Identification of the Potential LQT2 Carriers by Using the CAVIAR Method

L Restier Miron[1], J Fayn[1], G Millat[2], B Li[1],
C Rodriguez Lafrase[2], Ph Chevalier[2], P Rubel[1]

[1]INSERM ERM107, Lyon, France
[2]Hôpital Cardiologique de Lyon, Lyon, France

Abstract

The congenital Long QT Syndrome (LQTS) is an inherited disorder of ventricular repolarization that predisposes affected individuals to sudden death. The analysis of conventional ECG may be misleading, and we need non invasive markers to identify more accurately patients with these conditions. In the LQT2 syndrome, the fast-delayed rectifier current Ikr is altered and leads to a prolonged Action Potential Duration (APD) in mid-myocardial areas that in turn translates to a prolonged QT interval and a widened T-wave. The aim of this work was to find new ECG parameters which can be used to identify LQT2 carriers in a large population. The study population was composed of 67 normal healthy subjects and 11 known carriers of a HERG mutation (four different mutations and four families). For each subject a 12-lead digital ECG was recorded and analyzed by using the spatiotemporal CAVIAR method. With only one single CAVIAR measurement we could correctly classify 93.6% of the cases. This measurement, called ODIR, represents the difference in orientation between the T wave and the beginning of QRS. Its values are significantly higher in the LQT2 patients (m±SD=58.5° ±14.5°) than in the normal population (m±SD=21.6°±14.6°) with a p-value<10E-9. This study demonstrates that it could be possible to identify potential LQT2 carriers by analyzing spatiotemporal ECG waveform patterns of a conventional 12-lead ECG.

1. Introduction

Long QT syndrome (LQTS) results from structural abnormalities in the potassium or sodium channels of the heart, which predispose affected persons to an accelerated heart rhythm (arrhythmia). This can lead to sudden loss of consciousness and may cause sudden cardiac death in teenagers and young adults who are faced with stressors ranging from exercise to loud sounds. LQTS is a monogenic disorder with a prevalence of about 1 in 5,000 persons in the United States. More than 340 mutations in the genes responsible for 7 different forms of LQTS (LQT 1-7) have been described [1-3]. The symptoms, signs and treatment of the disease vary somewhat depending on the gene involved. LQT2 is caused by mutations in HERG (KCNE2) which encode for the α subunit of IKr channels [4] and leads to a prolongation of the action potential duration in all of the three ventricular cell types but mostly in M cells [5]. The patients with LQTS are usually identified by QT prolongation on the ECG during clinical evaluation of unexplained syncope, or fortuitously discovered in a family study when one family member has been identified with the syndrome. It is known that the diagnosis of this risky condition is a difficult one. Genetic screening of family members of a genotyped proband sometimes allows the identification of a large number of clinically symptomless relatives. It has also been reported that 32% carriers of LQTS-related mutations may have a QTc within normal limits [6]. Reliance on the computer-generated ECG diagnostic interpretation alone may fail to identify many at-risk family members. Thus, there is a need to develop noninvasive discriminate measurements of ventricular repolarization in large population.

The aim of this work was to identify new spatiotemporal phenotypic parameters which can be helpful for the diagnosis of LQT2 syndrome.

2. Methods

2.1. Study population

All subjects were recruited at the Hôpital Cardiologique of Lyon. After standard clinical investigation, 12-lead digital ECGs were recorded and analyzed using the CAVIAR (Comparative Analysis of ECG-VCG and their Interpretation with Auto-Reference to the patient) method [7,8].

2.1.1. LQT2 group

The LQT2 group included four unrelated families (5 women and 6 men) from 9 to 64 years (m±SD=36 ± 19

years), with a known clinical diagnosis of LQT2 (four mutations). They were recruited at the Consultation department and a written informed consent was obtained. One of them had symptoms (further syncope episodes) and 9 presented an abnormal T-wave on ECG recording.

2.1.2. Control group

A population of 91 volunteer normal healthy subjects with no medication and no personal or family history of syncope or cardiac events were recruited in the Consultation department among the medical staff. In this group, 24 subjects were excluded because their QRS duration was greater than 110 ms or because their QTc interval duration was greater than 450 ms. Finally, for the control group we retained 67 subjects composed of 44 women and 23 men (m±SD: 37 ± 10 years ; range: 19 to 61 years).

