



**PREVALENCE OF QT_c ALTERATIONS IN
NEONATES: AGE AND GENETIC
DETERMINANTS**

END OF TERM PROJECT

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ABBREVIATIONS

CAD: Coronary artery disease

ECG: Electrocardiogram

LQTS: Long QT syndrome

SCD: Sudden cardiac death

SIDS: Sudden infant death syndrome

SUDS: Sudden unexplained death syndrome

VT: Ventricular Tachycardia

WG: Weeks of gestation

ABSTRACT

Title: Prevalence of QTc alterations in neonates: age and genetic determinants.

Background: Long QT syndrome (LQTS) is an arrhythmogenic disease characterized by the presence of a prolonged QT interval on the ECG. LQTS is associated with sudden cardiac death in the young. LQTS is genetic; therefore other family members could be carriers of the same pathogenic genetic variants and be at risk of sudden death. Early identification of these individuals is essential to adopt protective therapies and prevent sudden death. LQTS is also one of the most important causes of sudden infant death syndrome (SIDS), death of a child in the first year of life, with a normal autopsy. Thus, in order to prevent SIDS, in recent years there has been an important impulse by the scientific community towards the implantation of a program of screening of newborns with an ECG, for the detection of this ECG abnormality. However, not all agree, and there is important controversy as to the clinical value, and cost-effectiveness of this approach. In order to shed some light into this subject we propose a broad analysis of this subject by performing an electrocardiographic screening of the newborn to identify abnormal prolongation of the QT interval. **Aims:** In healthy term neonates in Sant Joan de Déu hospital we propose to collect a 5 year prospective cohort in order to analyze the prevalence of prolongation of the QT interval on the ECG, the percentage of long QT which normalizes during the first year of life, and a potential genetic basis in patients who maintain a $QTc > 460$ at the twelfth month of life. **Methods:** We will perform four ECG, at 48 hours, first, sixth and twelfth month to an estimated 4.500 participants. The QTc will be measured by three different investigators. Genetic analyses will be performed with the use of Next Generation Sequencing technology. **Outcomes:** The results of this work will enable to assess the value of neonatal ECG screening in the detection of prolonged QT interval, the variability of the electrocardiographic parameter during the first months of development, and the prevalence of the genetic disease in the patient population. Finally, this work will set the basis towards defining whether there are benefits of implementing mandatory electrocardiographic screening in the newborn. **Keywords:** long QT, genetics, sudden infant death syndrome.

INTRODUCTION

SUDDEN UNEXPLAINED DEATH SYNDROME

Sudden unexplained death syndrome (SUDS) is a lethal condition that encloses several disorders leading to unexpected sudden death (SD). The victim has no prior history of heart disease and has been seen alive within 12 hours before death. In addition, after a complete autopsy, the cause of death remains unsolved (1). In adults (16 to 64 years old) the rate of SUDS is 11/100000 inhabitants. In younger people, (<16 years of age), the rate of SUDS per year is 7.5/100000. Despite that coronary artery disease (CAD) is the most common cause of SCD (2), this entity is infrequently present in the SCD of the young, and limited usually to premature coronary atherosclerosis of genetic origin. Thus, the most common causes of the SCD of the young include, non-atherosclerotic coronary artery disease, myocarditis, conduction system disease, cardiomyopathies (structural heart disease) and channelopathies (pure electrical diseases) (3).

In people under 35 years of age, primary inherited channelopathies are responsible for an important percentage of deaths (3–7). Four different channelopathies have been described to date: Long QT syndrome, Brugada syndrome, Catecholaminergic Polymorphic Ventricular Tachycardia and Short QT syndrome (4). In children and young adults SCD has an incidence of 2.5/100000 patient per year (8). Up to 35% of cases may actually be caused by pathogenic variants in ion channels (9).

SUDDEN INFANT DEATH SYNDROME

Sudden Infant Death Syndrome (SIDS) is defined as the sudden unexpected death of an infant <1 year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including a complete autopsy and review of the circumstances of death and the clinical history (10,11).

The median age for SIDS deaths is 11 weeks, and 90% are under 6 months of age (10). The death rate varies between different nations. Thus, in Japan the incidence is 0.09/1000 live births, in the Netherlands 0.1/1000, in Denmark 0.22/1000, in the USA 0.6/1000, and in New Zealand 0.8/1000 (12). SIDS is the leading cause of death in the first year of life (13). A large number of mechanisms have been proposed to be responsible for SIDS, such as male gender, premature birth, prone sleeping, soft bedding, maternal smoking and co-sleeping with postnatal smoking mothers whereas breastfeeding (14), room sharing, sleeping sack and pacifier use have been found to have a potentially protective effect. (14–16), but no clear correlation with pathological findings has been established (17). The syndrome is seen in all social groups but it is more prevalent in the socioeconomically deprived. Features common to such groups in the population- such as mothers who smoke, young mothers, and large families- are all associated with an increased risk (13). A genetic basis has been proposed in the last years as also being responsible for SIDS. In fact, a study reported that 50% of SIDS cases had a prolonged QT interval in the first week of life (18). In addition, several studies have shown that approximately 10%-20% of SIDS cases carried pathogenic variants which induce an abnormal electrical conduction (19–21).

The identification of prolonged QT interval in the newborn is hindered by the fact that the electrocardiographic pattern is labile during the first months of life due to development of sympathetic innervation of the heart, which continue to develop after birth and becomes functionally complete by approximately the sixth month of life. Thus, two mechanisms are currently proposed to increase the risk of arrhythmias linked to prolonged QT interval in the newborns. First, it may be due to developmental alterations in cardiac sympathetic innervation. The right and left sympathetic nerves may occasionally develop at different rates and lead temporarily to a harmful imbalance. A sudden increase in sympathetic activity may trigger lethal arrhythmias in such electrically unstable hearts. The second involves a genetic abnormality, a genetic variant which may cause congenital long-QT syndrome. This disorder is characterized by prolongation of the QT interval and a high risk of sudden death, mostly under stressful conditions but also during sleep; it is usually familial but may be sporadic. (18) Infants with these ecg patterns would be particularly vulnerable during the first year of

life, and their higher risk of SIDS could be identified by the observation of a prolonged QT interval.

