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**MOLECULAR AUTOPSY IN SUDDEN CARDIAC DEATH
AND GENETIC SCREEN IN
ARRHYTHMOGENIC CARDIAC SYNDROMES**

Tesi di Dottorato
Cettina Sortino

Coordinatore: *Prof.ssa Carla Giardano*

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PhD Thesis
Cettina Sortino

Director of PhD Programme: *Prof.ssa Carla Giordano*

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Abstract

Sudden cardiac death (SCD) is one of the most important mode of death in Western Countries and remains a major public health problems; is responsible for half of all deaths due to cardiovascular disease. New methods of preventing potentially fatal arrhythmias have been developed, and the accurate diagnosis of the causes of Sudden Cardiac Death is now of particular importance. In recent years researchers have identified the genetic background of many diseases involving the myocardium, and many Cardiomyopathies are considered to have a genetic origin. Autopsy and genetic family screening remains of great importance, not only in the research setting but also for clinical testing and should be performed when ever possible. The adequate assessment of Sudden Cardiac Death, including not only a protocol for heart examination and histological sampling, but also for toxicology and molecular genetic investigation.

Sudden deaths involving young individuals (< 35 years of age) remain unexplained following a complete medicolegal investigation that includes an autopsy. Cardiac channelopathies associated with structurally normal hearts such as long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome (BrS), leave no evidence to be found at autopsy, leaving investigators to only speculate that a lethal arrhythmia might lie at the heart of a sudden unexplained death (SUD).

In cases of autopsy negative SUD, continued investigation, through the use of a cardiological and genetic evaluation of first- or second-degree relatives and/or a molecular autopsy, may pinpoint the underlying mechanism attributing to the sudden death and allow for the identification of living family members with the pathogenic substrate that renders them vulnerable to an increased risk for cardiac events, including sudden death.¹

¹ Nicole J. Boczek, David J. Tester and Michael J. Ackerman. *The molecular autopsy: an indispensable step following sudden cardiac death in the young?*. *Herzschrittmacherther Elektrophysiol.* 2012 September ; 23(3): . doi:10.1007/s00399-012-0222-x.

Introduction

Sudden cardiac death (SCD) is defined as an unexpected and non-traumatic death of an individual who had been observed healthy in previous 6 hours of the death. In western countries, SCD underlies 20% of total mortality.² Although heart failure and coronary artery disease are the most prevalent substrates, epidemiological studies indicate that monogenic syndromes – called inherited arrhythmogenic diseases - also plays an important role in cardiac electrical instability. Thus, the inherited arrhythmogenic diseases have been defined broadly as 2 categories of pathologies: channelopathies and cardiomyopathies. Channelopathies are caused by pathogenic variations in genes encoding ion channels, and include Long QT Syndrome (LQTS), Brugada Syndrome (BrS), Short QT Syndrome (SQTS), and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT).³ Cardiomyopathies results from variations in genes encoding sarcomeric, cytoskeletal, and desmosomal proteins, and include Hypertrophic Cardiomyopathy (HCM), Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), and Dilated Cardiomyopathy (DCM).⁴

In Europe, sudden cardiac death (SCD) is one of the most common causes of death in infants, children and young adults and is relatively rare, with an incidence of 1 to 5 per 100.000 persons per year.⁵

Sudden death has been defined as *“a natural, unexpected fatal event occurring within one hour from the onset of symptoms in apparently healthy subject or whose disease was not so severe as to predict an abrupt outcome”*⁶

² Zipes DP, Camm AJ, Borggrefe M, et al. ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/ American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (writing committee to develop guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death): developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. *Circulation* 2006;114:e385– e484.

³ Priori SG, Aliot E, Blomstrom-Lundqvist C, et al. Task force on sudden cardiac death, European Society of Cardiology. *Europace* 2002;4:3–18.

⁴ O. Campuzano, C. Allegue, A. Fernandez, A. Iglesias, R. Brugada. *Determining the Pathogenicity of Genetic Variants Associated with Cardiac Channelopathies*. *Scientific Reports* 5:7953 DOI: 10.1038/srep07953.

⁵ S. Kaufenstein, N. Kiehne et al, 2009. *Cardiac gene defects can cause sudden cardiac death in young people*. *Deutsches Arzteblatt International* 2009; 106(4): 41-7.

⁶ Goldstein S (1982). The necessity of a uniform definition of sudden coronary death: witnessed death within 1 hour of the onset of acute symptoms. *Am Heart J* 103:156-159.

Sudden Arrhythmic Death Syndrome (SADS) is defined as sudden unexpected death with no previous cardiac history, last seen alive within 12 hours of being found dead and with no cause identifiable on postmortem examination (including negative toxicology and ideally an expert cardiac pathologist review)⁷; is responsible about 1 million deaths annually in the developed world, which makes arrhythmias as one of the most significant causes of death and disease in the general population.⁸

The underlying substrate varies: ischemic Heart disease in 75-80% cases; idiopathic cardiomyopathy in 10-15%; and 1-2% due to rare monogenic mutation in cardiac ion channel or associated proteins⁹.

Cardiomyopathy were defined as primary myocardial disorders of unknown cause: primary is used to describe diseases in which the heart is the sole or predominantly involved organ; secondary to describe diseases in which myocardial dysfunction is part of a systemic disorder.

Cardiomyopathies are grouped into specific morphological and functional phenotypes; each phenotype in then sub-classified into familial and non familial forms. The expert panel of the American Heart Association has suggested that ion channelopathies and disorders of conduction should also be considered as cardiomyopathies¹⁰.

When the cause is not readily identifiable by the common investigations (coronary angiography and transthoracic echocardiography or by autopsy) the cause of the cardiac arrest is termed “unexplained”. Unexplained cardiac arrest is defined as a cardiac arrest in the absence of coronary artery disease and overt structural heart disease, present in 5%-10% of cardiac arrest survivors. In the young, in up to 50% of sudden cardiac death cases, sudden death is the first and only clinical manifestation of an inherited cardiac disease that had remained undetected by conventional clinical investigations. The autopsy becomes the principal diagnostic tool because macroscopic and microscopic analyses

⁷L M Nunn, P D Lambiase, 2011. *Genetics and cardiovascular diseasedcauses and prevention of unexpected sudden adult death: the role of the SADS clinic*. Heart, vol. 97 pag1122-1127.

⁸Campuzano O. et al, 2010. *Genetics and cardiac channelopathies*. Genet Med.;12 (5):260-7.

⁹Chopra N. et al, 2011. “Genetics of sudden cardiac death syndromes”. Curr Opin Cardiol 26:196-203.

¹⁰Elliot P. et al, 2008. “Classification of the cardiomyopathies: a position statement from the European society of cardiology working group on myocardial and pericardial diseases”. European Heart Journal, 29, 270-276.

reveal the underlying cause of death. However, a significant number of sudden cardiac deaths remain unexplained.¹¹

A genetic contribution to cardiac arrest is more common in this population, most commonly attributed to an inherited ion channel abnormality leading to familial syncope and sudden death¹².

The goal of this study was to examine blood samples from patients with cardiac syndromes and formalin-fixed paraffin embedded tissue (FF-PET) cases of sudden cardiac death autopsy, to verify the corresponding genetic mutations and in particular the Ion channel disease.

Ion channels are integral membrane proteins that are responsible for ion conduction across the cell membrane. Ion channel subunits are multimeric proteins formed by different subunits, which are usually encoded by separate genes. Ion channel subunits are formed by pore-forming α -subunits, which mediate ion currents, and regulatory β -subunits. Channelopathy is thus defined as an inherited syndrome caused by mutations in genes encoding for ion channels, their subunits, or associated proteins. Genetic studies may be key to providing a lead toward an etiology for the unexplained symptoms.

There are several mutations that can affect the ion channels. The effects of these mutations can be grouped in three: alteration of the channel's permeability, alteration of the channel's activation, and dysfunction of the channel's inactivation.

Genetic testing is becoming an important tool for a personalized medical approach to Cardiomyopathies. Autopsy and genetic family screening remains of great importance, not only in the research setting but also for clinical testing and should be performed when ever possible¹³.

¹¹ 7. Maron BJ, Towbin JA, Thiene G, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation* 2006;113:1807–1816.

¹² Matthew T. et al., 2013. *Review Assessment of Genetic Causes of Cardiac Arrest*. Canadian Journal of Cardiology, vol. 29, pag. 100–110.

¹³ Sinagra G; Mestroni L. ; Camerini F., 2012. *Genetic Cardiomyopathies – a clinical approach*. Springer ISBN 978-88-470-2756-5

1. GUIDELINES FOR AUTOPSY INVESTIGATION OF SUDDEN CARDIAC DEATH (SCD) AND INDICATIONS FOR MOLECULAR AUTOPSY

The role of the autopsy in sudden death is most important to establish:

- 1) Whether the death is attributable to a cardiac disease or to other causes of sudden death;
- 2) The nature of the cardiac disease, and whether the mechanism was arrhythmic or mechanical;
- 3) Whether the cardiac condition causing sudden death may be inherited, requiring screening and counselling of the next of kin;
- 4) The possibility of toxic or illicit drug abuse and other unnatural deaths.¹⁴

Any potential source of information should be interrogated, preferentially before autopsy is carried out. Ideally, the following information is required:

- Age, gender, occupation, lifestyle
- Circumstances of death (date, time interval, place of death)
- Medical history;
- Family cardiac history.

All sudden death autopsies should be sequential structured examinations. They should specifically address the major causes of extra-cardiac sudden death. Principles and rules relating to autopsy procedures should adhere to the Recommendations on the Harmonisation of Medico Legal Autopsy Rules produced by the Committee of Ministers of the Council of Europe.¹⁵

¹⁵ Brinkmann B (1999) harmonization of medico legal autopsy rules. Committee of ministers. Council of Europe. Int J Legal Med 113:1-14

- *External examination of the body*

- Establish body weight and height (to correlate with heart weight and wall thickness);¹⁶⁻¹⁷
- Check for recent intravenous access, intubation, ECG pads, defibrillator and electrical burns, drain sites and traumatic lesions;
- Check for implantable cardioverter defibrillator (ICD)/ pacemaker, if in situ, see MDA Safety Notice 2002 for safe removal and interrogation;¹⁸

- *Exclusion of non-cardiac causes of sudden death*

Any natural sudden death can be considered cardiac in origin after the exclusion of non cardiac causes. Thus, a full autopsy with sequential approach should be always performed to exclude common and un-common extra-cardiac causes of sudden death (cerebral; respiratory; acute haemorrhagic shock; septic shock).

- *Search for cardiac causes of sudden death*

- Many cardiovascular diseases can cause SCD, either through an arrhythmic mechanism (electrical SCD) or by compromising the mechanical function of the heart (mechanical SCD). These disorders may affect the coronary arteries, the myocardium, the cardiac valves, the conducting system, the intra-pericardial aorta or the pulmonary artery, the integrity of which is essential for a regular heart function.
- The standard gross examination of the heart;
- The standard histologic examination of the heart Myocardium (in particular cases, Electron microscopy investigation;
- Further laboratory tests: molecular or toxicologic studies.

- *Molecular Pathology*

Molecular studies of SCD include both detection of viral genomes in inflammatory cardiomyopathies, and gene mutational analysis in both structural and non-structural genetically determined heart diseases¹⁹.

