Family A (Figure 1A, patient A2.21), 36 years of age, began experiencing palpitations at the age of 15; at the age of 24, this patient began experiencing palpitations followed by syncope triggered by emotional stress, sudden noise and upon awakening, being diagnosed with LQTS. The proband of Family B (Figure 1B, patient B2.1) was 42 years-old and experienced episodes of pre-syncope during physical exertion. The same patient sought medical attention and was diagnosed with LQTS after his cousin (#B2.9) experienced an episode of aborted sudden death (ASD) in a hospital environment. ECGs with measurements of the QT interval of the probands are shown in Figure 2. Patient A2.21 had QT_C = 560 ms (Figure 2A) and patient B2.1 had QT_C = 551 ms (Figure 2B). In the family history of proband A2.21, it was possible to identify 10 cases of sudden death at early ages, which is consistent with LQTS. (Figure 1A, highlighted in green). In Family B, there was only one case of aborted sudden death (#B2.9).

Clinical and electrocardiographic data of individuals from both families are presented in Table 1. Family A had a total of 9 women and 2 men, between 14 and 80 years of age. Family B comprised 17 individuals (7 males), between 5 months and 69 years of age. It was possible to identify 8 individuals with Schwartz score ≥ 4, four in Family A (A2.21, A2.23, A3.2, A3.3) and 4 in Family B (B1.1, B2.1, B2.9, B3.1). Two of these individuals (A2.21 and B2.9) had episodes of aborted sudden death (ASD) and were treated with ICD + beta-blocker. The others (A1.10, A2.23, A3.2, A3.3, B1.1) were asymptomatic and were treated with beta blockers alone. One case of ASD (B2.9) had QT_C = 448 ms. However, this ECG was recorded during treatment with beta-blockers, which can reduce the QT interval. Only one individual (A1.10) showed intermediate probability of LQTS (Schwartz score = 2.5) and borderline QT_C (450 ms). The analysis of the pedigree chart suggested that this individual would be carrying a mutation that causes LQTS, which was confirmed by a molecular analysis. Only one individual (A2.21) had a record of Torsades de Pointes.