LQT SYNDROME

Introduction

LQT syndrome is the most common inherited arrhythmogenic disease. LQTS is a rare cardiac channelopathy characterized by delayed ventricular repolarization, seen as a QT prolongation on the 12-lead electrocardiogram (ECG), in a structurally normal heart. The phenotype can range from asymptomatic individuals (the majority of patients) to ventricular tachyarrhythmias (*torsade de pointes*), and SCD (22). The confirmed clinical diagnosis requires ECG findings, symptoms (syncope) and family history of LQT syndrome or SCD (23). The incidence is estimated from 1:2000 to 1:5000 (24,25).

Etiology

The LQT syndrome is either acquired, which is iatrogenic and commonly induced by drugs, or congenital. A common cause of the acquired disease is the use of medications such as antiarrhythmics, antidepressants, and phenothiazines. In addition, electrolyte imbalance, such as hypokalemia, hypomagnesemia, and hypocalcemia, especially in the presence of predisposing medications, could cause LQT syndrome. (26)

Pathogenesis

To date, hundreds of pathogenic variants, in at least 17 genes LQT-associated genes, have been published (27). These are responsible for almost 75% of all LQT syndrome cases. Most of clinically definite LQT syndrome cases (70%) are caused by pathogenic variants in 3 main genes encoding ion channel proteins: *KCNQ1* –KvLQT1 or Kv7.1- (LQT type 1), *KCNH2* –HERG or Kv11.1- (LQT type 2), and *SCN5A* –Nav1.5- (LQT type 3). These three genes account for 65% of the cases. These are genes involved in the generation of the cardiac action potential, and will prolong repolarization by decreasing function (potassium channels) or by prolonging the entry of sodium ions to the interior of the cell, beyond the closing state in the sodium channel mutations. Thus, the pathogenesis of LQT syndrome could be summarized as mutations in K1+

channels resulting in decreased potassium outward current, whereas mutations in Na¹⁺ channels leading to inadequate closure of the channels and excessive sodium inward currents. The ensuing result is inadequate prolonged repolarization time, with increased time to reach the resting state of the action potential and prolongation of the QT interval. (26)

Current clinical guidelines recommend the screening of these three main genes (28). They also recommend genetic testing of relatives to identify individuals carriers at risk (29,30).

Despite that several LQT cohorts have been published, at least 30% of LQT cases continue to be genetically elusive and the real prevalence of these LQT-related genes remains to be clarified (27,31). The prevalence of genetic variants associated with LQT will likely increase in the upcoming years, thanks to recent developments in high-throughput genetic tools of massively parallel sequencing (Next Generation Sequencing -NGS-). The biomedical arena has been revolutionized by these new tools, which can quickly and cost-effectively perform large-scale sequencing (32). NGS surpasses Sanger technology in speed, cost and even in the ability to detect new forms of genetic variants. NGS can detect simple genetic variants (SNV) and insertions/deletions (*indels*) of single nucleotide polymorphisms (SNPs) and variations in the number of copies (CNVs). These technologies have been used with success in cardiac genetics, including in studies for the detection of variants associated with LQTS (33–35), as well as in SIDS (36,37).

Clinical manifestations

The clinical manifestations of LQTS fall under two main categories: the arrhythmic events and the ECG aspects.

The *arrhythmic events* are due to runs of torsades de pointes, which, according to their duration, produce syncope, cardiac arrest and –when it deteriorates into ventricular fibrillation- sudden death. The term torsade de pointes refers to a VT characterized by QRS complexes of changing amplitude that appear to twist around the isoelectric line and occur at rates of 200 to 250/min. (38)

Among untreated patients, the natural history is represented by the occurrence of a number of syncopal episodes, eventually leading to sudden death. Sudden death as a

first manifestation represents the main rationale for the treatment of asymptomatic patients. (28)

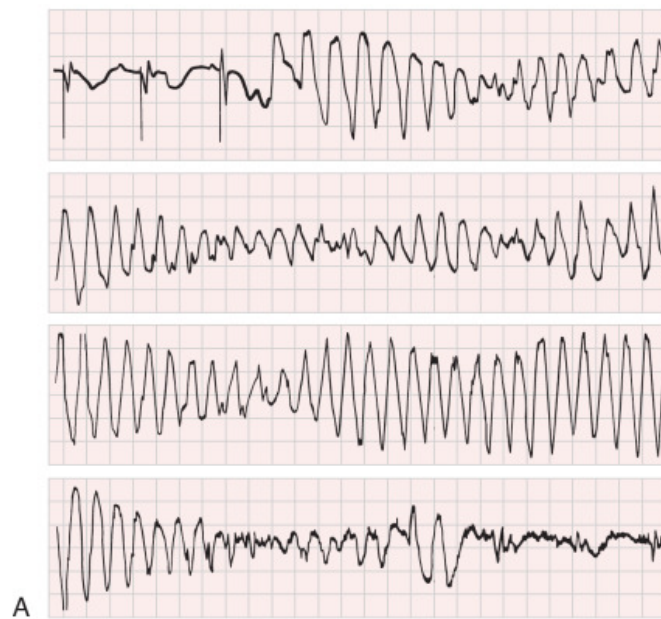


Figure 1: Torsades de pointes A. Continuous monitor lead recording. B. Tracing from a young boy with congenital long-QT syndrome.

The *ECG alterations* are important and numerous. While the prolongation of the QT interval is the hallmark of LQTS, it is not always present. Ventricular repolarization is not only prolonged but often presents bizarre morphologic alterations, some of which tend to be gene-specific. Macroscopic T-wave alternans is the most distinctive ECG pattern of LQTS, and is a marker of high cardiac electrical instability. (28) The specific T-wave morphology for each type of LQTS is commented in the follow section.

Genotype-Phenotype Correlation in LQT Syndrome

Several genotype-phenotype correlation studies have been performed to identify the genetic determinants of triggering events, electrocardiographic phenotype, and response to therapy.