¹⁶ Kitzman DW, Scholz DG, Hagen PT, et al. Age-related changes in normal human hearts during the first 10 decades of life. Part II (maturity): a quantitative anatomic study of 765 specimens from subjects 20 to 99 years old. *Mayo Clin Proc* 63:137-146.

¹⁷ Schulz DM, Giordano DA (1962) hearts of infants and children: weights and measurements. *Arch Pathol* 73:464-471.

¹⁸

¹⁹ 5. Chugh SS, Senashova O, Watts A, et al. Postmortem molecular screening in unexplained sudden death. *J Am Coll Cardiol* 2004;43:1625–1629.

For these purposes: 10 ml of EDTA blood and 5g of heart and spleen tissues are either frozen and stored at -80°C, or alternatively stored in RNA *later* at 4°C for up to e weeks.

- *Formulation of diagnosis*

The report should conclude with a clear clinicopathological summary. As far as possible this should relate the pathological findings to the clinical history, the circumstances of the death and any investigation performed close to the time of the death. In the majority of SCDs, a clear pathological cause can be identified, albeit with varying degrees of confidence. Wherever possible, the most likely underlying cause should be stated and the need for familial clinical screening and genetic analysis clearly indicated. It is important to accept that different degrees of certainty exist in defining the cause-effect relationship between the cardiovascular substrate and the sudden death event. Finally, there are myocardial diseases in which the border between physiological and pathological changes is poorly defined.

Death that remain unexplained after careful macroscopic, microscopic and laboratory investigation should be classified as sudden arrhythmic death syndrome. There is increasing evidence that SCD in these instances might be due to inherited ion channel disorders, such as long QT and short QT syndromes, Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia sick sinus syndrome, which present with well-defined abnormalities of basal ECG. In this setting, the availability of ECG tracing may be crucial for the diagnosis and molecular studies are essential. First degree relatives should undergo clinical screening and subsequent genetic analysis when indicated.

New methods of preventing potentially fatal arrhythmias have been developed and the accurate diagnosis of SCD is now of particular importance. The guidelines represent the minimum standards of practice that should be adopted throughout the European Union and elsewhere.

A careful cardiac investigation during the autopsy can provide a definitive diagnosis in structural heart diseases, be it by the identification of a culprit coronary lesion or heart muscle disease, which explain the episode. However, a significant number of SCD cases remain unexplained after a comprehensive medicolegal investigation. These autopsies are usually labeled as negative, natural, or arrhythmogenic. In these negative autopsies,

especially in the young, a genetic disease causing an electrical disturbance (ion channelopathy) should be immediately suspected. Because of the familial nature of the disease, the identification of these genetic defects carry important implications for relatives who are at potential risk of also having a fatal cardiac condition.²⁰

Key principles of postmortem investigations of sudden unexpected death in the young adapted from “Postmortem in sudden unexpected death in the young: guidelines on autopsy practice,” devised by TRAGADY - Trans-Tasman Response Against Sudden Death in the Young - and endorsed by the Royal College of Pathologists of Australasia.²¹

1. All cases of sudden unexpected or unexplained death in the young (age group of 0–40 years) should have an autopsy
2. A full postmortem examination should be completed
3. The investigation, ideally lead by a pathologist, should involve a team approach <ul style="list-style-type: none"> a. A person designated to liaise with the family b. Specialist cardiology involvement with the family when noncardiac causes are excluded c. Laboratories with molecular genetics, toxicology, and metabolic expertise
4. A detailed antecedent clinical history must be obtained <ul style="list-style-type: none"> a. Circumstances of the death—detailed review of the date, time, place, and activity surrounding the death (i.e., at home, work, or on the athletic field, at rest or during exercise or emotional excitement). Was the death witnessed? Document any associated seizures, prodromal symptoms b. Past medical history—Document the general health status, including previous significant illness or events such as syncope, seizures, epilepsy, palpitations, and respiratory or neurological disease. Retrieve results of any prior investigations (e.g., ECG, EEG, CT, or MRI) c. Previous surgical procedures or interventions—Document details of current medications, in particular those that are known to be pro-arrhythmic (see: www.qt drugs.org)
5. A detailed and relevant family history must be obtained <ul style="list-style-type: none"> a. Family history—Document any family history of premature death (explained or unexplained death, SIDS, unexplained drowning, or unusual motor vehicle accidents), seizures, or syncope. Be sure to include a detailed description of the date, time, place, and activity surrounding such events, if available b. Diagnosed disorders—Document any family history of clinical diagnosis of potentially arrhythmic disorders, including LQTS, CPVT, BrS, familial cardiomyopathy, or other cardiac conditions
6. Skilled macroscopic and microscopic examination of the organs is required, particularly of the heart (especially right ventricular muscle) and the brain. This may require some specimens to be examined by other specialists
7. Adequate histological material be obtained for review or, if necessary, referral
8. Tissue or blood suitable for DNA extraction must be obtained
9. Results, including photography, must be documented clearly
10. Results must be described and annotated in a standard fashion which will allow epidemiological data gathering

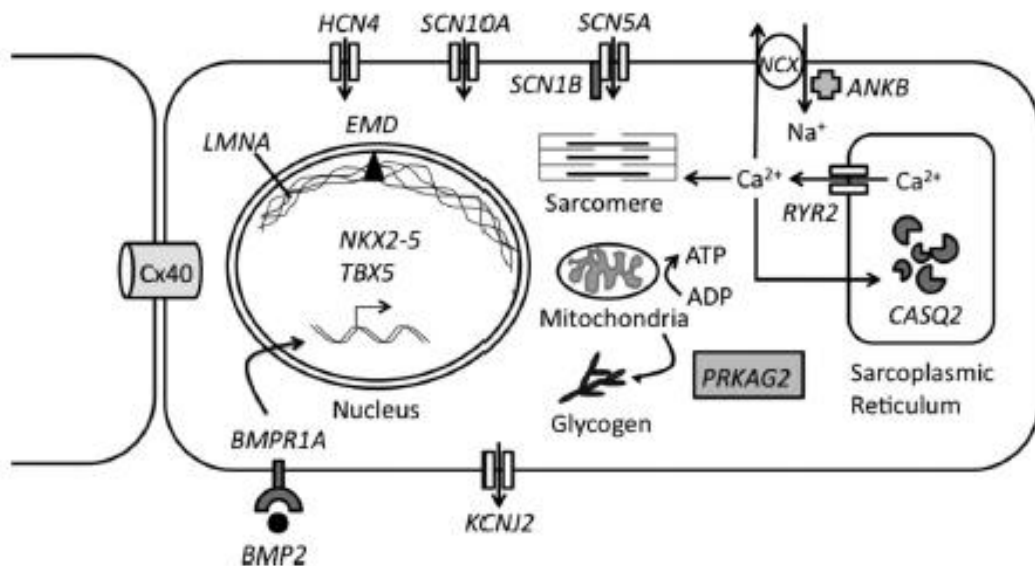
²⁰ O. Campuzano, P. Beltrán-Alvarez, A. Iglesias, F. Scornik, G. Pérez, R. Brugada. *Genetics and cardiac channelopathies*. Genetics in medicine – Vol. 12, Number 5, May 2010.

²¹ Tragady. [Accessed June 18, 2012] Post-mortem in sudden unexpected death in the young: guidelines on autopsy practice Sydney: Royal College of Pathologists of Australasia. 2008. [http://www.rcpa.edu.au/applications/DocumentLibraryManager2/upload/SUDYbestpracticedocumentendorsedMay2708%20\(2\).pdf](http://www.rcpa.edu.au/applications/DocumentLibraryManager2/upload/SUDYbestpracticedocumentendorsedMay2708%20(2).pdf).

2. ION CHANNEL DISEASES: GENETICS AND CARDIAC CHANNELOPATHIES.

Inherited cardiac diseases associated with SCD can be classified into two broad groups:

1. Primary electrical diseases, or channelopathies, in which the arrhythmogenic substrate is found in the electrical properties of the heart, and
2. Cardiomyopathies, in which structural abnormalities such as hypertrophy, dilatation, or fatty and/or fibrotic infiltration, are responsible for inducing the arrhythmia.



1. Cardiac conduction system cell. genes identified in human cardiac conduction system disease

2.1 Diseases of Automaticity

Normal heart rhythm (sinus rhythm) depends on regular activity of the sinoatrial node (SAN), a heterogeneous collection of specialized myocytes in the right atrium. SAN cells, in general, possess a unique electrophysiological profile that promotes spontaneous electrical activity (automaticity).²²

Cells in the pacemaker and conduction system possess the defining characteristic of automaticity, an ability to generate an action potential in the absence of an external stimulus. Automaticity is required for cardiac pacemaking and depends on a unique ion channel expression profile conducive to spontaneous Action Potential generation.²³

The human sinoatrial node (SAN) is a crescent-shaped, intramural structure with its head located subepicardially at the junction of the right atrium and the superior vena cava and its tail extending 10 to 20 mm along the crista terminalis. The SAN has complex 3-dimensional tissue architecture with central and peripheral components made up of distinct ion channel and gap junction expression profiles. Central and peripheral cells have different action potential characteristics and conduction properties (Figure 2). The central SAN, the site of dominant pacemaking, is electronically insulated from the hyperpolarizing atrial myocardium through the differential expression of connexins and ion channels. Peripheral SAN cells are electrophysiologically intermediate between central cells and atrial cardiomyocytes.

²² SathyaD.Unudurthi, RoseanneM.Wolf and ThomasJ.Hund. *Role of sinoatrial node architecture in maintaining a balanced source-sink relationship and synchronous cardiac pacemaking.* FrontiersinPhysiology | CardiacElectrophysiology November2014, Volume5, Article 446.

²³ Verkerk AO, Wilders R. Pacemaker activity of the human sinoatrial node: effects of HCN4mutations on the hyperpolarization-activated current. *Europace.* 2014 Mar;16(3):384-95.