A molecular analysis was capable of detecting variations previously described of gene KCNH2 in probands of both families. In the proband of Family A, gene variants A1692G and G1714A were found, both in exon 8. In the proband of Family B, gene variants C1467T, C1600T, A1692G and T1956C were found. All variants found are single nucleotide polymorphisms (SNPs) and the patients are heterozygous at all *loci*. However, gene variants G1714A (Family A) and C1600T (Family B), both in exon 8 of the KCNH2 gene, are non-synonymous (missense) SNPs and were previously described as associated with LQTS. The identification of these gene variants is highlighted in Figure 3. The other variants found are common polymorphisms and their clinical relevance has not been demonstrated[12].
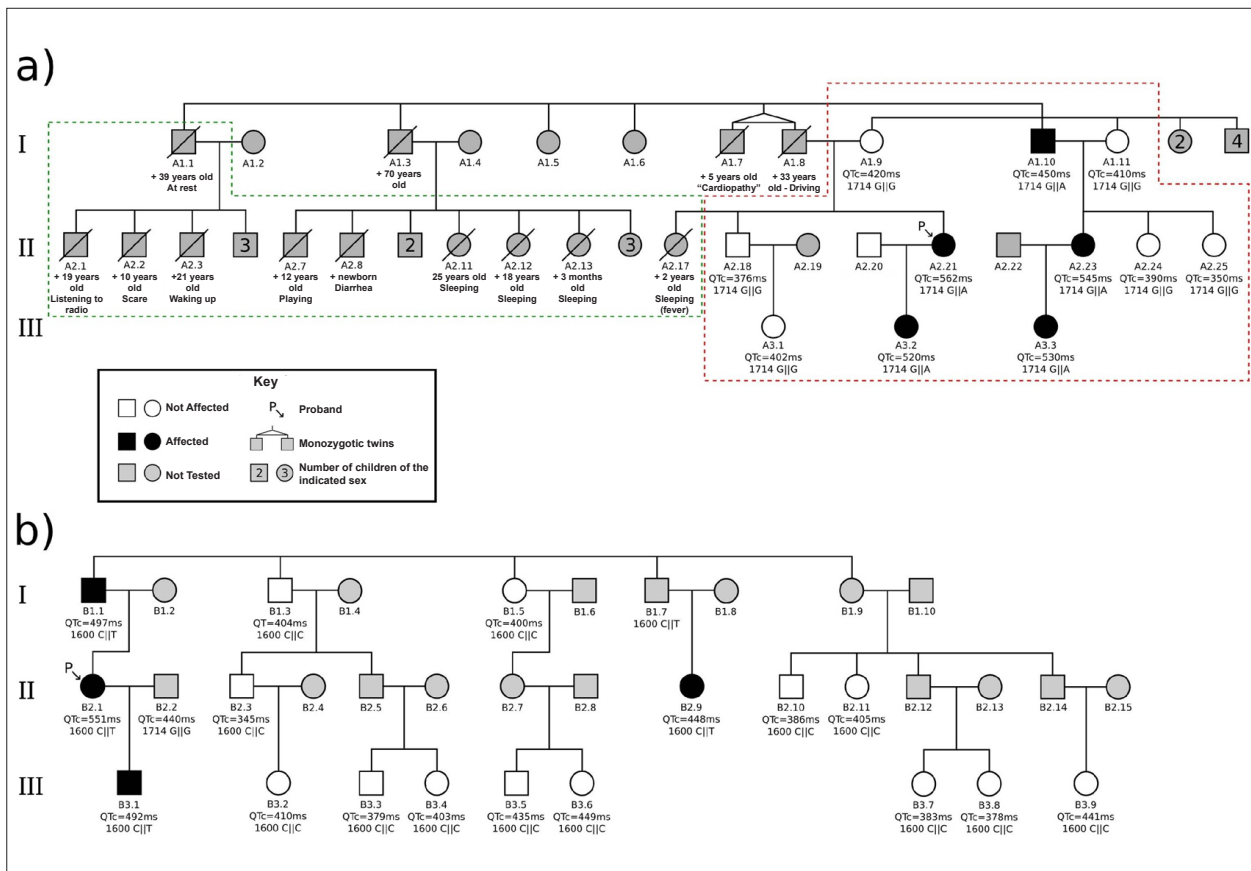
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Figure 1 - Pedigree charts of the families studied. The probands are indicated by arrows and the letter P. The Roman numerals on the left represent the generations. Siblings of the same sex that are not clinically relevant are represented by a square or circle with a number inside. A) Family A - sudden death cases are highlighted by the green dashed line. The age and cause or circumstances of death are shown below each deceased individual. Individuals included in the project are highlighted by the red dashed line. The QT_C interval and genotype at position 1714 are shown below each individual. b) Family B - the genotype at position 1600 and the QT_C of individuals investigated is presented as described above.
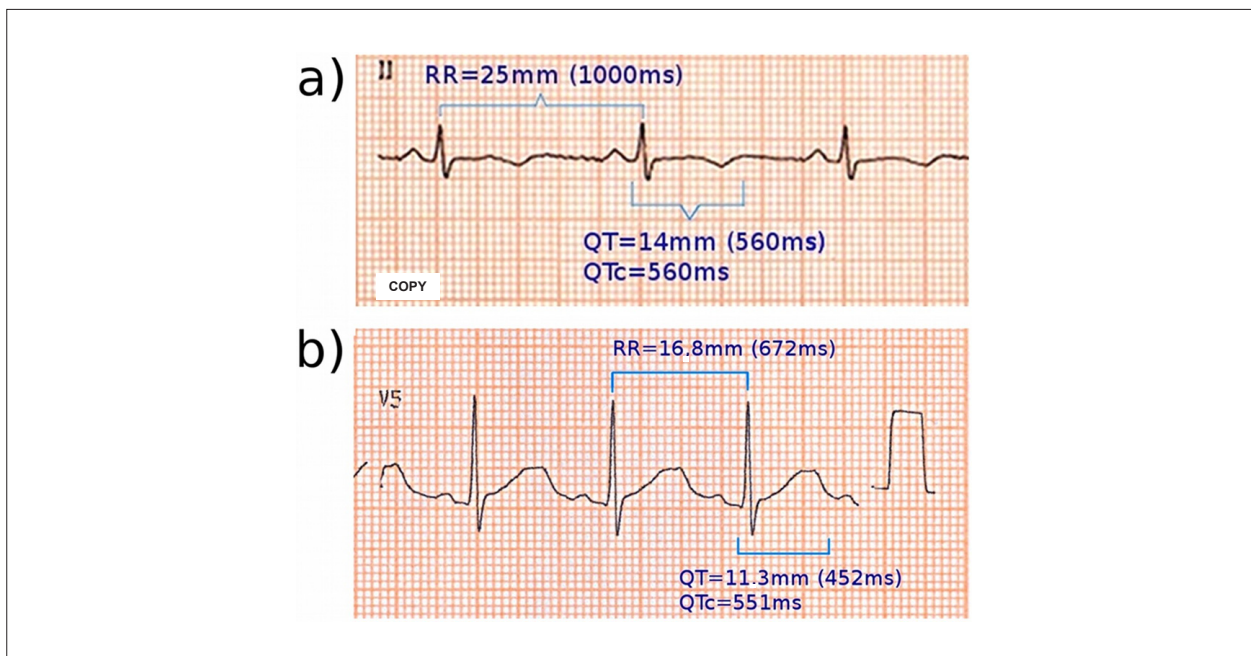