-LQT1: In general, individuals with LQT1 exhibit symptoms during physical activity, especially in swimming activities, emotional stress could be another trigger. The T wave usually begins just after the QRS, becoming long and broad based. Patients tend to respond to beta blocker therapy.

-LQT2: The most common trigger is emotional stress, followed by sleep or auditory stimulation. The T wave tends to be notched and bifid. Patients typically respond to beta blocker therapy.

-LQT3: Most events occur during sleep, suggesting that they are at higher risk during periods of slow heart rates. LQT3 patients show a very late T wave with a prolonged ST segment. Beta blockers have not been shown to be as beneficial, however, they are also first line of therapy. Preliminary data suggest that LQT3 patients might benefit from Na¹⁺ channel blockers, such as mexiletine. (39)

Mutations also carry prognostic significance. In general, patients with LQT1 and LQT2 have a higher risk of cardiac events than patients with LQT3. The latter, despite having fewer events, have a relatively higher mortality, which indicates higher lethality of the events. (26)

Diagnosis

Typical cases usually present no diagnostic difficulty for physicians who are aware of the disease. However, borderline cases are more complex and require the evaluation of multiple variables besides clinical history and ECG. For that reason, a diagnostic score known as Schwartz score was presented in 1993. (23)

TABLE 2. 1993 LQTS Diagnostic Criteria

	Points
ECG findings*	
A. QT _c †	
≥480 msec ^{1/2}	3
460-470 msec ^{1/2}	2
450 msec ^{1/2} (in males)	1
B. Torsade de pointes‡	2
C. T-Wave alternans	1
D. Notched T wave in three leads	1
E. Low heart rate for age§	0.5
Clinical history	
A. Syncope‡	
With stress	2
Without stress	1
B. Congenital deafness	0.5
Family history 	
A. Family members with definite LQTS#	1
B. Unexplained sudden cardiac death below age 30 among immediate family members	0.5

Scoring:

≤1 point: low probability of LQTS

2-3 points: intermediate probability of LQTS

≥4 points: high probability of LQTS

LQTS, long QT syndrome.

*In the absence of medications or disorders known to affect these electrocardiographic features.

†QT_c calculated by Bazett's formula, where $QT_c = QT / \sqrt{RR}$.

‡Mutually exclusive.

§Resting heart rate below the second percentile for age.²⁵

||The same family member cannot be counted in A and B.

#Definite LQTS is defined by an LQTS score ≥4.

Figure 2: Diagnostic Criteria of LQTS (23)

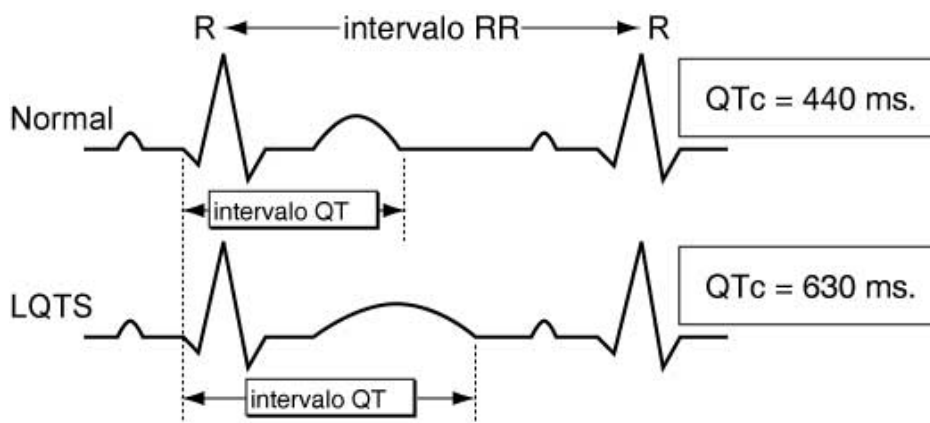


Figure 3: Long QT (40)

Treatment

The current treatment of long QT syndrome, according to the European society of cardiology, is explained in the following table. (41)

Risk stratification and management in Long QT Syndrome

Recommendations	Class ^a	Level ^b
The following lifestyle changes are recommended in all patients with a diagnosis of LQTS: (a) Avoidance of QT-prolonging drugs (http://www.crediblemeds.org). (b) Correction of electrolyte abnormalities (hypokalaemia, hypomagnesaemia, hypocalcaemia) that may occur during diarrhoea, vomiting or metabolic conditions. (c) Avoidance of genotype-specific triggers for arrhythmias (strenuous swimming, especially in LQTS1, and exposure to loud noises in LQTS2 patients).	I	B
Beta-blockers are recommended in patients with a clinical diagnosis of LQTS.	I	B
ICD implantation with the use of beta-blockers is recommended in LQTS patients with previous cardiac arrest.	I	B
Beta-blockers should be considered in carriers of a causative LQTS mutation and normal QT interval.	IIa	B
ICD implantation in addition to beta-blockers should be considered in LQTS patients who experienced syncope and/or VT while receiving an adequate dose of beta-blockers.	IIa	B
Left cardiac sympathetic denervation should be considered in patients with symptomatic LQTS when (a) Beta-blockers are either not effective, not tolerated or contraindicated; (b) ICD therapy is contraindicated or refused; (c) Patients on beta-blockers with an ICD experience multiple shocks.	IIa	C
Sodium channel blockers (mexiletine, flecainide or ranolazine) may be considered as add-on therapy to shorten the QT interval in LQTS3 patients with a QTc > 500 ms.	IIb	C
Implant of an ICD may be considered in addition to beta-blocker therapy in asymptomatic carriers of a pathogenic mutation in <i>KCNH2</i> or <i>SCN5A</i> when QTc is > 500 ms.	IIb	C

EPS = electrophysiological study; ICD = implantable cardioverter defibrillator; LQTS = long QT syndrome; LQTS1 = long QT syndrome type 1; LQTS2 = long QT syndrome type 2; LQTS3 = long QT syndrome type 3; PVS = programmed ventricular stimulation; QTc = corrected QT; VT = ventricular tachycardia; SCD = sudden cardiac death.

^aClass of recommendation.

^bLevel of evidence.