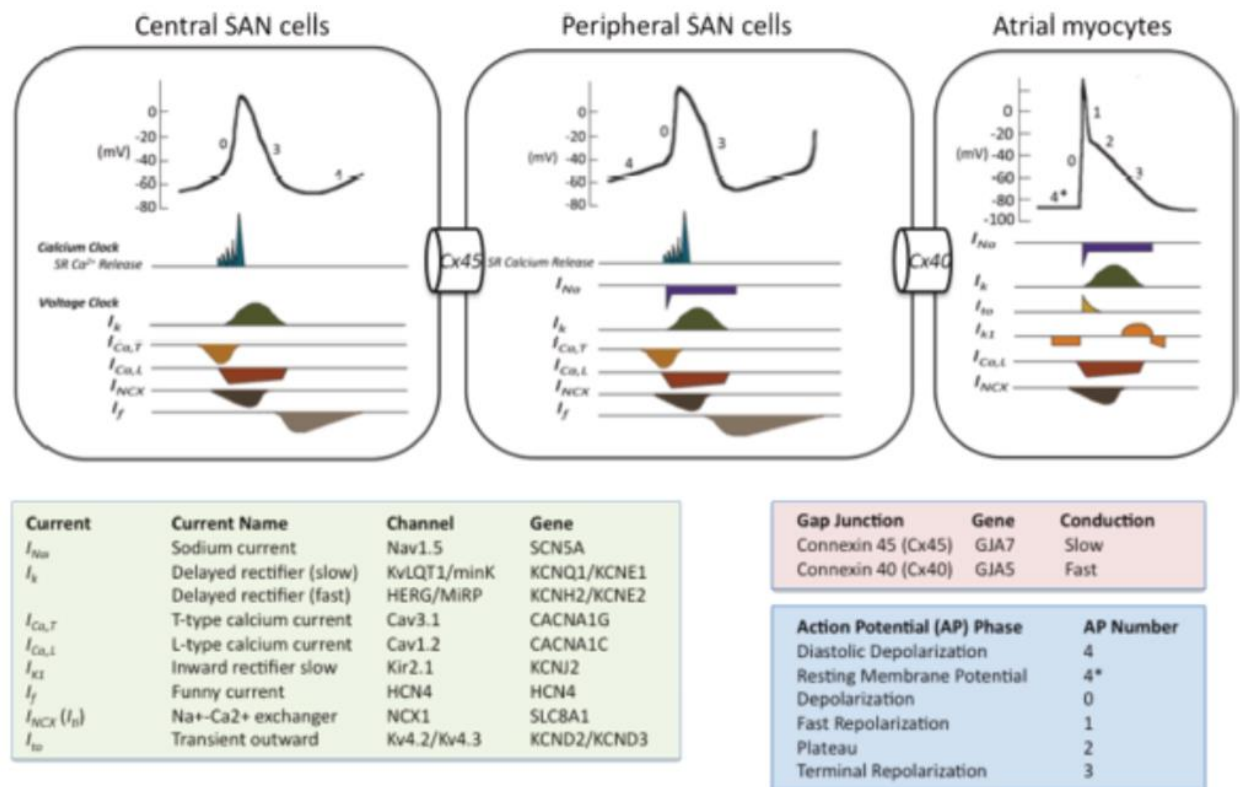


Figure 2 Electrophysiological heterogeneity of the sinoatrial node (SAN)

Human mutations affecting the *voltage clock* (SCN5A and HCN4), calcium clock (RYR2 and CASQ2), or both mechanisms (ANKK) have been identified that negatively affect sinus node function.²⁴

Among the important currents contributing to diastolic depolarization is the hyperpolarization-activated “funny” current (I_f) (due primarily to HCN4 in SAN cells), with permeability to both Na⁺ and K⁺ and biophysical properties that make it a depolarizing current during diastole. It activates as the membrane potential approaches its max diastolic value and helps to spontaneously depolarize the membrane.²⁵ At the same time, a low level of Ca²⁺ release from sarcoplasmic reticulum ryanodine receptor

²⁴ David S. Park, MD, PhD; Glenn I. Fishman, MD. *The Cardiac Conduction System*. Circulation. 2011;123:904-915.

²⁵ DiFrancesco D. The role of the funny current in pacemaker activity. *Circ Res*. 2010 Feb 19;106(3):434-46.

Ca²⁺ release channels likely promotes a depolarizing current via the Na⁺/Ca²⁺ exchanger (Lakatta et al., 2010).

2.2 HCN4 Diseases of Automaticity

Hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels contribute to the pacemaker current (I_f) that is responsible for generating and regulating heart rhythm. Loss-of-function mutations of HCN4, the major HCN channel subunit in pacemaker cells, cause bradycardia. Moreover, cardiac tachyarrhythmias have also been shown to be associated with dysfunctional HCN4 channels.²⁶ Although the pathogenic role of HCN channel mutations in cardiac tachyarrhythmias remains to be determined, one of the clues can be found in the suggested function of I_f in preventing bradycardia-induced ventricular arrhythmias by inhibiting early after-depolarization.²⁷

The pacemaker, or funny current, I_f, is generated by the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel. The term funny current stems from the unique characteristics of HCN channels, which include permeability to K⁺ and Na⁺, activation at hyperpolarized membrane potentials, and modulation by cAMP. The biophysical properties of HCN channels make them ideally suited to function as modulators of the pacemaker potential.²⁸ First, because they are activated at hyperpolarized membrane potentials (between -65 and -40 mV), their slow inward current contributes to diastolic depolarization; second, the main cardiac HCN channel (HCN4) is cAMP responsive, allowing I_f to be modulated by autonomic stimulation²⁹.

²⁶ Baruscotti M, Bottelli G, Milanese R, DiFrancesco JC, DiFrancesco D. HCN-related channelopathies. *Pflugers Arch*. 2010 Jul;460(2):405-15.

²⁷ Scicchitano P, Carbonara S, Ricci G, Mandurino C, Locorotondo M, Bulzis G, Gesualdo M, Zito A, Carbonara R, Dentamaro I, Riccioni G, Ciccone MM. HCN channels and heart rate. *Molecules*. 2012 Apr 5;17(4):4225-35.

²⁸ Milano A, Vermeer AM, Lodder EM et al. HCN4 mutations in multiple families with bradycardia and left ventricular noncompaction cardiomyopathy. *J Am Coll Cardiol*. 2014 Aug 26;64(8):745-56.

²⁹ DiFrancesco D. funny channel gene mutations associated with arrhythmias. *J Physiol*. 2013 Sep 1;591(Pt17):4117-24.

2.3 Sick sinus syndrome

Sick sinus syndrome refers to a collection of disorders marked by the heart's inability to perform its pacemaking function. Intrinsic causes include degenerative fibrosis, ion channel dysfunction, and remodeling of the sinoatrial node.³⁰

The hyperpolarization-activated 'funny' current, *I_f*, plays an important modulating role in the pacemaker activity of the human sinoatrial node (SAN). *I_f* is carried by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, which are tetramers built of four HCN subunits. In human SAN, HCN4 is the most abundant of the four isoforms of the HCN family (Figure 3). Since 2003, several loss-of-function mutations in the HCN4 gene, which encodes the HCN4 protein, or in the KCNE2 gene, which encodes the MiRP1 accessory b-subunit, have been associated with sinus node dysfunction.³¹

The SAN dysfunction is characterized by an atrial rate that is inappropriate for physiological requirements and presents itself with a combination of symptoms such as dizziness, fatigue, and syncope.³²

³⁰ Duhme N, Schweizer PA, Thomas D, et al. Altered HCN4 channel C-linker interaction is associated with familial tachycardia-bradycardia syndrome and atrial fibrillation. *Eur Heart J*. 2013 Sep;34(35):2768-75. Epub 2012 Nov 23.

³¹ Arie O, Verkerk and Ronald Wilders. Pacemaker activity of the human sinoatrial node: effects of HCN4 mutations on the hyperpolarization-activated current. *European Society of Cardiology* (2014) 16, 384–395.

³² Dobrzynski H, Boyett MR, Anderson RH. New insights into pacemaker activity: promoting understanding of sick sinus syndrome. *Circulation* 2007;115:1921–32.

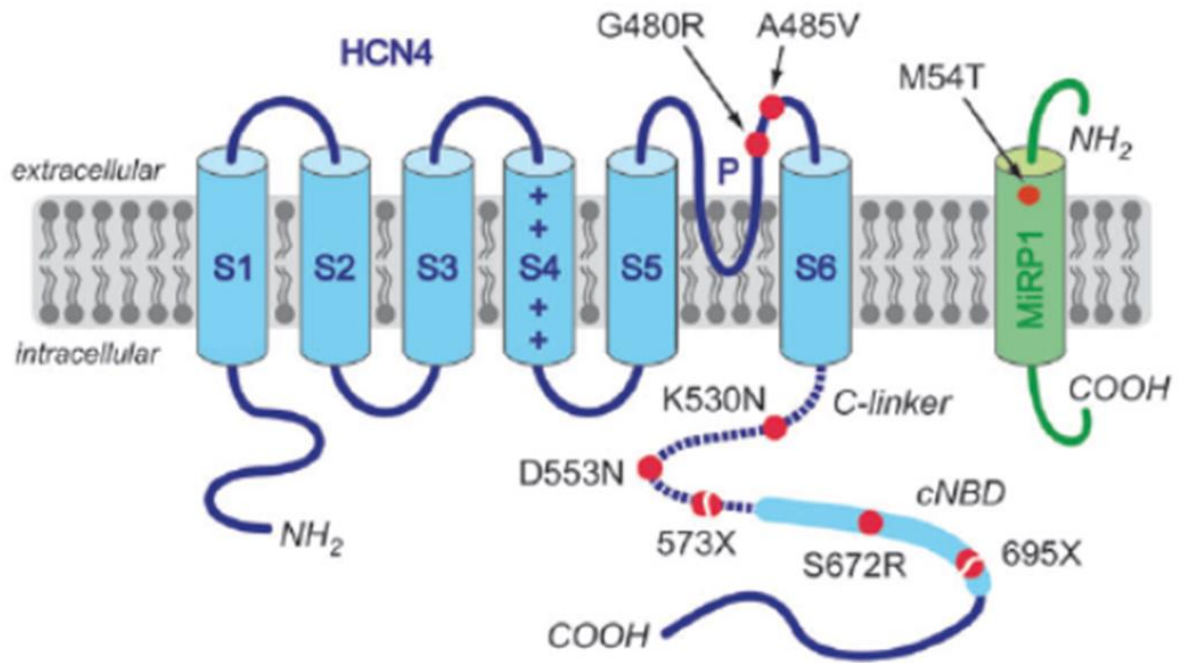
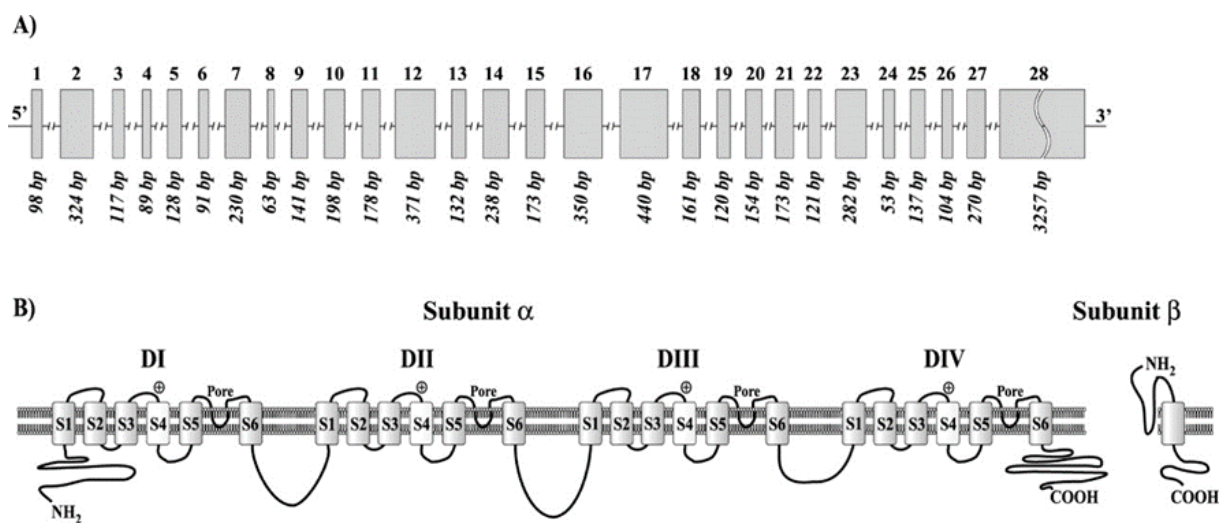


Figure 3 Mutations in HCN4 and MiRP1 associated with sinus bradycardia

3. DISEASES ASSOCIATED WITH SODIUM CHANNEL DYSFUNCTION

The α -subunit of the cardiac isoform of the sodium channel is known as *Nav1.5* and is encoded by the *SCN5A* gene. *Nav1.5* is a membrane protein that contains 2015 or 2016 residues (depending on the splice variant) for an approximate molecular weight of 227 kDa. It consists of four homologous domains, known as DI to DIV, joined by so-called linkers.³³ These three linkers in addition to the N-terminus and the C-terminus of the protein are cytoplasmic. Each domain, DI to DIV, contains six transmembrane helices (namely S1–S6) linked by intracellular or extracellular loops. S5 and S6 in each of the domains form the pore-lining helices, and the extracellular loop between them is the longest among extracellular segments of *Nav1.5*.³⁴



Malfunctioning of cardiac Na^+ channels is the cause of some primary arrhythmia syndromes.³⁵ Among others, four diseases have been associated to genetic mutations that affect Na^+ channel function: Brugada syndrome (BrS), Lev-Lene`gre syndrome (familial

³³ Bennett PB, Yazawa K, Makita N, George AL Jr. Molecular mechanism for an inherited cardiac arrhythmia. *Nature* 1995;376:683– 685.