2.2. ECG recordings

Two or three consecutive standard digital 12-lead ECG recordings were collected from each subject. The serial ECGs were recorded successively without changing the electrode placement. All ECGs were recorded using a digital electrocardiograph (Cardiette ar2100view, ET-Medical Devices) with a sampling rate of 1000 Hz and analyzed at 500 samples/sec. Recorded ECGs were then transmitted in the SCP-ECG standard format for further analysis.

2.3. CAVIAR ECG analysis

The digital ECG recordings were analyzed in a fully automatic manner using the CAVIAR system [7,8]. Spatiotemporal ECG analysis was performed by using the previously reported CAVIAR analysis method which consists in the representation of the recorded ECG signals into a three dimensional reference frame that is intrinsically linked to each individual's Purkinje conduction system. This new reference frame consists in the preferential plane (U, V) scanned by the cardiac electrical field, in the W axis perpendicular to this plane and in the center of gravity G of the spatial representations of the QRS and T waves. As a result, the CAVIAR method allows a quantitative characterization and an optimal display of the spatiotemporal patterns of the QRS and T waves, independent of the recording conditions (electrode positions, anatomic position of the heart in the thorax). We studied a set of 33 CAVIAR measurements of various types including the time interval durations, the spatial amplitude of the QRS and T-wave, the morphology and the planarity of QRS (initial and terminal parts) and of the T-wave, and the spatial orientation differences between QRS (initial and terminal sector) and the T-wave.

2.4. Statistical analysis

The comparison of the spatiotemporal CAVIAR measurements between the two groups was performed using a stepwise discriminant multivariate analysis (BMDP New System 2.0). All data are expressed as mean ± SD. For means comparison we used a Mann-Whitney non parametric test for independent samples.

2.5. Mutational analysis

DNA was extracted from peripheral-blood lymphocytes according to standard procedures. Primer pairs for KCNQ1, KCNH2 and SCN5A amplification were used [9,10]. Single-strand conformational polymorphism analysis, denaturing high performance liquid chromatography (Wave Transgenomics), or both were performed with amplified genomic DNA. For the samples of abnormal patterns, the precise sequences of the mutations were determined by sequence analysis.

3. Results

3.1. Genetic results

In family #1, an LQT3 mutation was discovered in a 37 years old woman after several syncopal events. Surprisingly, her children and her husband were carriers of a LQT2 mutation and were thus included in this study. Two of them were on beta blocker medication. In family #2, the proband was diagnosticated with LQTS at age 47 after a cardiac arrest. His mother and brother were found to be carriers of the same mutation. Because the proband and his mother have a cardiac conduction disorder with a QRS>110 ms, they were excluded from the study. In this family the only subject which was included had no clinical events and no medication. In family #3, the proband was a woman who suffered from syncopal events when she was 28 years old. Her father, brother and her aunt were carriers of the same LQT2 mutation. She was receiving a beta-blocker therapy at the time of the study. In family #4, two relatives were diagnosticated with LQTS after the sudden death of a 22 years old woman. Her sister and father were genotyped with a LQT2 mutation. They had no clinical events but they were treated with beta-blocker.

3.2. ECG measurements

Table 1 shows the statistical results of three of the standard ECG measurements. There were no significant differences between the measurements of these two groups, except for the QTc (which was calculated using the Bazett's formula). The QTc values ranged from 385 to 465 ms for the control group and from 353 to516 ms for the LQT2 group.

Table 1. Standard ECG parameters differences

Group	QTc (ms) m±SD	HR (bpm) m±SD	QRS (ms) m±SD
Control	420.10±16.91*	68.22±9.68	87.43±7.10
LQT2	439.82±42.63*	68.00±13.12	88.91±11.91

* $p < 0.01$.