Figure 2 - Electrocardiograms of the probands of families A (patient A2.21) and B (patient B2.1).

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Table 1 - Clinical and electrocardiographic characteristics of the individuals investigated in families A and B

Individual	Sex	Age	Clinical presentation	HR (bpm)	QT$_c$ (ms)	Morphology of the ST segment	Documented arrhythmia	SS
A1.9	F	62	Asymptomatic	55	420	Normal	-	1.5
A1.10	M	80	Asymptomatic	65	450	Normal	-	2.5
A1.11	F	65	Asymptomatic	75	410	Normal	-	1
A2.18	M	39	Asymptomatic	56	376	Normal	-	1
A2.21	F	36	Syncope (auditory stimulus); ASD with the use of ATB	48	562	Biphasic T in DII, aVF, aVR, V1 to V4	TdP	5.5
A2.23	F	38	Asymptomatic	67	545	Normal	-	4
A2.24	F	44	Asymptomatic	99	390	Flattened T	-	1
A2.25	F	39	Asymptomatic	60	350	Biphasic T V1 to V4	-	1
A3.1	F	10	Asymptomatic	84	402	Normal	-	1
A3.2	F	14	Asymptomatic	54	520	Biphasic T V3 to V5	-	5.5
A3.3	F	15	Asymptomatic	68	530	Prominent U waves	-	4
B1.1	M	69	Asymptomatic	61	497	Flattened T wave	-	4
B1.3	M	67	Asymptomatic	66	404*	Normal	AF	1
B1.5	F	65	Asymptomatic	68	400	Normal	-	1
B2.1	F	42	Presyncope on exertion	89	551	Flattened T waves; notches in aVF, V2, V3, prominent U waves.	NSSVT	5
B2.3	M	30	Asymptomatic	54	345	Normal	-	1
B2.9	F	37	ASD with the use of ATB	59	448†	Flattened T; notches in V2, V4, V5.	-	4
B2.10	M	33	Asymptomatic	61	386	Normal	-	1
B2.11	F	39	Asymptomatic	83	405	Normal	-	1
B3.1	M	15	Pre-syncope with emotional stress	52	492	Bradycardia. Slowly ascending T. Prominent U waves.	-	5
B3.2	F	1 to 4m	Asymptomatic	106	410	Normal	-	1
B3.3	M	11	Asymptomatic	71	379	Normal	-	1
B3.4	F	5	Asymptomatic	86	403	Normal	-	1
B3.5	M	11	Asymptomatic	75	435	Normal	-	1
B3.6	F	8	Asymptomatic	91	449	Normal	-	1
B3.7	F	9	Asymptomatic	84	383	Normal	-	1
B3.8	F	13	Asymptomatic	61	378	Normal	-	1
B3.9	F	5m	Asymptomatic	126	441	Normal	-	1

The numbering of individuals follows the standard adopted in the pedigree charts (Figure 1); the probands are highlighted in bold; SS - Schwartz Score; ATB - Antibiotic; ASD - Aborted Sudden Death; AF - Atrial fibrillation; NSST - Non-Sustained Supraventricular Tachycardia; (*) the QT interval was used instead of the QT$_c$ interval due to the presence of AF; (†) EGG recorded during treatment with beta-blockers.

Missense variants C1600T and G1714A were investigated in the relatives of probands A2.21 and B2.1, respectively. The results of this genotyping are shown in Figures 1A and 1B. In Family A, the average QT$_c$ in patients carrying the gene variant was 521 ± 42 ms, compared to an average QT$_c$ of 391 ± 21 ms in non-carriers. In Family B, the average QT$_c$ interval in patients carrying the gene variant was 497 ± 42 ms, compared to an average QT$_c$ of 404 ± 29 ms in non-carriers. Therefore, it is possible to see that all carriers of the C1600T and G1714A alleles have a prolongation of the QT$_c$ interval, confirming the clinical relevance of the variants found.