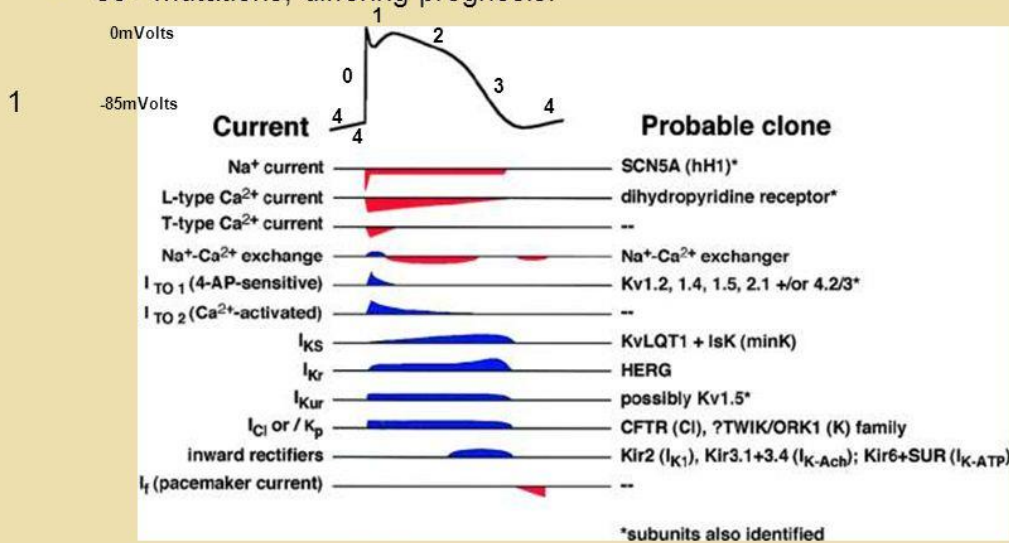
³⁴ 9. Benson DW, Wang DW, Dyment M, et al. Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (*SCN5A*). *J Clin Invest* 2003;112:1019 –1028.

³⁵ Tan HL, Bink-Boelkens MT, Bezzina CR, et al. A sodium-channel mutation causes isolated cardiac conduction disease. *Nature* 2001;409:1043–1047.

progressive conduction disease), long QT syndrome (LQTS), and familial atrial fibrillation (AF). Certain mutations can give rise to different phenotypes with enhanced or reduced channel function and even combinations of phenotypes.

SCN5A gene

- Codes for cardiac sodium channel that opens during phase 2 of the action potential. In Brugada, it opens poorly in RV epicardial cells.
- Autosomal dominant inheritance
- 20-30% of cases have anbl SCN5A gene.
- 80+ mutations, differing prognosis.



Priori, S. G. et al. Circulation 1999;99:674-681

3.1 Brugada syndrome

Brugada syndrome (BrS) was described 20 years ago as a new clinical entity characterized by the presence of a typical electrocardiographic (ECG) pattern (right bundle branch block and persistent ST -segment elevation in right precordial leads) and associated with a high risk of sudden cardiac death (SCD)³⁶.

Currently, it is believed to be responsible for 12% of SCD cases and 20% of SCD in patients with structurally normal hearts. Patients may suffer syncope or SCD secondary to polymorphic ventricular tachycardia (PVT)/ventricular fibrillation (VF). However, the majority of patients remain completely asymptomatic. Some of the arrhythmias may occur after large meals, during rest, or while sleeping, believed to be due to high vagal tone. The symptoms usually appear around 40 years of age; however, there are reports of patients affected from ages 1 to 84.³⁷ Males are more often symptomatic than females, probably from the influence of hormones and gender distribution of ion channels across the heart.³⁸

There is little information regarding the pediatric population, but studies performed in children have failed to identify a male predominance, perhaps due to low levels of testosterone in children of both genders.³⁹ The prevalence of the disease is difficult to estimate because the pattern is not always recognized or because it may transiently normalize. Nevertheless, global prevalence varies from 5 to 20 in every 10,000, and it is considered endemic in Southeast Asian countries, where the prevalence is higher.⁴⁰

Molecular Mechanism - the characteristic right precordial ST -segment elevation in the ECG is not well understood. Currently there are two mechanisms that may explain the ECG alteration; neither mechanism has been conclusively confirmed, nor are they

³⁶ Gussak I, Brugada P, Brugada J, et al. Idiopathic short QT interval: a new clinical syndrome? *Cardiology* 2000;94:99 –102.

³⁷ Sheikh AS, Ranjan K. Brugada syndrome: a review of the literature. *Clin Med.* 2014 Oct;14(5):482-9. doi: 10.7861/clinmedicine.14-5-482.

³⁸ Grant AO, Carboni MP, Neplioueva V, et al. Long QT syndrome, Brugada syndrome, and conduction system disease are linked to a single sodium channel mutation. *J Clin Invest* 2002;110:1201–1209.

³⁹ Brugada R, Campuzano O, Sarquella-Brugada G, Brugada J, Brugada P. Brugada syndrome. *Methodist DeBakey Cardiovasc J.* 2014 Jan-Mar;10(1):25-8.

⁴⁰ R. Brugada, O. Campuzano, G. Sarquella-Brugada, J. Brugada, P. Brugada. *Brugada syndrome*. houstonmethodist.org/debakey-journal. MDCVJ | X (1) 2014.

mutually exclusive.⁴¹ The first hypothesis, repolarization, focuses on the presence of transmural voltage gradients due to heterogeneity in action potential duration between the RV epicardium and endocardium (disequilibrium between INa and Ito). This generates transmural dispersion of repolarization and causes the ST-segment elevation.⁴²

The second hypothesis, depolarization, involves preferential conduction slowing in the RV outflow tract, leading to ST-segment elevation in the right precordial leads. Regional differences in conduction velocity in the RV epicardium would be aggravated by INa reduction and trigger the occurrence of epicardial reentrant excitation waves. Additionally, in 2009, Boukens et al. suggested that the embryological development of the right ventricle could explain the electrophysiological heterogeneity in the ventricular myocardium, including the RV outflow tract, which could provide the arrhythmogenic substrate.⁴³

Genetics - Brugada syndrome is a disease with an autosomal dominant pattern of transmission. Incomplete penetrance is frequent in families, and the disease can be sporadic in up to 60% of patients. In 1998, the first pathogenic mutation in the SCN5A gene was identified. This gene encodes the alpha subunit of the cardiac sodium channel (Nav1.5). Since then, more than 350 pathogenic mutations in several genes have been published (Table 1).⁴⁴

⁴¹ Mashar M, Kwok AJ, Pinder R, Sabir I. The Brugada syndrome revisited. *Trends Cardiovasc Med*. 2014 Jul;24(5):191-6. doi: 10.1016/j.tcm.2013.11.001. Epub 2013 Nov 11.

⁴² Brugada P, Brugada J, Roy D. Brugada syndrome 1992-2012: 20 years of scientific excitement, and more. *Eur Heart J*. 2013 Dec;34(47):3610-5. doi: 10.1093/eurheartj/eht113. Epub 2013 Nov 1.

⁴³ Arbelo E, Brugada J. Risk stratification and treatment of brugada syndrome. *Curr Cardiol Rep*. 2014 Jul;16(7):508. doi: 10.1007/s11886-014-0508-1.

⁴⁴ Barajas-Martínez H, Hu D, Antzelevitch C. Genetic and molecular basis for sodium channel-mediated Brugada syndrome. *Arch Cardiol Mex*. 2013 Oct-Dec;83(4):295-302. doi: 10.1016/j.acmx.2013.10.001. Epub 2013 Nov 21.

CHANNEL	GENE	PROTEIN
SODIUM	<i>SCN5A</i> <i>GPD1-L</i> <i>SCN1B</i> <i>SCN3B</i>	Nav1.5 glycerol-3-P-DH-1 Navβ1 Navβ3
	<i>SCN2B</i> <i>RANGRF</i> <i>SLMAP</i>	Navβ2 RAN-G-release factor (or MOG1) sarcolemma associated protein
POTASSIUM	<i>KCNE3</i>	MIRP2
	<i>KCNJ8</i>	Kv6.1 Kir6.1
	<i>HCN4</i>	hyperpolarization cyclic nucleotide-gated 4
	<i>KCNE5</i>	K voltage-gated subfamily E member 1 like
	<i>KCND3</i>	Kv4.3 Kir4.3
CALCIUM	<i>CACNA1C</i>	Cav1.2
	<i>CACNB2B</i>	voltage-dependent β-2
	<i>CACNA2D1</i>	voltage-dependent α2/δ1
	<i>TRPM4</i>	transient receptor potential M4

Table 1. Genes associated with Brugada syndrome.

These genes encode subunits of cardiac sodium, potassium, and calcium channels as well as genes involved in the trafficking or regulation of these channels. Despite the high number of gene mutations, only about 35% of BrS patients have been determined to have a genetic cause. Of them, nearly 30% carry a pathogenic mutation in the *SCN5A* gene.⁴⁵ All other genes together are responsible for about 5% of all BrS cases. Therefore, 65% of cases do not have a genetic origin.

Several factors could explain the high number of BrS patients without genetic alteration after genetic screening. For example, copy number variations have already been reported in *SCN5A*. In addition, pathogenic mutations associated with BrS could be localized in unknown genes, or the disease could be related to epigenetic factors, mainly DNA methylation, post-translational modifications, and RNA mechanisms. All these factors

⁴⁵ Liu M, Yang KC, Dudley SC Jr. Cardiac sodium channel mutations: why so many phenotypes? *Nat Rev Cardiol.* 2014 Oct;11(10):607-15. doi: 10.1038/nrcardio.2014.85. Epub 2014 Jun 24.

could also explain, at least in part, incomplete penetrance and variable expressivity characteristics in BrS families.⁴⁶

Risk stratification - It is well accepted that the etiology of BrS is multifactorial, involving genetic, environmental, and hormonal components that contribute to its phenotype manifestation. In addition, some clinical features have been identified as high-risk markers in BrS. It is established that symptomatic patients with recurrent syncope, agonal respiration during sleep, or unknown seizures are at risk of sudden death and need ICD. However, a debate is still ongoing on the value of risk stratification parameters, such as electrophysiological inducibility, in asymptomatic patients. Some will argue that it has no value, while others will claim that the electrophysiology study (EPS) enables the identification of a subgroup of asymptomatic patients at higher risk who will benefit from ICD implantation.