3.3. CAVIAR measurements and statistical analysis

Control versus LQT2 discriminant analysis results show that the most relevant CAVIAR measurement which best classifies the subjects is ODIR. This variable measures the orientation difference (OD) between the preferential planes described by the cardiac electrical field vector during the initial depolarization phase and the repolarization phase. The ODIR values were significantly higher in the LQT2 group (m±SD=58.5 ± 14.5°, range from 28.8 to 78.0°) than in the Control group (m±SD=21.6 ± 14.6°, range from 2.7 to 70.8°) with a p-value $p<0.001$. Using only this CAVIAR measurement, we found that a cut off ODIR value of 45° allows to correctly classify 93.6% of the subjects. The sensitivity and the specificity were respectively 90.9% and 95.5%. There were only five misclassified subjects in our study population. Four of them were from the control group and one was a LQT2 carrier. For these cases, the ODIR value ranged from 52.36° to 70.84° for the normal subjects and was 28.83°for the LQT2 subject. To understand this erroneous classification, ECGs were reexamined and new interpretations brought up. It appears that one of the normal subjects presented negative T-waves on two leads and another one had notched T-waves. The two other subjects had respectively an isolated micro-voltage and abnormal Q-waves. These findings may explain the abnormal ODIR values found with the CAVIAR method. Furthermore, on the surface ECG of the LQT2 misclassified patient, a notched QRS complex in lead II may be an explanation for an abnormal spatial orientation of the QRS wave.

4. Discussion and conclusions

Long QT syndrome is characterized by a wide genetic heterogeneity and a puzzled relationship between QT interval prolongation and the symptoms. Given both the rarity and the variable clinical presentation of this disorder, genotype-phenotype relationships are difficult to clarify. Vincent et al (1992) have examined the QT interval distribution in families that were genotyped for the chromosome 11p15.5 locus for LQTS (LQT1). Having categorized each patient as a carrier or a non carrier of this LQTS locus, this study demonstrated substantial erroneous patient classification on the basis of a simple measurement of the QT interval with a cutoff value of 440 ms. Large studies using standard ECG failed to detect a difference in QT interval measurements between LQT1, LQT2 and LQT3 phenotype [12,13]. Because failure to identify individuals at risk could prove to be a fatal mistake, the investigation of the accuracy of quantitative methods such as CAVIAR is critical. In the attempt to provide a new and more powerful method to quantify repolarization, Day et al proposed to use an index of abnormal repolarization based on the QT dispersion defined as the difference between the longest and the shortest QT interval measured on a 12 lead ECG. However, this method is affected by the usual QT measurement limitations such as the difficulty in identifying the end of the T wave and the confounding role of the U waves. We feel that a novel method for a clinically useful determination of ventricular repolarization should integrate automated quantification of spatial dispersion. De Ambroggi et al (1986) have applied principal component analysis to the study of ventricular repolarization in LQTS patients. They have shown that this method distinguishes between normal and abnormal repolarization pattern with a sensitivity of 83%.

The CAVIAR spatiotemporal representations display a peculiar spatial orientation of the T wave in LQT2 patients (Fig. 1) that support these findings (ODIR mean values are significantly different between the two groups). We think that the quantitative analysis made by the CAVIAR method may obviate the limitations of subjective interpretation of the QT interval by the clinicians. According to our results, a patient with an ODIR > 45°, even if he has no clinical events or a QTc < 460 ms, should be considered as a potential LQTS carrier and genotype analysis should be done. Our results also suggest that specific spatiotemporal ECG waveform patterns of a conventional 12-lead ECG may identify potential LQT2 carriers independently of gender, age and beta blocker therapy. It is tempting to speculate that the CAVIAR method applied to standard 12-lead ECG may yield new non invasive markers in different LQTS populations and may help in identifying LQTS patients at higher risk of arrhythmias. If it is possible to identify ECG parameters that are really capable to provide complete discrimination, inexpensive diagnostic analysis might be widely deployable in clinical settings.

Study limitations

Our study has several limitations. First, the number of LQT2 patients is relatively small and our results should be considered as preliminary. To confirm our findings, recruitment of additional LQT2 patients is a priority. Five subjects were missclassified using our reference value of 45° for ODIR. Four were normal subjects and

one a LQT2 carrier. For this latter, only identification of the gene of the proband was performed. We believed that a full genotype analysis should have been realized, because a simple polymorphism or a second mutation in an other loci than LQT2 could have explained the erroneous classification. Furthermore, regarding the difficulty to identify precisely an abnormal ECG, it would have been preferable to perform a full genotype analysis for all the subjects of the control group. Despite these limitations, we believe that our results are encouraging enough to foster additional CAVIAR analysis of ECG in larger populations of LQTS patients.