Discussion

In this work we sequenced all the coding regions of genes KCNQ1, KCNH2 and SCN5A, to identify the gene variants associated with LQTS in two families containing members diagnosed with the disease. The gene variants in LQTS are
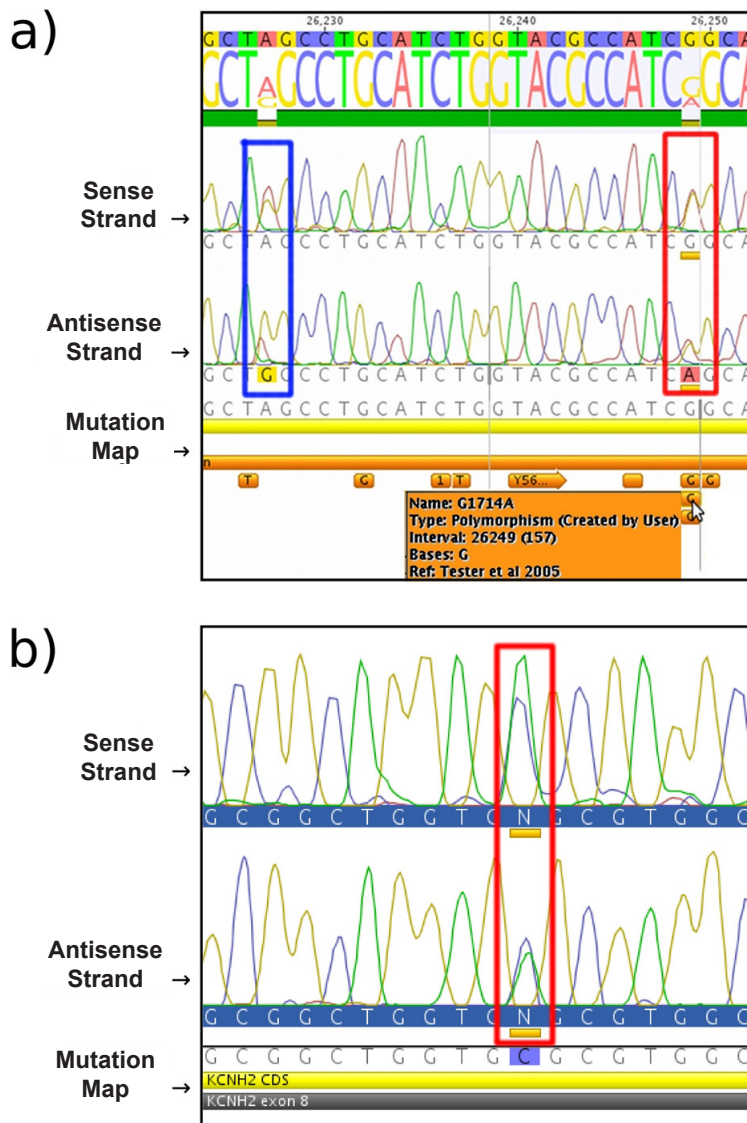
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Figure 3 - *Identification of gene variants by sequencing and analysis with the Geneious Pro™ software. The electropherograms for the sequencing of exon 8 of gene KCNH2 (antisense and sense strands) of the probands were aligned with the map of mutations already described (see Methods). The sense and antisense strands and the map of mutations are indicated. A) Family A - the A1692G variant is highlighted by a blue rectangle and the G1714A variant by a red rectangle. b) Family B - the C1600T variant is highlighted by a red rectangle.*

often screened using the SSCP (single strand conformation polymorphism) technique, which requires more manual work and is less sensitive than sequencing.

The difficulties in implementing, in a routine, a highly complex molecular diagnostic method, such as the LQTS, led us to develop, by using the Geneious Pro™ software, a sequence of steps to accurately identify new and already described mutations. The sequence manipulation and annotation features of this software were used to develop mutation maps indicating the locations with mutations described, allowing the alignment of sequences of different genes obtained from patients with built maps of genes

KCNQ1, KCNH2 and SCN5A, facilitating the identification of differences between both sequences. The alignment performed in this program also allows eliminating sequencing artifacts. If a gene variant found was previously described as associated with LQTS, bibliographic references of the same will be readily available in the markings on the map. In addition, the program organizes the files internally in a folder system, in order for the several original sequence files to be centralized and indexed, which is crucial when one takes into account the enormous volume of information generated for each patient. Thus, the methodology developed allows analyzing the sequences in a way that is efficient and easy to

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use, reducing the chance of misinterpretation and allowing the technical training of personnel for future implementation of a routine of this methodology in the public health system.