Figure 4: Treatment of LQTS (41)

JUSTIFICATION OF THE PROJECT

Detection of potentially lethal diseases in the first year of life requires neonatal screening. Several investigators have advocated the inclusion of an electrocardiogram in neonatal screening, with the argument that the abnormal findings may carry a lethal outcome, and that early identification may potentially prevent SCD (42). However, only few studies have been published assessing the value of neonatal QTc screening. One of these studies, the largest one to date, showed a prevalence of long QTc (defined as >440 msec) of 2,5%. After genetic analysis, it was determined that the prevalence of the genetic disease was 1/2000, much larger than previously suspected (25). It has to be noted that this study mainly analyzed white European neonates. Despite this limitation, it became the most important argument on the value of neonatal ecg. Since then there has been a steady impulse in the medical community for the ecg to become routine in neonatal screening. Not everybody agrees though, and it has become an area of active health policy discussion, concerned about the economic, clinical, social, even psychological implications of having a borderline QTc.

The present project intends to shed some light in this ongoing argument. We propose to perform a prospective, investigation on the QT interval in a consecutive cohort of newborns, to assess the change in the electrocardiogram until the first year of life, when it is considered that the autonomic nervous system is completely mature and the QTc is stabilized. In addition, we plan to perform a comprehensive family and genetic analysis in those individuals with a prolong QT interval, with the use of a custom made genetic panel with high throughput genetic tools. This work will provide us the prevalence of long QT, the changes on the electrocardiogram over the first year of life, and the prevalence of genetic disease associated with prolonged QT interval. The results of this work will determine whether neonatal ecg screening should be adopted as a routine test. If that is the case, we will have the evidence substantiating when, in the first year of life, it is the best time to obtain the ecg. In addition, this work will provide the evidence on whether genetic testing may be beneficial in a subgroup of patients with prolong QT interval and his family members.

HYPOTHESIS

We are taking advantage of the availability of a mixed population who give birth in one community hospital to answer one of the key questions in neonatal screening: the need to perform an electrocardiogram to detect abnormal QT prolongation potentially associated with sudden cardiac death.

Hypothesis 1: In our mixed cohort, the global prevalence of prolongation of the QT interval on the electrocardiogram at 48 hours of life is 10% if the cutoff is QTc 440 msec and 6% in QTc>470 msec.

Hypothesis 2: A neonatal QTc < 460 msec will completely normalize during follow-up.

Hypothesis 3: The QTc>460 msec at twelfth month, is the result of a genetic predisposition in 75%.

OBJECTIVES

Objective 1: To analyze the prevalence of prolongation of the QT interval on the electrocardiogram at 48 hours of life in healthy term neonates in Sant Joan de Déu hospital.

Objective 2: To analyze the percentage of long QT which normalize the first year of life.

Objective 3: To analyze whether patients with $QTc > 460$ msec at twelfth month have a genetic basis for the electrocardiographic alteration.

METHODS

STUDY DESIGN:

This is a prospective cohort observational study, in which we will perform four ECGs during the first year of life to 1.500 neonates born in Sant Joan de Déu hospital in Barcelona per year. The first ECG will be realized to all neonates at the first 48 hours of life, following by an ECG at the first, sixth, and twelfth month of life. It is estimated, according to previously published studies that 75% of cases with QTc 460-485 msec will normalize after the first year.

The protocol includes also a genetic analysis of those neonates who show a QTc>460 msec at twelfth month in order to identify a potential genetic alteration associated with the long QT syndrome.

POPULATION:

The study population is based on Caucasian healthy term neonates who are borned in hospital Sant Joan de Déu in Barcelona. It is estimated that the study will include at least 1.500 neonates per year. This has been calculated based on the number of infants born at this center after having excluded those who are not candidates because of following criteria. We will recruit neonates during three years, therefore, it will be included about 4.500 neonates.

INCLUSION AND EXCLUSION CRITERIA:

Inclusion criteria:

- Healthy term neonates (37-42WG)
- Caucasian ethnicity

Exclusion criteria:

- Pre-term neonates (<37WG)
- Post-term neonates (>42WG)

- Neonates with any disease which may alter ionic balance and therefore QT interval (cardiovascular, renal, neurological disease, digestive, etc)
- Healthy term neonates who have an unreadable ECG
- Known genetic syndromes or malformations other than long QT syndrome
- Lack of informed consent
- Familiar antecedents of long QT syndrome

SAMPLE:

The sample size is calculated by GRANMO program

Sample size for hypothesis 1:

1. A sample size of **139** subjects randomly selected will suffice to estimate with a 95% confidence and a precision +/- 5 percent units, a population percentage of long QT>440 msec considered to be around 10%. It has been anticipated a replacement rate of 0%.

1. A sample size of **87** subjects randomly selected will suffice to estimate with a 95% confidence and a precision +/- 5 percent units, a population percentage of long QT>470 msec considered to be around 6%. It has been anticipated a replacement rate of 0%.

Sample size for hypothesis 2:

2. Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, **369** subjects are necessary to recognize as statistically significant a difference consisting in an initial proportion of long QT 0.06 and a final proportion of 0.02. It has been anticipated a drop-out rate of 20%.

Sample size for hypothesis 3:

3. A sample size of **289** subjects randomly selected will suffice to estimate with a 95% confidence and a precision +/- 5 percent units, a population percentage considered to be around 75%. It has been anticipated a replacement rate of 0%.

Our sample sizes are much smaller than the quantity of patients we really study. We will study up to 4.500 newborns which will allow to have a higher statistical power. Thus, we will be able to detect smaller differences in the variables of interest.

VARIABLES:

Hypothesis 1: Dichotomic variable

QTc 440 msec	YES
	NO
QTc 470 msec	YES
	NO

QTc 440 msec and QTc 470 msec will be calculated using the Bazett formula at 48 hours of life (see measurements).

We have used the cutoff of 440 msec as this is considered the highest normal QTc in the population. Below this value the diagnosis of long QT cannot be considered. On the other hand, we have used the cutoff of 470 msec as above this value, the diagnosis has to be always considered. The value 440-460 msec are considered borderline at present.