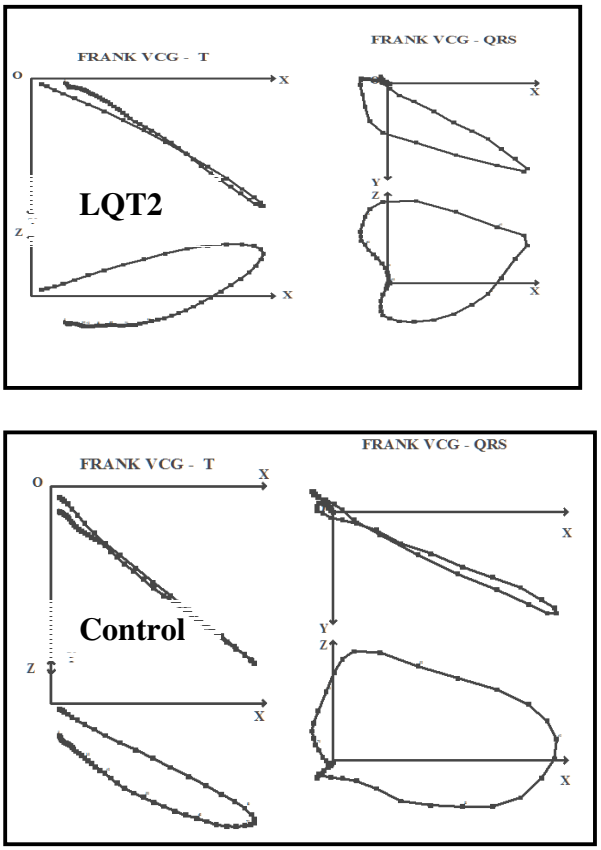

Fig. 1. Frank representations of the QRS and T waves of the ECG tracings of a LQT2 patient and of a normal subject showing the spatial orientation differences between the T-wave and the QRS complex

Acknowledgements

This work was supported by Medtronics grant to LRM. We also wish to thank R. Perraudin and E Froidefond, for laboratory assistance.

References

[1] Li H, Fuentes-Garcia J, Towbin JA. Current Concepts in Long QT Syndrome. Pediatr Cardiol 2000;21:542-550.

[2] Delisle BP, Anson BD, Rajamani S, January CT. Biology of Cardiac Arrhythmias. Ion Channel Protein Trafficking. Circ Res 2004:1418-28.

[3] Kass RS and Moss AJ. Long QT syndrome: novel insights into the mechanisms of cardiac arrhythmias. J Clin Invest 2003;112:810-5.

[4] Shimizu W, Antzelevitch C. Sodium channel block with mexiletine is effective in reducing dispersion of repolarization and preventing torsade de pointes in LQT2 and LQT3 models of the long-QT syndrome. Circulation 1997;96:2038-47.

[5] Seemann G, Weiβ DL, Sachse FB, Dössel O. Simulation of the Long-QT Syndrome in a model of Human Myocardium. Computers in Cardiology 2003;30:287-90.

[6] Priori SG. Inherited Arrhythmogenic Diseases. The Complexity Beyond Monogenic Disorders. Circ Res 2004:140-5.

[7] Fayn J, Rubel P. CAVIAR, a serial ECG processing system for the Comparative Analysis of ECG-VCGs and their Interpretation with Auto-Reference to the patient. J Electrocardiol. 1988;21 Suppl:173-6.

[8] Fayn J, Hamidi S, Maison-Blanche P et al. Quantitative assessment of changes in the repolarization phase in Holter recordings using CAVIAR. In Murray A, Arzbaecher R, editors. Computers in Cardiology. Los Alamitos: IEEE Computer Society Press, 1992:175-8.

[9] Splawski I, Shen J, Timothy KW et al. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. Genomics 1998;51:86-97.

[10] Syrris P, Murray A, Carter ND et al. Mutation detection in long QT syndrome: a comprehensive set of primers and PCR conditions. J Med Genet 2001;38:705-10.

[11] Němec J, Buncová M, Bulcová V et al. Heart Rate Dependence of the QT Interval Duration: Differences Among Congenital Long QT Syndrome Subtypes. J Cardiovasc Electrophysiol, 2004;15:550-6.

[12] Schwartz PJ, Priori SG, Spazzolini C et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. Circulation 2001;103:89-95.

[13] Zhang L, Timothy KW, Vincent GM et al. Spectrum of ST-T-wave patterns and repolarization parameters in congenital long-QT syndrome: ECG findings identify genotypes. Circulation 2000;102:2849-55.

Address for correspondence

Lioara Restier Miron.
INSERM ERM107.
Hopital Cardiologique de Lyon
28, avenue Doyen Lépine 69677 Bron France
lioara.restier@univ-lyon1.fr