The molecular analysis methodology established by us was capable of identifying gene variants previously associated with the disease, in two families selected by the clinical criteria already defined. Both families investigated had variants associated with LQTS in the KCNH2 gene, which allowed classifying both patients as having type-2 LQTS. This gene encodes the alpha subunit of potassium channel $K_V 11.1$, which is responsible for the I_{Kr} current in the cardiac action potential[26]. The G1714A variant found in Family A has been previously described as associated with LQTS[27], and it causes the substitution of glycine for serine at position 572 of the protein, which corresponds to the pore. However, the electrophysiological consequences of the mutation for the ion channel activity have not been investigated. The presence of gene variant G1714A in the family described here strengthens the evidence in favor of the pathogenicity of this change. Gene variant C1600T, found in Family B, causes the substitution of arginine for cysteine, at position 534 of the polypeptide that corresponds to the voltage sensor of the same ion channel[28]. Moreover, in contrast to the previous variant, the effects of this change were electrophysiologically investigated by Nakajima et al[29], who demonstrated a reduction in the threshold of channel activation, accelerated activation and inactivation, and reduced inactivation of the channel at rest.

Interestingly, the genotype identified was a subtype 2 - the second most frequent one, accounting for about 30% of cases. Moreover, although these families were carriers of the same subtype, and the gene variants were fairly close (in the same exon, by the way), the clinical presentation of the disease differed between them, perhaps due to changes in different regions of the ion channel. The association between the region changed in the protein structure and the severity of the disease was investigated by Moss et al[19]. The authors demonstrated that mutations in the pore region of the channel encoded by the KCNH2 gene are associated with a much higher risk - about three times higher - of arrhythmogenic events. A study by Shimizu et al[30] found similar results. In accordance with such studies, we observed much greater severity of the illness in Family A (mutation in the S5-pore region) than in Family B (mutation in the S4 transmembrane segment - voltage sensor). However, another factor described that results in greater severity in the clinical presentation is the presence of more than one mutation causing LQTS (compound mutations), which occurs in about 2% of cases[31]. Since the gene variants in genes less commonly associated with LQTS (LQTS types 4-12) were not investigated, we cannot exclude the possibility of Family A being a case of this type.

Asymptomatic carriers were found, but it was not possible to find silent carriers with normal ECG. Silent carriers are described in LQTS[4,5,32], being more common in LQTS[14]. Individual A1.10 illustrates the complexity of the disease: although he carries the variant associated with disease, his electrocardiogram shows only a borderline prolongation of the QT_C interval (450 ms) and its Schwartz Score is equal to 2.5. Several factors may influence the QT interval - some diseases, the serum concentrations of calcium and potassium ions and several drugs -, thereby influencing the LQTS presentation. Furthermore, some recent studies have demonstrated that common polymorphisms in genes of cardiac adrenergic receptors can modulate the duration of the QT interval, and the severity of the clinical presentation, among patients with the same mutation in the KCNQ1 gene[33]. Therefore, the investigation of genetic variants in LQTS may help identify asymptomatic carriers with borderline QT_C, and it may be useful in genetic counseling.

Conclusion

The molecular investigation of two families clinically diagnosed with LQTS was undertaken, and two distinct genetic variants previously associated with the disease were found. A methodology was implemented to amplify and sequence the complete coding region of genes KCNQ1, KCNH2 and SCN5A, and a simplified computer method was developed to analyze the sequences, with the purpose of investigating the gene variants, by using features of the Geneious Pro™ software, which facilitates the training of technical personnel for future application of this methodology to the diagnosis routine.

References

1. Ward OC. A new familial cardiac syndrome in children. J Ir Med Assoc. 1964;54:103-6.

2. Jervell A, Lange-Nielsen F. Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death. Am Heart J. 1957;54(1):59-68.

3. Schwartz PJ, Stramba-Badiale M, Crotti L, Pedrazzini M, Besana A, Bosi G, et al. Prevalence of the congenital long-QT syndrome. Circulation. 2009;120(18):1761-7.

4. Moss AJ, Schwartz PJ, Crampton RS, Locati E, Carleen E. The long QT syndrome: a prospective international study. Circulation. 1985;71(1):17–21.