Hypothesis 2: Dichotomic variable

QTc<460 msec	YES
	NO

Following current electrocardiographic criteria, with QTc borderline at 440-460 msec, the cutoff of 460 msec will define a population at risk of long QT. Considering that prolonged QT at birth may shorten during the first year of life, the QTc in cases with borderline high QTc will completely normalize.

These variables will be assessed at

- QTc < 48 hours of life
- QTc 1 month of life
- QTc 6 month of life
- QTc 12 month of life

Hypothesis 3: Dichotomic variable

Known genetic alteration	YES
	NO

There are 17 genes associated with long QT syndrome, at present (Annex 1). These cases will be analyzed using next generation sequencing technologies. We will proceed with bioinformatics analyses to identify pathogenicity score associated with the variant. If a variant is considered pathogenic the case will be classified as a potential case of long QT syndrome.

STATISTICAL ANALYSIS:

UNIVARIATE ANALYSIS

The results are expressed as frequencies/percentages for categorical variables and as mean +/- SD or median (quartiles) for continuous variables depending on whether or not they were normally distributed.

BIVARIATE ANALYSIS

To analyze the prevalence of prolongation of the QT interval on the electrocardiogram in healthy term neonates in Sant Joan de Déu hospital, the percentage of long QT which normalize the first year of life, and whether patients with QTc>460msec have a genetic basis for the electrocardiographic alteration, the statistic methods used will be χ^2 test, as all the variables are qualitative.

A multivariate analysis is not necessary because with the exclusion criteria we have controlled the possible confusion factors.

MEASUREMENTS:

ECG recording

Twelve-lead ECG will be performed following pediatric cardiologist European recommendations about ECG in infants (25 mm/s y 10 mm/mV). Value of interval QT will be measured by at least two expert pediatric cardiologists. If there is discrepancy of 10 ms or more between both expert pediatric cardiologists, a third expert pediatric cardiologist will measure the value. The QT intervals of 3 consecutive beats will be measured from the onset of the Q wave to the end of the T wave in lead V5. If the QT interval cannot be measured because of instability of isoelectronic levels in lead V5, the QT intervals in lead II will be measured.

The value will be corrected (Bazett: $QTc = QT/\sqrt{RR'}$) (QTc). The QT/RR data of each of 3 consecutive beats will be corrected, and the mean values of the 3 consecutive QTc will be used. We will use a neonatal ECG (CardioSoft®) already available in the center participating in the project.

Genetic analysis

A genetic study of individuals with potential risk of genetic abnormalities associated with SCD has been approved by the local institutional review board guidelines and ethics committee, following the Helsinki II declaration. The parents/legal tutor of neonates and family members will give written informed consent before inclusion in the study. (Annex 2)

Genetic analysis of all genes associated with LQT syndrome will be able to identify pathogenic variants in nearly 70% of cases carrying a prolonged QTc on the electrocardiogram. We expect to identify more potentially pathogenic variants because we will perform a comprehensive genetic analysis including associated genes but also candidate genes. Thus, we expect to identify genetic variants in at least 90% of analyzed individuals.

Genomic DNA will be extracted from using Chemagic® (Chemagen Systems, Germany). Integrity/quality/quantity will be measured using Nanodrop (Thermo Scientific), and Qubit® 2.0 (Invitrogen). DNA will be sonicated (Bioruptor, Durviz) and analyzed Bioanalyzer (Agilent). After that, sample libraries will be prepared following the SureSelect XT Target Enrichment System for Illumina Paired-End Sequencing Library protocol (Agilent Technologies, Inc., USA). We will use a custom candidate gene resequencing panel containing 77 genes. Our panel includes current genes associated or candidates for LQT syndrome which could better define the global genetic risk of cardiac arrest. The panel contains the following genes: *ABCC9*, *ACTC1*, *ACTN2*, *AKAP9*, *ANK2*, *BAG3*, *CACNA1C*, *CACNA2D1*, *CACNB2*, *CALM1*, *CALM2*, *CALM3*, *CASQ2*, *CAV3*, *CRYAB*, *CSRP3*, *DES*, *DSC2*, *DSG2*, *DSP*, *FKTN*, *GPD1L*, *HCN4*, *JPH2*, *JUP*, *KCND3*, *KCNE1*, *KCNE1L*, *KCNE2*, *KCNE3*, *KCNH2*, *KCNJ2*, *KCNJ5*, *KCNJ8*, *KCNQ1*, *LAMP2*, *LDB3*, *LMNA*, *MYBPC3*, *MYH6*, *MYH7*, *MYL2*, *MYL3*, *MYOZ2*, *MYPN*, *NEBL*, *NEXN*, *NOS1AP*, *PDLIM3*, *PKP2*, *PLN*, *PRKAG2*, *RANGRF*, *RBM20*, *RYR2*, *SCN1B*, *SCN2B*, *SCN4B*, *SCN5A*, *SCN10A*, *SGCD*, *SLMAP*, *SNTA1*, *TAZ*, *TCAP*, *TGFB3*, *TMEM43*, *TMPO*, *TNNC1*, *TNNI3*, *TNNT2*, *TP63*, *TPM1*, *TRDN*, *TRPM4*, *TTN*, and *VCL*. This panel will be used to detect SNVs, indels, and CNVs.

The bioinformatic secondary analysis of the genetic panel data includes proper alignment methods and variant call with GEM MAPPER software. Our in-house developed secondary analysis first involves trimming of the FASTQ files. Trimmed reads will then be mapped with GEM III. Output from mapping steps will be joined and sorted, and unique and properly mapping read pairs selected. Finally, cleaned BAM file will be annotated with SAMtools v.1.1, GATK v3.2 together with an ad hoc developed method to generate the first raw VCF files. For the secondary analysis after exome resequencing, reads will be transferred on .cs fasta and .qual files (SOLID5500) to an offline cluster to be processed using BioScope® (Life Technologies) and GATK. Variants will be annotated with dbSNP IDs and the 1000 Genomes browser, Exome Variant Server, ExAP, our in-home database IDs and Ensembl information, if available.