Other modulating factors also have been investigated. For example, genetic studies have reported that compound pathogenic mutations in BrS patients cause more severe phenotype and that common polymorphisms may modulate the effect caused by pathogenic mutations. In addition, it recently has been published that pathogenic mutations in combination with common single nucleotide polymorphisms could increase the risk of arrhythmias in BrS patients, though at present genetics are not useful in risk stratification. At this time, genetic screening is only recommended as a diagnostic tool.⁴⁷

⁴⁶ Miller D, Wang L, Zhong J. Sodium channels, cardiac arrhythmia, and therapeutic strategy. *Adv Pharmacol.* 2014;70:367-92. doi: 10.1016/B978-0-12-417197-8.00012-2.Review.

⁴⁷ R. Brugada, O. Campuzano, G. Sarquella-Brugada, J. Brugada, P. Brugada. Brugada syndrome. *houstonmethodist.org/debakey-journal. MDCVJ | X (1) 2014.*

3.2 *Lev-Lenegre syndrome*

Lev-Lenegre syndrome is a progressive cardiac conduction disease characterized by gradually developed ventricular fibrosis of the conduction system, which may lead to arrhythmias or asystolia. The first locus for the disease was reported in 1995, on chromosome 19q13.2–13.3.⁴⁸ In 1999, the first mutations causing the disease were associated with SCN5A.24 Mutations in SCN5A lead to a reduction in Na⁺ current, reducing the velocity of the impulse conduction.¹¹

Electrocardiographically, both Lenegre and Lev disease are characterized by chronic conduction delay through the His-Purkinje system, resulting in partial or complete AV-block and right or left bundle branch block.⁴⁹ In both diseases a sclerodegenerative process causes fibrosis of the His-Purkinje fibres. The severity and extent of the fibrosis in these diseases, however, is different. In Lenegre disease, a diffuse fibrotic degeneration is limited to the conduction fibres, while in Lev disease the sclerodegenerative abnormalities affect both the specialized conduction system and the fibrous skeleton of the heart. An inherited component may be involved in both diseases. However, particularly Lev disease may be a variation of the normal ageing process.⁵⁰

⁴⁸ Brink PA, Ferreira A, Moolman JC, Weymar HW, van der Merwe PL, Corfield VA. *Gene for progressive familial heart block type I maps to chromosome 19q13*. *Circulation* 1995;91:1633–1640.

⁴⁹ Lev M, Cuadros H, Milton HP. *Interruption of the atrioventricular bundle with congenital atrioventricular block*. *Circulation* 1971;43:703e10.

⁵⁰ Harrison's principles of internal medicine, online edition, <http://harrisons.accessmedicine.com>

3.3 Long QT Syndrome

The prevalence of congenital LQTS is reported to be approximately 1/2,000. Syncope is generally the most commonly encountered first episode in LQTS patients, and aborted cardiac arrest/sudden cardiac death (ACA/SCD) is rare (1–3%).

Of the patients who eventually become symptomatic, 50% experience their first cardiac event by the age of 12, and 90% by the age of 40. LQTS is also known as an etiology of sudden infant death syndrome (SIDS) and in approximately 10% of SIDS cases the infant carried a mutation in a LQTS-causing gene.⁵¹

The most frequent LQTS subtypes are type 1 (LQT1), type 2 (LQT2) and type 3 (LQT3). The subdivision is based on the underlying genetic substrate, with the potassium channel genes, KCNQ1, KCNH2, and the sodium channel gene, SCN5A, as the involved genes. Specific triggers of symptoms are known in these major LQTS subtypes.⁵² For example in LQT1, cardiac events occur during physical exertion or emotional stress, typically during swimming, whereas auditory stimulation such as an alarm clock or a telephone bell is a typical trigger for arrhythmic events in LQT2.⁵³ Emotional stress, as well as the postpartum period, are also frequently observed triggers of arrhythmia in LQT2. More recently, a history of epilepsy has been reported to be more common with LQT2 (39%) than with other subtypes of LQTS (10%, $P < 0.001$), possibly because KCNH2 is expressed in the brain as well. Obviously in such cases, the differential diagnosis of LQTS and epilepsy is important for treatment, because LQTS is often mistreated as epilepsy because of generalized seizures secondary to torsades de pointes. In LQT3, symptoms are most frequently observed during rest or at night.⁵⁴

Diagnosis - shows the LQTS diagnostic scoring system (updated in 2011), which includes symptoms, family history and ECG findings.¹⁶ Patients with a Schwartz score ≥ 3.5 points in the absence of a secondary cause for QT prolongation are diagnosed as LQTS.

⁵¹ Benito B, Brugada R, Perich RM, et al. A mutation in the sodium channel is responsible for the association of long QT syndrome and familial atrial fibrillation. *Heart Rhythm* 2008;5:1434–1440.

⁵² Barsheshet A, Dotsenko O, Goldenberg I. Congenital long QT syndromes: prevalence, pathophysiology and management. *Paediatr Drugs*. 2014 Dec;16(6):447-56.

⁵³ Adler A, Uppal S, Orr N, Alzoughool F, Gollob MH. First report of a large duplication of the KCNQ1 gene in a patient with long QT syndrome. *Can J Cardiol*. 2014 Oct;30(10):1249.e5-7.

⁵⁴ Campuzano O, Sarquella-Brugada G, Mademont-Soler I, et al. Identification of Genetic Alterations, as Causative Genetic Defects in Long QT Syndrome, Using Next Generation Sequencing Technology. *PLoS One*. 2014 Dec 10;9(12):e114894.

Typical LQTS cases can be readily diagnosed with this scoring system, whereas latent LQTS patients with normal QTc at rest, found in 36% of LQT1, 19% of LQT2 and 10% of LQT3, respectively, may show a non-diagnostic score of 1–3 points. In such cases, serial ECGs, 24-h Holter recordings, and an exercise or epinephrine test are recommended to reveal subclinical QT prolongation.⁵⁵

Table 1. Schwartz Score (Updated in 2011)¹⁶	
ECG findings	Points
QTc	
>480ms	3
460–470ms	2
450 (male) ms	1
4-min recovery QTc after exercise test \geq 480ms	1
Torsades de pointes	2
T-wave alternans	1
Notched T wave in 3 leads	1
Low heart rate for age	0.5
Clinical history	
Syncope	
With stress	2
Without stress	1
Congenital deafness	0.5
Family history	
A. Family members with definite LQTS	1
B. Unexplained sudden cardiac death <age 30 among immediate family members	0.5

3 LQTS Diagnostic criteria

The QT interval reflects repolarization of the ventricular action potentials orchestrated by various cardiac ion currents, including sodium, calcium and potassium currents. When a decreased potassium current (loss-of-function) or increased sodium or calcium current (gain-of-function) is caused by a mutation in an ion channel or axillary protein, a

⁵⁵ Tester DJ, Ackerman MJ. Genetics of long QT syndrome. *Methodist Debaque Cardiovasc J.* 2014 Jan-Mar;10(1):29-33.

prolongation of the action potential duration occurs, which manifests on the 12-lead ECG as QT prolongation.⁵⁶

In 1995 and 1996, causal gene mutations of familial LQTS in 3 genes (KCNQ1, KCNH2 and SCN5A) were discovered. To date, mutations in 15 different genes have been reported in LQTS, but KCNQ1, KCNH2 and SCN5A remain numerically the major genes in LQTS and comprise more than 90% of genotype-positive cases of LQTS.

LQTS type	Gene	Protein	Current	Frequency (%)
Romano-Ward				
LQT1	KCNQ1	Kv7.1	↓ I _{Ks}	40–55
LQT2	KCNH2	Kv11.1	↓ I _{Kr}	30–45
LQT3	SCN5A	Nav1.5	↑ I _{Na}	5–10
LQT4	ANKB	Ankyrin	↓ Coordination of Ncx, Na/K ATPase	Rare
LQT5	KCNE1	MinK	↓ I _{Ks}	Rare
LQT6	KCNE2	MiRP1	↓ I _{Kr}	Rare
LQT7	KCNJ2	Kir2.1	↓ I _{K1}	Rare
LQT8	CACNA1C	Cav1.2	↑ I _{Ca}	Rare
LQT9	CAV3	Caveolin 3	↑ I _{Na}	Rare
LQT10	SCN4B	Sodium channel β4-subunit	↑ I _{Na}	Very rare
LQT11	AKAP9	Yotiao	↓ I _{Ks}	Very rare
LQT12	SNTA1	Syntrophin-α1	↑ I _{Na}	Very rare
LQT13	KCNJ5	Kir3.4	↓ I _{K,Ach}	Very rare
LQT14	CALM1	Calmodulin 1	Dysfunctional Ca ²⁺ signaling	Rare
LQT15	CALM2	Calmodulin 2		Rare
Jervell and Lange-Nielsen				
JLN1	KCNQ1	Kv7.1	↓ I _{Ks}	Rare
JLN2	KCNE1	MinK	↓ I _{Ks}	Rare

Figure 5. congenital LQTS genes and affected Ion Current

A causal mutation is found in approximately 75% of LQTS patients with a Schwartz score ≥ 4 , but the genetic background of the other 25% of patients remain elusive.

In approximately 85% of genotype-positive cases of LQTS, the patient carries a mutation inherited from one of the parents and in the remaining 15% a de novo mutation is pertinent. In genotyped LQTS patients, approximately 50% have no lifetime symptoms, and 10–50% of such patients show no apparent QT prolongation. Compound mutations

⁵⁶ Harmer SC, Mohal JS, Royal AA, McKenna WJ, Lambiase PD, Tinker A. Cellular mechanisms underlying the increased disease severity seen for patients with long QT syndrome caused by compound mutations in KCNQ1. *Biochem J.* 2014 Aug 15;462(1):133-42

(ie, ≥ 2 mutations) are found in 10% of genotype-positive patients⁴⁷ and the clinical manifestation of disease in such patients is often more severe.

As for minor LQTS subtypes with severe clinical phenotypes, mutations in KCNQ1 or KCNE1 present as homozygous or compound heterozygous mutations in Jervell and Lange-Nielsen syndrome. KCNQ1 mutations are much more prevalent (90%) than KCNE1 mutations (10%). Calmodulin de novo mutations were reported in infant cases of recurrent cardiac arrest by means of exome sequencing in the parents-child trio.

Since the beginning of research into the genetic background in LQTS, linkage analysis and the candidate gene approach have been used, assuming LQTS to be a monogenic model. Later, it became evident from research into large LQTS families that LQTS actually shows incomplete penetrance with variable expressivity. In other words, different clinical phenotypes (sudden death, syncope, asymptomatic) are observed in family members carrying the same familial mutation, which may be caused by a more complex genetic model involving multiple genetic and environmental factors affecting disease development.⁵⁷

Modifier genes are common genetic variants found in more than 1% of affected individuals, which have an influence on susceptibility to disease. They have less effect on disease compared with a causal gene mutation, but by summing up the effects of such modifier genes, the disease severity varies. In LQTS, variants in the coding regions of LQTS-related genes such as KCNE1 D85N, KCNH2 K897T, and SCN5A H558R are known to influence the QT interval. Single nucleotide polymorphisms (SNPs) in non-coding regions (intron, 3'UTR) of KCNQ1 are also reported as an important QT-interval modifier. Furthermore, genome-wide association studies have revealed SNPs in NOS1AP as a modulator of QT interval in LQTS patients, as well as in the general population. An effort to find any SNPs affecting QT interval of the Caucasian general population continues by larger international consortia. This field is expected to expand to further understand the complexity of genotype-phenotype relationship in LQTS. However, it remains currently at the research level, which requires careful interpretation by experts.