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5. Schwartz PJ, Moss AJ, Vincent GM, Crampton RS. Diagnostic criteria for the long QT syndrome: an update. Circulation. 1993;88(2):782-4.

6. Hofman N, Wilde AAM, Kääb S, van Langen IM, Tanck MWT, Mannens MAM, et al. Diagnostic criteria for congenital long QT syndrome in the era of molecular genetics: do we need a scoring system? Eur Heart J. 2007;28(5):575-80.

7. Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell. 1995;80(5):805-11.

8. Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell. 1995;80(5):795-803.

9. Mohler PJ, Schott JJ, Gramolini AO, Dilly KW, Guatimosim S, duBell WH, et al. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. Nature. 2003;421(6923):634-9.

10. Kaufman ES. Mechanisms and clinical management of inherited channelopathies: long QT syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, and short QT syndrome. Heart Rhythm. 2009;6(8 Suppl):S51-5.

11. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation. 2000;102(10):1178-85.

12. Priori SG, Napolitano C, Wilson J, deGiuli L, Rosetti S. Inherited Arrythmias Database [documento na Internet]. IRCCS Fondazione Salvatore Maugeri; 2000. Atualizado em 2 de março de 2010. [Citado em 2010 maio05]. Disponível em http://www.fsm.it/cardmoc/.

13. Ackerman MJ. Cardiac causes of sudden unexpected death in children and their relationship to seizures and syncope: genetic testing for cardiac electropathies. Semin Pediatr Neurol. 2005;12(1):52-8.

14. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, et al. Risk stratification in the long-QT syndrome. N Engl J Med. 2003;348(19):1866-74.

15. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. Circulation. 2001;103(1):89-95.

16. Khositseth A, Tester DJ, Will ML, Bell CM, Ackerman MJ. Identification of a common genetic substrate underlying postpartum cardiac events in congenital long QT syndrome. Heart Rhythm. 2004;1(1):60-4.

17. Shimizu W, Antzelevitch C. Differential effects of beta-adrenergic agonists and antagonists in LQT1, LQT2 and LQT3 models of the long QT syndrome. J Am Coll Cardiol. 2000;35(3):778-86.

18. Priori SG, Napolitano C, Schwartz PJ, Grillo M, Bloise R, Ronchetti E, et al. Association of long QT syndrome loci and cardiac events among patients treated with beta-blockers. JAMA. 2004;292(11):1341-4.

19. Moss AJ, Zareba W, Kaufman ES, Gartman E, Peterson DR, Benhorin J, et al. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. Circulation. 2002;105(7):794-9.

20. Postema PG, Jong JSSGD, der Bilt IACV, Wilde AAM. Accurate electrocardiographic assessment of the QT interval: teach the tangent. Heart Rhythm. 2008;5(7):1015-8.

21. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16(3):1215.

22. Wang Q, Li Z, Shen J, Keating MT. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. Genomics. 1996;34(1):9-16.

23. Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. Genomics. 1998;51(1):86-97.

24. Syrris P, Murray A, Carter ND, McKenna WM, Jeffery S. Mutation detection in long QT syndrome: a comprehensive set of primers and PCR conditions. J Med Genet. 2001;38(10):705-10.

25. Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, et al. Geneious, v. 4.8; 2009.[Acesso em 2010 mar 23] Disponível em http://www.geneious.com/.

26. January CT, Gong Q, Zhou Z. Long QT syndrome: cellular basis and arrhythmia mechanism in LQT2. J Cardiovasc Electrophysiol. 2000;11(12):1413-8.

27. 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(5):507-17.

28. Itoh T, Tanaka T, Nagai R, Kamiya T, Sawayama T, Nakayama T, et al. Genomic organization and mutational analysis of HERG, a gene responsible for familial long QT syndrome. Hum Genet. 1998;102(4):435-9.

29. Nakajima T, Furukawa T, Hirano Y, Tanaka T, Sakurada H, Takahashi T, et al. Voltage-shift of the current activation in HERG S4 mutation (R534C) in LQT2. Cardiovasc Res. 1999;44(2):283-93.

30. Shimizu W, Horie M, Ohno S, Takenaka K, Yamaguchi M, Shimizu M, 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(1):117-25.

31. Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC. Compound mutations: a common cause of severe long-QT syndrome. Circulation. 2004;109(15):1834-41.

32. Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. Circulation. 1999;99(4):529-33.

33. Brink PA, Schwartz PJ. Of founder populations, long QT syndrome, and destiny. Heart Rhythm. 2009;6(11 Suppl):S25-S33.