Tertiary analysis starts with both the .bam files and the list of variants detected. Reads will be visualized and analyzed by dedicated software. Variations identified after candidate-gene panel or whole exome sequencing will be compared with 1000 control alleles of the same population, 1000 Genomes Project database, Exome Variant Server, ExAP and HapMap Project database. Putative deleteriousness will be evaluated in silico (Condel, PROVEAN, PolyPhen2, and Mutation Taster).

Variants will be confirmed by an alternative approach (Sanger sequencing or MLPA/HRM). This tiered approach, which complements thorough candidate-gene analysis with in-depth genetic investigation of novel genes and gene-disease associations, will be determinant to unravel the genetic architecture of LQT syndrome.

ETHICAL CONSIDERATIONS

This study will be conducted according to the ethical principles established by World Medical Association in the *Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects*. The research protocol must be presented and submitted for consideration, guidance and approval by the *Clinical Research Ethical Committee (CEIC, "Comitè Ètic d'Investigació Clínica")* at Hospital Sant Joan de Déu before the study begins, and at the end of the study, the final report must also be submitted to the CEIC.

According to "*Ley Orgánica 15/1999 de Protección de Datos de Carácter Personal*", personal and clinical information of participants will be confidential and only used for the purpose of the research. Moreover, all data will be analyzed anonymously. Participants will have to sign voluntarily the informed consent (*Annex 2*) before being included in the study after receiving the appropriate information about procedures, according to "*Ley 41/2002 Básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica*".

The present research protocol includes genetic analyses, which will be performed according to "*Ley 14/2007, de 3 de julio de investigación biomédica*". Before performing the genetic analyses, the participants legal representatives will have signed the voluntarily informed consent before enter in the study that includes the consent for the genetic analysis. (*Annex 2*)

We have to take into account other ethical aspects related about the family information during the follow-up and what to do with those neonates who have an ECG at high risk of SIDS at the beginning of the follow-up. The family will be informed about the results of every ECG performed during the first year of life, after the analysis of each one. It could be argued if it is worth to inform the family due to the concern raised. However, the disease may be associated with sudden cardiac death, and the disease may be treated to prevent events. Thus, the results will be communicated to

the patient if there is a doubt on the presence of the disease, for close follow-up. Neonates who have high probability of LQTS during follow-up, for instance, at 6 months, will be started on treatment at that time, without awaiting for the twelfth month ECG.

STRENGTHS AND LIMITATIONS

STRENGTHS

This study incorporates a global genetic investigation of all genes previously associated with long QT syndrome, with the use of ultrasequencing. This study will provide not only potentially causal variants associated with the electrocardiographic pattern but also modulating variants of the phenotype.

The ECG is a safe and non-invasive procedure with no known risks.

Our research team is also competitive to carry on this study. All investigators, except the medical student, have experience in research and clinical practice.

LIMITATIONS

The first limitation that may be considered is the loss of follow up. As we have to follow up our patients during one year, it could be possible that some of them, for any reason, have to drop out the study. We have the sufficient number of births to achieve statistical significance.

Another limitation to be taken into account is the information bias. In order to avoid this type of bias the value of interval QT will be measured by at least two expert pediatric cardiologists. If there is a discrepancy of 10 ms or more between both expert pediatric cardiologists, a third expert pediatric cardiologist will measure the value.

This study could be broaden with the investigation of ethnic background. We think it could be interesting to investigate the prevalence of QTc abnormalities in the different ethnicities, its changes in the first year of life, and the prevalence of potential genetic variants according to ethnicity. At present it is becoming more and more complex to define the ethnicity in the patient population, as the individuals are of mixed ethnic backgrounds. As a result, it may be necessary work with several ethnicities. Therefore, if we had included the investigation of ethnic background in the present protocol, the statistical power to draw any meaningful conclusions may not be sufficient. In order to

provide an important proof of concept on the role of modulating factors according to ethnicity, it is required the use of larger and multicentric cohorts and this will be the follow project of our research group.

WORK PLAN

The research team includes different specialists. Each of them will have a task assigned during the different stages of this study. There will be two pediatric cardiologists, a last year medical student, nurses, geneticists, bioinformaticians and molecular biologists. A statistician will be recruited for the statistical analysis.

Stage 1: INITIAL COORDINATION

This was an all-member meeting to start the project, define the roles of each participant, and to create a chronogram clarifying the different stages of the study. This type of coordination meetings will be repeated during the study to debate if there are any problems and also if any modification needs to be done. The whole research team will keep in contact via e-mail, just in case there is a need for improvisation and an extraordinary meeting is needed to be organized.

Stage 2: BIBLIOGRAPHY RESEARCH

Our objective and hypotheses are based upon the previous knowledge we obtain from other studies done in this field. So this part was performed by the whole team, and took us over 3 months to plan our study, trying to avoid the problems other authors may have encountered in similar studies.

Stage 3: PROTOCOL DEVELOPMENT

Once the bibliography research was done, the whole protocol for the study was written by the last year medical student. Then we asked approval from ethical committee (*CEIC*). It took about 3 months approximately.

Stage 4: DATA COLLECTION

If the study is approved to be done, the data collection will start. The nurses will perform the several ECG to all participants, starting with the first one before the 48 hours of life, and followed by first, sixth and twelfth month ecg. Each participant legal representative will have signed the informed consent before the performing of the

first ECG. The two pediatric cardiologists will read each ECG, and they will register the results in the data collection document (Annex 3). The doctors will inform the family about the results. We will be performing ECG during a recruitment period of three years, therefore, ECG will be performed during four years and this stage will take us four years.

Stage 5: GENETIC ANALYSIS

This stage overlaps the previous one. We will carry out a genetic analysis to those participants who have a $QTc > 460$ msec at twelfth month. The role of geneticists, bioinformaticians and molecular biologists is reflected in this part.

Stage 6: DATA ORGANIZATION AND STATISTICAL ANALYSIS

Once the data collection is finished, the whole data will be organized in a database by the research team. Then, the statistician will perform the statistical analysis. This stage will last about 3 months.