⁵⁷ Kolder IC, Tanck MW, Postema PG, et al. Analysis for Genetic Modifiers of Disease Severity in Patients with Long QT Syndrome Type 2. *Circ Cardiovasc Genet*. 2015 Mar 3.

Beta-blocker therapy is the first choice for LQTS. It dramatically decreases event rates, from 0.97 to 0.31 events per patient per year. Recurrent events were often seen in patients with a history of ACA or in patients who are non-compliant with β -blocker therapy. In LQT1 and LQT2, propranolol ($2\text{--}4\text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and nadolol ($1\text{--}1.5\text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) have been shown to be much more effective than metoprolol in suppressing recurrent cardiac events. Therefore, metoprolol should probably not be prescribed in symptomatic LQTS patients. Atenolol, not included in the aforementioned study, appears to be less effective, according to a study performed in (only) 28 genotyped patients with a median follow-up of 46 months.

LCSD is a surgical procedure to ablate the lower two-thirds of the left stellate ganglion together with the thoracic ganglia T2–T4 to denervate cardiac sympathetic innervation to the heart. It is currently used as an adjunctive therapy in symptomatic patients who are refractory to β -blocker therapy. LCSD has been shown to reduce cardiac events significantly in LQTS with a rather high long-term cardiac-event-free survival (46%/5 years, 91 59%/2 years). In patients with a history of syncope, post-LCSD QTc <500 ms predicts efficacy of LCSD.

However, those with persistent QTc prolongation ($\geq 500\text{ ms}$) have a high chance of SCD and need to be protected with an ICD. A known complication of this procedure is Horner's syndrome, but in most cases, it is only transiently observed after the surgery and the patient recovers afterwards.

Use of an ICD should be regarded as adjunctive therapy in LQTS. An ICD is recommended only for patients who have frequent syncopal episodes despite being on maximal doses of β -blocker (and eventually other additional pharmacological therapies) or at high risk of recurrent ACA/SCD, such as patients who have a history of ACA, symptomatic infant cases (<1 year of age) or those with J-LNS.

In LQTS genetics, next-generation sequencing enables us to detect SNPs in coding/non-coding regions of (LQTS-related) genes that modify the QT interval. Currently, analysis and interpretation of results by next-generation sequencing remains at the research level while advances in the understanding of genotype-phenotype relationships, including

LQTS causal genes as well as SNPs, are expected to guide us further to genetically-guided personalized treatment in the future.⁵⁸

⁵⁸Yuka Mizusawa, Minoru Horie, Arthur AM Wilde. *Genetic and Clinical Advances in Congenital Long QT Syndrome*. Circulation Journal Vol.78, December 2014.

4. DISEASES ASSOCIATED WITH POTASSIUM CHANNEL DYSFUNCTION

The potassium channels most relevant to heart diseases are the slowly activating delayed rectifier cardiac K^+ channel and the voltage-gated inwardly rectifying K^+ channel. The α -subunits of the cardiac isoforms of these two potassium channels are known as KvLQT1 and KCNH2, respectively. Potassium channels allow repolarization currents to counteract the preceding depolarization process.⁵⁹ Mutations in the genes that encode the K^+ channels may give rise to three types of disease: LQTS, short QT syndrome (SQTS), and Atrial Fibrillation.⁶⁰

Loss-of-function mutations of potassium channel genes (KCNQ1, KCNH2, KCNE1, KCNE2, KCNJ2, and KCNJ5) in LQTS reduce the repolarizing currents (IKr, IKs, and IKir) required to terminate the cardiac action potential, leading to a prolongation of the QT interval. Gain-of-function mutations in calcium channel (CACNA1C) and sodium channel genes (SCN5A and SCN4B) in LQTS cause delayed channel closing and inactivation, responsible for prolonged inward currents and depolarization with a resultant increased QT interval. By contrast, loss-of-function mutations in calcium channel genes (CACNA1C, CACNB2, and CACNA2D1) and gain-of-function mutations in potassium channel genes (KCNH2, KCNQ1, and KCNJ2) enhance repolarization, resulting in the abnormal shortening of the cardiac action potential in short QT syndrome. Loss-of-function mutations in sodium channel genes have been identified to cause Brugada syndrome, familia atrial fibrillation, sick sinus syndrome, familial heart block, and atrial standstill. It is noteworthy that both gain-of-function mutations (which decrease action potential duration) and loss-of-function mutations (which increase action potential duration) in potassium channel genes predispose to atrial fibrillation. This demonstrates a precise atrial electrophysiological balance in which minor disturbances in either direction can cause atrial fibrillation.⁶¹

⁵⁹ Ravens U, Cerbai E. *Role of potassium currents in cardiac arrhythmias*. *Europace* 2008;10:1133–1137.

⁶⁰ Jing L, Brownson K, Patwardhan A. Role of slow delayed rectifying potassium current in dynamics of repolarization and electrical memory in swine ventricles. *J Physiol Sci*. 2014 May;64(3):185-93.

⁶¹ Schmitt N, Grunnet M, Olesen SP. Cardiac potassium channel subtypes: new roles in repolarization and arrhythmia. *Physiol Rev*. 2014 Apr;94(2):609-53.

4.1 Atrial fibrillation

AF is the most common arrhythmia observed in clinical practice. Several loci have been mapped and most of them encode for subunits of potassium channels.

Identification of KCNQ1 as responsible for the disease provided the first link of the genetic for of the arrhythmia with an ion channelopathy.⁶²

A gain of function in I_{Ks} current when the mutated channel was expressed explained well the shortening of the action potential duration and effective refractory period, which are thought to be the culprits of the disease.⁶³

Defects in genes encoding for potassium currents were further confirmed responsible for AF with the identification of gain of function mutations in KCNE2, in KCNH2 (causing AF and short-QT-syndrome) and in Kir2.1.⁶⁴ A genetic defect has been described in KCNE3; however, the functional analysis did not demonstrate a different biophysical effect caused by the mutant genetic defect, indicating that it could be a rare polymorphism.⁶⁵ In summary, the biophysical findings therefore indicated a role of gain-of-function mutations in potassium channels in AF highlighting the pathophysiological role of shortened atrial action potentials.⁶⁶ When Olson et al. described a loss of function mutation in KCNA5, the gene that encodes Kv1.5, the debate became more stimulating because it initiated the hypothesis that a prolongation of the action potential can also be a basic mechanism for development of AF.⁶⁷

⁶² Zhao J, Liu T, Li G. Relationship between two arrhythmias: sinus node dysfunction and atrial fibrillation. *Arch Med Res.* 2014 May;45(4):351-5. doi: 10.1016/j.arcmed.2014.04.005. Epub 2014 May 11.

⁶³ Roberts JD, Gollob MH. A contemporary review on the genetic basis of atrial fibrillation. *Methodist Debaque Cardiovasc J.* 2014 Jan-Mar;10(1):18-24.

⁶⁴ Olesen MS, Nielsen MW, Haunsø S, Svendsen JH. Atrial fibrillation: the role of common and rare genetic variants. *Eur J Hum Genet.* 2014 Mar;22(3):297-306. doi: 10.1038/ejhg.2013.139. Epub 2013 Jul 10. Review.

⁶⁵ Tucker NR, Ellinor PT. Emerging directions in the genetics of atrial fibrillation. *Circ Res.* 2014 Apr 25;114(9):1469-82. doi: 10.1161/CIRCRESAHA.114.302225.

⁶⁶ Hong K, Xiong Q. Genetic basis of atrial fibrillation. *Curr Opin Cardiol.* 2014 May;29(3):220-6

⁶⁷ Munger TM, Wu LQ, Shen WK. Atrial fibrillation. *J Biomed Res.* 2014 Jan;28(1):1-17. doi: 10.7555/JBR.28.20130191. Epub 2013 Dec 28. Review.

5. DISEASES ASSOCIATED WITH CALCIUM CHANNEL DYSFUNCTION

The voltage-gated calcium channel subunit alpha, Cav1.2, encoded by *CACNA1C*, is a membrane protein with a similar topology to the subunit of the sodium channel: it consists of four homologous domains (DI to DIV), joined by cytosolic linkers. Each domain also contains six transmembrane helices. On the other hand, the Ryanodine receptor 2, encoded by *RyR2*, and the calsequestrin 2, encoded by the *RyR2* and *CASQ2*, respectively, locate at the sarcoplasmic reticulum.

Calcium ions are involved in phase 2 of cardiac action potential and also increase the output of calcium from the sarcoplasmic reticulum, which functions primarily as a storehouse of calcium in the skeletal muscle, to trigger cardiac contraction. The role of calcium channels in inherited arrhythmia syndromes has only recently been demonstrated. To date, a Ca²⁺ channel dysfunction can produce three different diseases—a combination of BrS with shorter than normal QT interval, Timothy syndrome (TS), and Polymorphic Ventricular Tachycardia.

5.1 Catecholaminergic polymorphic ventricular tachycardia (CPVT) is characterized by the development of bidirectional polymorphic ventricular tachycardia upon exposure to adrenergic stimulation in an otherwise normal heart. Experiencing emotional or physical stress can induce dizziness, syncope, and/or sudden cardiac death in patients with CPVT.⁶⁸ Manifestations occur in childhood or adolescence, with the average onset at age 7-9 years⁴⁹). CPVT can be inherited in an autosomal-dominant or recessive manner. The autosomal-dominant form of CPVT (CPVT type 1) is caused by gain-of-function mutations in RYR2, the gene that encodes the cardiac ryanodine receptor 2 (RYR2), a major component of RYR2 channels.⁶⁹ RYR2 channels mediate calcium release from the SR into the cytosol upon cell membrane depolarization. Defective closure of RYR2 channels results in intracellular calcium leakage from the SR, which leads to increased potential for delayed after-depolarizations and subsequent ventricular tachycardia⁵⁰).⁷⁰

⁶⁸ Priori SG, Napolitano C, Tiso N, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2001;103:196–200.

⁶⁹ Lahat H, Eldar M, Levy-Nissenbaum E, et al. Autosomal recessive catecholamine - or exercise-induced polymorphic ventricular tachycardia: clinical features and assignment of the disease gene to chromosome 1p13–21. *Circulation* 2001;103:2822–2827.

⁷⁰ June-Bum Kim, MD, PhD. *Channelopathies*. Korean J Pediatr 2014;57(1):1-18.

6. MOLECULAR GENETIC RESULTS

Were examined for a cardiac channel diseases, 9 cases of SUD (Sudden Unexplained Death) occurring in the Institute of legal medicine of Palermo, and 3 cases of clinical arrhythmias diseases occurring in the department of Clinical Cardiology in Palermo Hospital.

Of these, 12 (10 male and 2 female). Polymerase chain reaction, and DNA sequencing were used for a comprehensive mutational analysis of the 3 major channelopathy susceptibility genes (SCN5A, KCNQ1 and HCN4) and in one clinical case a targeted analysis of the arrhythmogenic right ventricular dysplasia/cardiomyopathy associated (ARVD/C)-, DSP-encoded cardiac desmosome junctions.