Stage 7: FINAL ARTICLE ELABORATION AND PUBLICATION OF RESULTS

This will be done by the research team once the data have been analyzed and concluded. The final article will be published in different medical journals in order to make a correct diffusion of the results. This part will finally take over 3 more months.

CHRONOGRAM

	JUNE 2015	JULY-SEPTEMBER 2015	OCTOBER-DECEMBER 2015	JANUARY 2016 – DECEMBER 2019	JANUARY-MARCH 2020	APRIL – JUNE 2020
STAGE 1: Coordination meeting						
STAGE 2: Bibliography research						
STAGE 3: Protocol development						
STAGE 4: Data collection						
STAGE 5: Genetic analysis						
STAGE 6: Data organization and statistical analysis						
STAGE 7: Final article elaboration and publication of results						

BUDGET

The research team will be in charge for most of the tasks in this study like the bibliography research, performing and reading the ECG, writing, publication. The only extra personnel needed will be a statistician.

The statistician will be hired for the data analysis, which is estimated to take about 30 hours of work. For rate of 25€ per hour, it will be of a total cost of 750 €.

Various coordination meetings will take place. We calculate that a total of 7 meetings will be needed, but probably some more extraordinary meetings will be necessary. We estimate an approximate cost of 50€ per meeting (includes team members' transport), and therefore a total cost of 350€ for the 7 meetings.

Consumable material needed for this project is an ECG machine, which is already in the hospital, and hence, it is at no cost for us. But, we calculate that the paper and the patches to perform the ECG will cost approximately 1€ per ECG. Therefore, during 4 years we will perform 4 ECG to 4.500 participants, that means a total of 18.000 ECG and a total cost of 18.000€.

We calculate that per 1.500 participants we will do 30 genetic analyses. Therefore, during all the study, 90 genetic analyses will be done. Each genetic analysis has a cost of 700€, hence, the total cost of the genetic analysis will be 63.000€.

The writing and the diffusion of the final article is a task of the research team, but the cost of peer reviewing and publication in the scientific journals goes up to 2.000€.

EXPENSES	COSTS (€)
7 coordination meetings	350€
Statistician service	750€
18.000 ECG	18.000€
90 genetic analysis	63.000€
Writing and diffusion of the final article	2.000€
TOTAL	84.100€

HEALTH CARE IMPACT

Sudden infant death syndrome is a tragic event in the families, for the unexplained cause as well as for the implications that the death can have for the family members, as some of these SIDS cases are of genetic origin.

This is the first comprehensive study of QT prolongation in neonates, which will enable the investigation of the prevalence of long QT syndrome, as well as the variability in the QT interval in the neonatal population.

This information will be key to further understand whether all neonates should undergo electrocardiographic analysis as part of the neonatal screening, who should be closely followed during the upcoming months, and whether there is a genetic predisposition to prolonged QT in the population.

This work will provide clinical guidelines to undertake preventive measures in patients identified with a borderline QT interval, and also to undertake protective therapies in family members at risk.

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ANNEX 1: GENETICS OF LONG QT SYNDROME

LQTS	Gen	RefSeq Gene ID	Protein	Chromosome location	Affected current	Inheritance	Incidence
LQTS1	KCNQ1	3784	Kv7.1	11p15.5	I _{Ks}	AD and AR	30-35%
LQTS2	KCNH2	3757	Kv11.1	7q36.1	I _{Kr}	AD	25-40%
LQTS3	SCN5A	6331	Na _v 1.5	3p21	I _{Na}	AD	5-10%
LQTS4 (Ankyrin-B Syndrome)	ANK2	287	Ankyrin-B	4q25-q27	I _{CaL} and I _{Na}	AD	<1%
LQTS5	KCNE1	3753	Mink	21q22.12	I _{Ks} and I _{Kr}	AD and AR	<1%
LQTS6	KCNE2	9992	Mirp1	21q22.12	I _{Kr} and I _{Ks}	AD	<1%
LQTS7 (ATS)	KCNJ2	3759	Kir2.1	17q24.3	I _{K1}	AD	<1%
LQTS8 (TS)	CACNA1C	775	Ca _v 1.2	12p13.3	I _{CaL}	AD	rare
LQTS9	CAV3	859	Caveolin	3p25	I _{Na} and I _{K1}	AD	rare
LQTS10	SCN4B	6330	Na _v β4	11q23.3	I _{Na}	AD	rare
LQTS11	AKAP9	10142	Yotiao	7q21-q22	I _{Ks}	AD	rare
LQTS12	SNTA1	20648	α-sintrofyn	20q11.2	I _{Na}	AD	rare
LQTS13	KCNJ5	3762	Kir3.4	11q24	I _{KAch}	AD	rare
LQTS14	RYR2	6262	Ryanodine Receptor 2	1q43	I _{Ca,release}	AD	rare
LQTS15	CALM1 CALM2	801 805	Calmodulin	14q32.11 2p21	multiple	AD	rare
LQTS16	SCN1B	6324	Na _v β1b	19q13.1	I _{Na}	AD	rare

ANNEX 2: INFORMED CONSENT

CONSENTIMENT PER L'ESTUDI DEL PAPER DE LA GENÈTICA EN LA DETECCIÓ PRECOÇ I PREVENCIÓ DE LA MORT SOBTADA NEONATAL MITJANÇANT L'ÚS DE L'ELECTROCARDIOGRAMA I L'ESTUDI GENÈTIC D'ULTRASEQÜENCIACIÓ MASSIVA

FINALITAT

Li agraïm la seva col·laboració en l'estudi del paper de la genètica en la detecció precoç i prevenció de la mort sobtada (MS) neonatal mitjançant l'ús de l'electrocardiograma (ECG) i l'estudi genètic d'ultraseqüenciació massiva (NGS). La seva participació contribuirà a millorar els coneixements que tenim sobre les malalties del cor. Als darrers anys s'està produint un avanç important en la investigació dels factors genètics de les malalties cardíques.