SUDDEN UNEXPLAINED DEATH						
	Case	Gender	Age	Molecular Autopsy	Dna source	Mutation analysis technique
1	54.09	M	35	HCN4	formalin-fixed paraffin embedded tissue (FF-PET)	PCR DNA sequencing
2	88.09	M	44	HCN4	formalin-fixed paraffin embedded tissue (FF-PET)	PCR DNA sequencing
3	67.11	F	19	HCN4	formalin-fixed paraffin embedded tissue (FF-PET)	PCR DNA sequencing
4	10.12	M	25	HCN4	formalin-fixed paraffin embedded tissue (FF-PET)	PCR DNA sequencing
5	11.12	M	25	HCN4	formalin-fixed paraffin embedded tissue (FF-PET)	PCR DNA sequencing
6	15.12	F	25	HCN4	formalin-fixed paraffin embedded tissue (FF-PET)	PCR DNA sequencing
7	S01	M	20	SCN5A KCNQ1 HCN4	Autopsy blood	PCR DNA sequencing
8	S02	M	25	SCN5A KCNQ1 HCN4	Autopsy blood	PCR DNA sequencing
9	S03	M	17	SCN5A	Autopsy blood	PCR DNA sequencing

ARRHYTHMOGENIC CARDIAC SYNDROME						
Case	Gender	Age	Arrhythmogenic Diseases	Genetic Screen	Dna source	method
C01.13	M	25	ECG alterations ICD (Implantable Cardioverter Defibrillator)	SCN5A KCNQ1 HCN4	Fresh Blood in EDTA	PCR DNA sequencing
C02.13	M	17	ECG alterations ICD (Implantable Cardioverter Defibrillator)	SCN5A KCNQ1 HCN4	Fresh Blood in EDTA	PCR DNA sequencing
C03.13	M	21	Signs of Right ventricular failure No ECG alteration	DSP	Fresh Blood in EDTA	PCR DNA sequencing

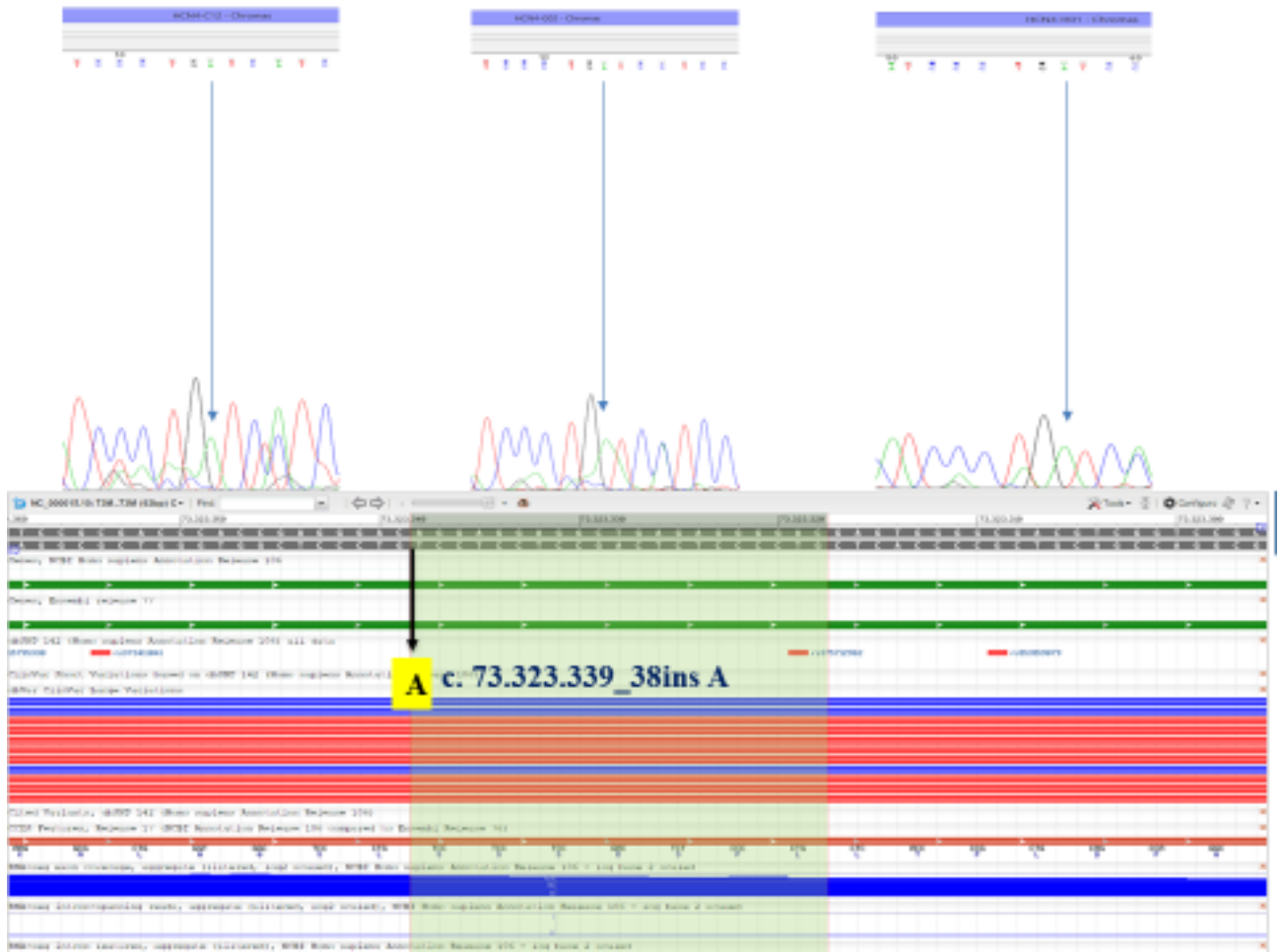
Genetic analysis of the HCN4 gene (exon 8) revealed the identical mutation (insertion) in 3 cases of autopsy-negative SUD; this mutation is unknown in Literature and probably is implicated in the susceptibility of action potential, generated by sino-atrial node, and cause of death may be due to lethal arrhythmias.

Gene: HCN4	Case	Chr Position	VARIANT ID	VARIANT TYPE	MOLECULAR CONSEQUENCE	CLINICAL CONSEQUENCE
Ex 8e	15.12 (C12)	Chr 15 73.323.339_38insA	Unknown	Insertion	susceptibility of SUD?	Sudden Death
Ex 8e	54.09 (G02)	Chr 15 73.323.339_38insA	Unknown	Insertion	susceptibility of SUD?	Sudden Death
Ex 8e	67.11 (H1)	Chr 15 73.323.339_38insA	Unknown	Insertion	susceptibility of SUD?	Sudden Death

HCN4 Ex8 Chr 15
Case 15.12 (C12)

HCN4 Ex8 Chr 15
Case 54.09 (G02)

HCN4 Ex8 Chr 15
Case 67.11 (H1)



In the exon 8, we found other single nucleotide variant with heterozygous polymorphism

Gene: HCN4	Case	Chr Position	Variant ID	Variant Type	Molecular consequence
Ex 8e	10.12 (B07)	Chr 15 73.323.336 A>C p. Ser=Ser	Unknown	Heterozygous polymorphism	Missense
Ex 8e	15.12 (C12)	Chr 15 73.323.336 A>C p. Ser=Ser	Unknown	Heterozygous polymorphism	Missense
Ex 8e	54.09 (G02)	Chr 15 73.323.292 G>A	rs 369485237	Single Nucleotide Variant	Missense
Ex 8e	67.11 (H1)	Chr 15 73.323.292 G>A	rs 369485237	Single Nucleotide Variant	Missense

Another interesting results after genetic analysis of the HCN4 gene (intron 7) revealed the identical mutation in 3 cases (2 autopsy-negative SUD and 1 clinic arrhythmias treated with implantable cardioverter defibrillator); this mutation is known in Literature and your position is most important because involved a intron sequence, between exon 7 and exon 8, and probably this intron variant region can play an important role in generation of two different spliced mRNA and/or the insertion of a stop codon. This mutation is probably involved in susceptibility of action potential, generated by seno-atrial node, and to produce a pathogenic and lethal arrhythmias. In the future the Splicing analysis are important for the classification of this polimorfisms as pathogenic variant.

Gene: HCN4	Case	Chr Position
Intron 7a	15.12	Chr 15 73.324.294G>A
Intron 7a	54.09	Chr 15 73.324.294G>A
Intron 7a	C02.13	Chr 15 73.324.294G>A

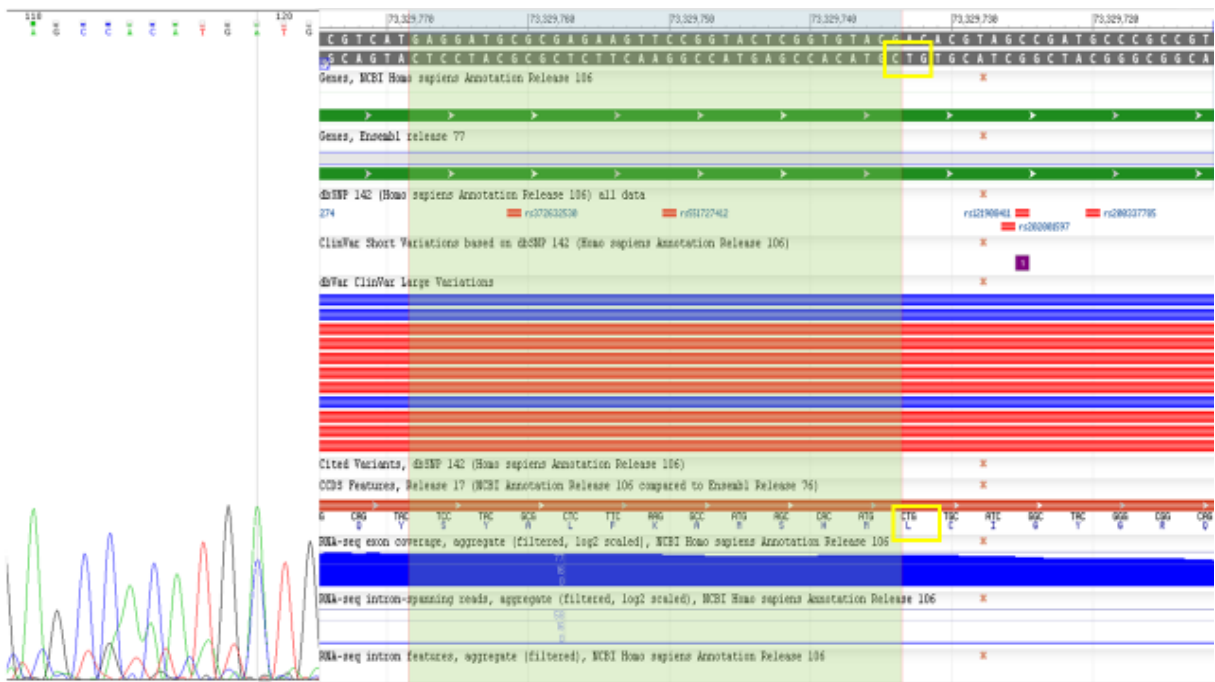
Case	Variant ID	Molecular consequence	Worst Clinical significance	Clinical Consequence
15.12 (C08)	rs481579 Single nucleotide variant	Intron variant	Unknown	Sudden Death
54.09 (F09)	rs481579 Single nucleotide variant	Intron variant	Unknown	Sudden Death
C02.13 (H5)	rs481579 Single nucleotide variant	Intron variant	Unknown	Pathogenic arrhythmias treated with ICD implantable

HCN4 Int. 7a Case 15.12 (C08)	HCN4 Int. 7a Case 54.09 (F09)	HCN4 Int. 7a Case C02.13 (H5)
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In HCN4-gene screen we found other heterozygous polymorphism unknown.