Per realitzar aquests estudis és necessari recollir una mostra de sang o saliva del pacient per tal d'obtenir ADN. L'ADN està present a totes les seves cèl·lules i porta un codi en forma de "gens" que determina les seves característiques físiques personals, com el color dels ulls, de la pell, etc. Aquests gens també poden influir en el risc de que algunes persones tinguin més risc que altres de desenvolupar certes malalties.

L'objectiu principal d'aquest estudi és esbrinar si existeixen factors genètics que fan que algunes persones, des del naixement, siguin més vulnerables que altres de patir un allargament de l'interval QT a l'ECG, i això fa que estiguin a risc de patir una MS. Si això fos així, al ser una patologia amb causa genètica i per tant heretable, la resta de persones de la família (pares, germans, fills, cosins...) també podrien tenir un risc més alt que la població general, pel que se'ls oferirà la possibilitat de fer-se un estudi genètic per esbrinar-ho.

Les mostres seran codificades per tal de garantir la confidencialitat de les dades personals. Únicament les persones relacionades directament amb l'estudi podran tenir accés als detalls de les mostres biològiques. Posteriorment, la mostra serà traslladada al Centre de Genètica Cardiovascular (CGC), Universitat de Girona-IDIBGI, per realitzar els estudis genètics detallats. Tota la informació personal que es reculli o es generi per l'estudi quedarà protegida d'acord amb la legislació vigent (LOPD, Ley Orgánica 15/1999). V1: 23/03/2015.

DESCRIPCIÓ DEL PROCÉS

Durant la participació a l'estudi:

-L'informarem sobre els objectius del projecte i respondrem els dubtes que vostè pugui plantejar.

-Per la participació en el nostre estudi no rebrà cap recompensa econòmica o de cap altre tipus.

-Les dades enregistrades en aquest arxiu seran susceptibles a ser tractades estadísticament pels objectius d'investigació científica que es descriuen més endavant.

- Les dades podran ser proporcionades i tractades, de forma anònima, per terceres persones que podran fer-ho exclusivament pels seus objectius d'investigació científica.
- Al pacient se li prendrà una mostra biològica de sang per tal d'extreure ADN.
- El CGC es compromet a que tota la informació i totes les mostres rebudes siguin anonimitzades mitjançant codificació prèvia al seu enviament a investigadors externs. D'aquesta manera la identitat del donant serà anònima per aquests investigadors.
- Els productes obtinguts de les mostres podran ser utilitzats en estudis d'investigació Biomèdica realitzats per altres centres, nacionals o estrangers, sempre que: 1) hagin estat considerats d'interès científic, 2) que compleixin els requisits establerts pels comitès externs Científic i d'experts Assessors en qüestions ètiques, Econòmiques, Mediambiental, Jurídiques i Socials.
- Vostè té dret a sol·licitar l'eliminació total de les mostres donades i de la informació relacionada amb les mateixes que en aquell moment estiguin emmagatzemades al CGC.

Per contactar amb aquets responsables pot dirigir-se a:

Dr. Oscar Campuzano Larrea

Centre Genètica Cardiovascular-IdIBGi

Carrer Pic de Peguera nº11, 17003, Girona (Espanya)

oscar@brugada.org

DECLARACIÓ DEL DONANT

He estat informat pel professional de salut a sota esmentat:

- Sobre els avantatges i inconvenients d'aquest procediment.
- Sobre el lloc d'obtenció, emmagatzemament i el procés que seguiran les dades i les mostres.
- Sobre la finalitat per la qual s'utilitzaran les mostres i dades (estudis genètics, de salut pública o estadístics, que aconsegueixin tots els requisits que exigeixen la llei, el Comitè d'experts Assessors en qüestions ètiques, Econòmiques, Mediambiental, Jurídiques i Socials, i el Comitè Científic).
- Que les mostres i dades seran proporcionats de forma anonimitzada als investigadors que treballin amb elles.
- Que en qualsevol moment puc revocar el meu consentiment i sol·licitar l'eliminació de les dades i mostres que romanguin emmagatzemades al CGC.
- Que en qualsevol moment puc sol·licitar informació genètica sobre els estudis pels quals s'han utilitzat els productes de les mostres d'assaig.
- Que he comprès la informació rebuda i he pogut formular totes les preguntes que he cregut oportunes.V1: 23/03/2015

DECLARACIÓ DEL PROFESSIONAL DE SALUT DE QUE HA INFORMAT DEGUDAMENT AL DONANT

Nom del metge

Signatura del metge

PACIENT

Nom del pacient

Signatura del pacient

MENOR O PACIENT AMB CAPACITATS MENTALS DISMINUÏDES

Nom del menor/ o pacient discapacitat mental

Nom del familiar/tutor/ o representant legal (cal especificar-ne la relació)

Signatura del familiar/tutor/ o representant legal

PACIENT DIFUNT

Nom del difunt

Nom del familiar/tutor/ o representant legal (cal especificar-ne la relació)

Signatura del familiar/tutor/ o representant legal

ANNEX 3: DATA COLLECTING

PAPER DE LA GENÈTICA EN LA DETECCIÓ PRECOÇ I PREVENCIÓ DE LA MORT SOBTADA NEONATAL MITJANÇANT L'ÚS DE L'ECG I L'ESTUDI GENÈTIC D'ULTRASEQUÈNCIACIÓ MASSIVA



HOSPITAL MATERNOINFANTIL
UNIVERSITAT DE BARCELONA

ID del pacient: _____

Sexe: masculí femení

Ètnia: _____

Edat gestacional: _____

Pes al nèixer: _____ g

Longitud al nèixer: _____ cm

Algun problema durant l'embaràs? Sí Quin? _____

No

Algun problema durant el part? Sí Quin? _____

No

La mare té alguna patologia? Sí Quina? _____

No

Hi ha alguna patologia a la família? Sí Quina? _____

No

Valor del QT llarg a l'ECG: 48 hores: _____ msec

1 mes: _____ msec

6 mesos: _____ msec

1 any: _____ msec

Triple Screening: TN: _____

PAPP-A: _____

Beta hCG lliure o hCG: _____

AFP: _____

Inhibina A: _____

Prova del taló: Fenilcetonúria: sí no

Hipotiroidisme congènit: sí no

Fibrosis quística: sí no