Gene:	Case	Chr Position	Variant ID	Variant Type	Worst Clinical Significance	Clinical Consequence
HCN4	11.12 (D09)	Chr 15 73.329.734A>C p.Met>Leu	Unknown	Heterozygous polymorphism	Unknown	Sudden Death



Gene: HCN4	Case	Chr Position	Variant ID	Variant Type	Worst Clinical Significance	Clinical Consequence
Ex 8a	88.09 (A09)	Chr 15 73.323.866C/A p.Glu/Lys	Unknown	Heterozygous polymorphism	Unknown	Sudden Death



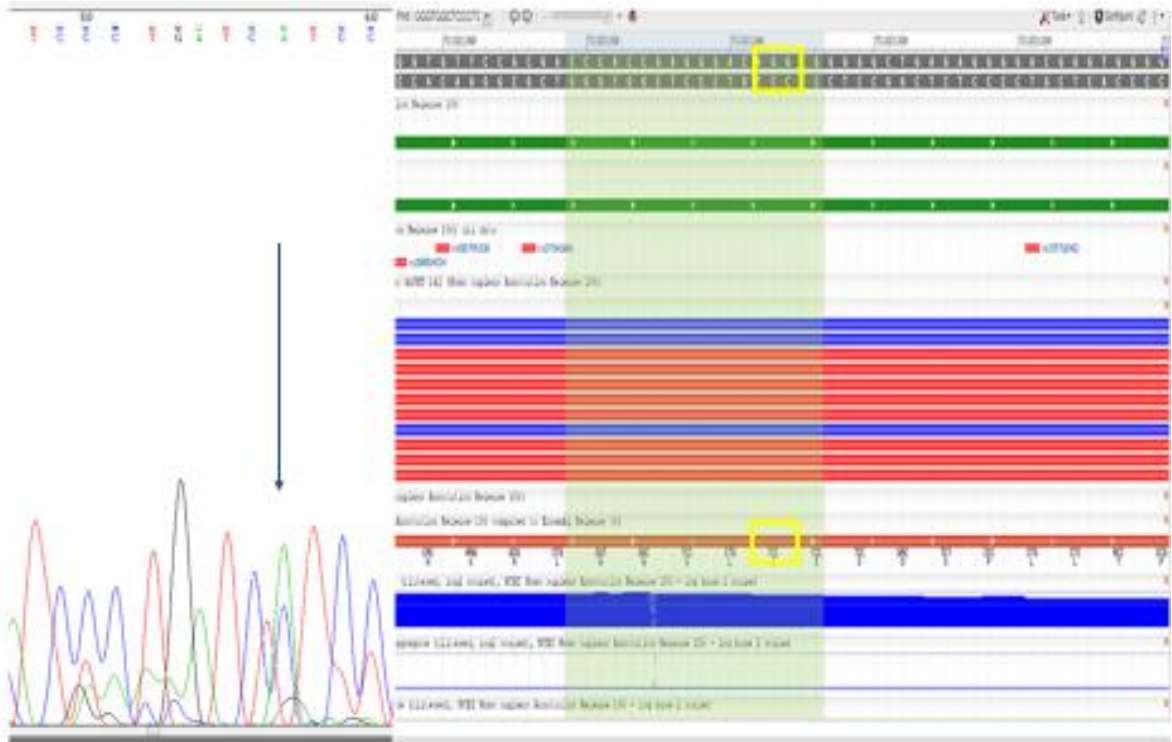
Gene: HCN4	Case	Chr Position	Variant ID	Variant Type	Worst Clinical Significance	Clinical Consequence
Ex 8b	C02.13 (B06)	Chr 15 73.323.699C/A p.His/Gln	Unknown	Heterozygous polymorphism	Unknown	pathogenic arrhythmias treated with ICD implantable



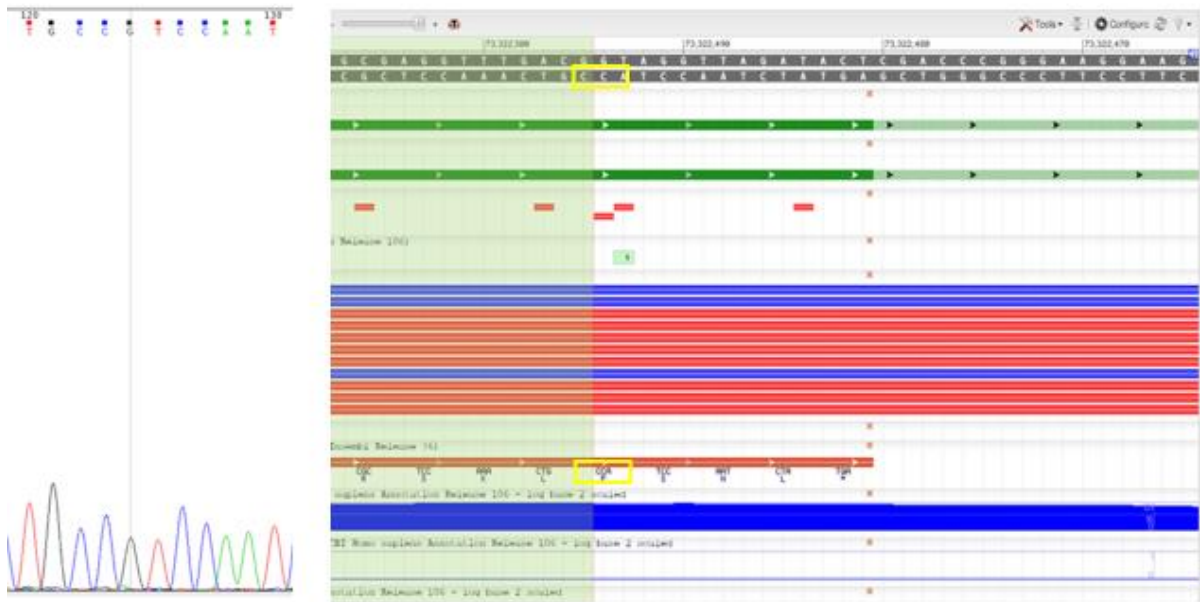
Gene: HCN4	Case	Chr Position	Variant ID	Variant Type	Worst Clinical Significance	Clinical Consequence
Ex 8e	10.12 (B07)	Chr 15 73.323.336A>C p.Ser=Ser	Unknown	Heterozygous polymorphism	Missense	Sudden Death



Gene: HCN4	Case	Chr Position	Variant ID	Variant Type	Worst Clinical Significance	Clinical Consequence
Ex 8e	15.12 (C12)	Chr 15 73.323.336A>C p. Tyr>Ser	Unknown	Heterozygous polymorphism	Unknown	Sudden Death



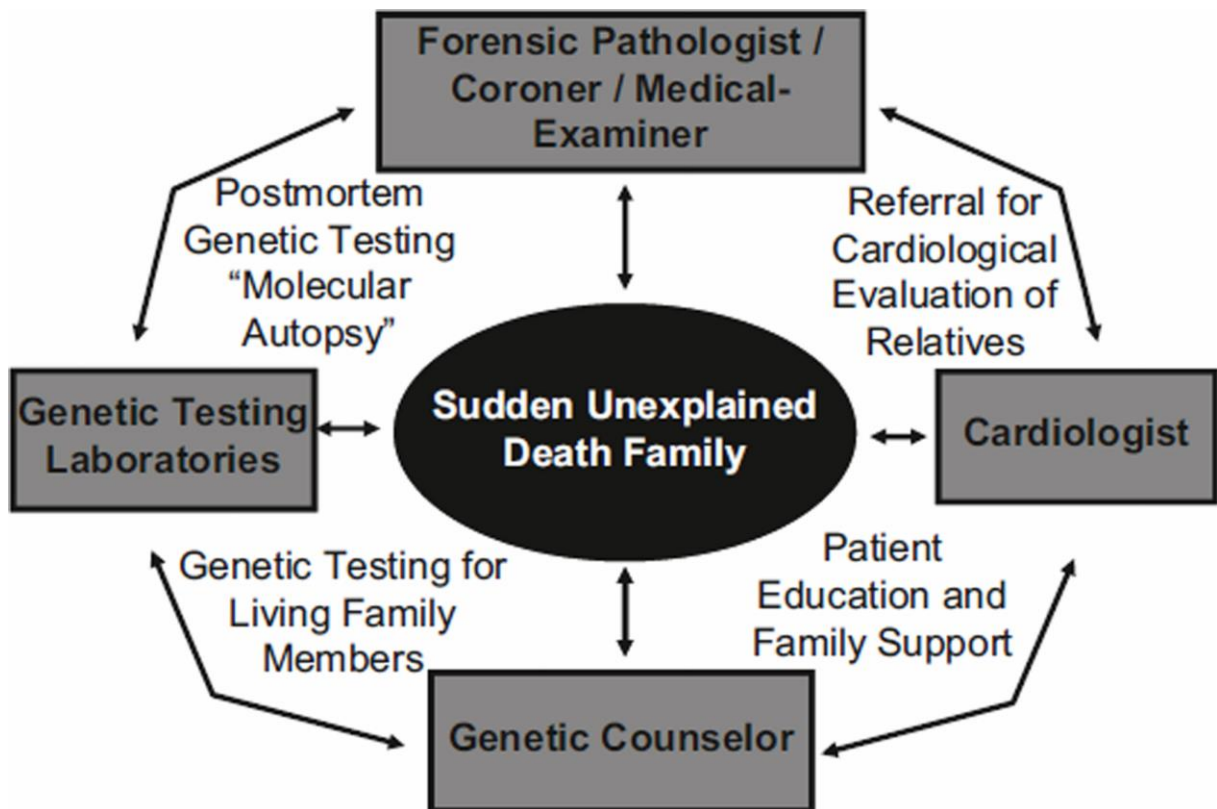
Gene: HCN4	Case	Chr Position	Variant ID	Variant Type	Worst Clinical Significance	Clinical Consequence
Ex 8I	54.09 (G05)	Chr 15 73.322.494G>A	rs529004	Heterozygous polymorphism	Synonymous variant	Sudden Death



7. CONCLUSION

More than one-fourth of autopsy-negative SUD cases may stem from channelopathic mutations. A cardiac channel molecular autopsy should be considered as a standard part of the evaluation of autopsy-negative SUD, especially among subjects with exercise-induced SUD, and those with a positive personal or family history of cardiac events.

The evaluation of SUD should be an interdisciplinary collaboration between an expert pathologist/medical examiner/coroner, cardiologist, and colleagues equipped with expertise in molecular genetic counseling.



4 Multidisciplinary approach to the evaluation of sudden unexplained death (SUD). *Herzschrittmacherther Elektrophysiol.* 2012 September ; 23(3).

All families in which a sudden unexplained death has occurred require targeted and standardized clinical testing in an attempt to identify relatives who may be at-risk of having the same inherited heart disease and therefore also predisposed to an increased risk of SCD.

Our results we propose future challenges consist in: the study of genotype/phenotype correlations, the phenotype interpretation of all these variants for its clinical translation; the routinely implementation of molecular autopsy in cases of autopsy-negative, and at the same time the genetic study of family members, in addition to clinical cases of arrhythmogenic cardiomyopathy, to prevent other cases of SUD.

Next Generation Sequencing technology can be used efficiently to analyse the rest of the genes associated with the arrhythmogenic disease.

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