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REVIEW

Basic science for the clinician

Genetics of sudden death: focus on inherited channelopathies

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Since the discovery of the genetic bases of the long QT syndrome, several new genetically mediated arrhythmias have been described, defining a new group of syndromes, called inherited arrhythmogenic diseases. This allowed clarifying the substrate of several cases of juvenile sudden death, previously defined as 'idiopathic ventricular fibrillation'. Studies derived from this field also contributed to advance the field of electrophysiology, elucidating some of the mechanisms that regulate the cardiac electrical properties of the heart. Recently, new genes and new proteins have been called into play, expanding the knowledge on the complexity of the regulatory processes modulating the cardiac action potential. Moreover, the collaboration between clinicians and basic scientists opened new approaches in the management of patients affected by genetic arrhythmias. This body of knowledge has then moved into the realization that genetic variations may also influence the predisposition to acquired cardiac diseases. The new exciting challenges that investigators are now facing are connected to the possibility of expanding the field towards the use of these information to shape a newer vision in the management and cure of patients.

Keywords Sudden cardiac death • Inherited arrhythmias • Channelopathies • Genetics • GWAS

Introduction

Sudden cardiac death (SCD) is the most common cause of death in the Western countries and, in the USA, it accounts for 250 000–400 000 cases annually.[1] Coronary artery disease and heart failure are the prevalent underlying substrates. However, in the last decade, the genetic bases of a sizable proportion of ventricular fibrillation episodes occurring in young individuals with normal heart and no coronary artery disease have been discovered, leading to the definition of a new group of syndromes, the inherited arrhythmogenic diseases (IADs) (*Table 1*).[2] This field has rapidly expanded, with the discovery of an increasing number of new genes associated with cardiac arrhythmias. Molecular biology and basic electrophysiology studies contributed to the development of a new conceptual approach for the management of patients that includes genotype-based risk stratification[3] and mutation-specific therapies.[4] Stemming from the achievements in the field of IADs, recent studies have demonstrated how the genetic background namely the presence/absence of common genetic variations called polymorphisms modulates the risk of SCD also in acquired cardiac diseases.[5–8]

In this review, we will focus on the newest advancements in the field of IADs and on the role that genetics may play in modulating the risk of SCD also in acquired conditions.

Cardiac channelopathies

Mutations on the genes that encode for the cardiac ionic channels can disrupt the fine balance of ionic currents that shape the action potential and cause life-threatening arrhythmias in the absence of structural heart defects. The diseases caused by genetic abnormalities that disrupt the electrical component of cardiac function have been named 'channelopathies'. In the past two decades, a growing number of channelopathies have been described, attributing several cases of unexplained arrhythmias in young individuals to distinct heritable conditions such as the long QT syndrome (LQTS) the short QT syndrome (SQTS), the Brugada syndrome (BrS) and catecholaminergic polymorphic ventricular tachycardia (CPVT)[2,9] (*Figure 1*). In the next section, we will recapitulate the newest discoveries about the genetic bases of these diseases and their clinical relevance.

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Table 1 Genetic loci and genes associated with inherited arrhythmogenic diseases

Gene symbol	Locus name	Chromosomal locus	Inheritance	Protein	Functional effect	Phenotype
KCNQ1	LQT1	11p15.5	AD	I_{Ks} potassium channel α-subunit (KvLQT1)	Loss of function	Long QT
	JLN1		AR		Loss of function	Long QT, deafness
	SQT2		AD		Gain of function	Short QT
KCNH2	LQT2	7q35–q36	AD	I_{Kr} potassium channel α-subunit (HERG)	Loss of function	Long QT
	SQT1		AD		Gain of function	Short QT
SCN5A	LQT3	3p21	AD	Cardiac sodium channel α-subunit (Nav 1.5)	Gain of function	Long QT
	BrS1		AD		Loss of function	Brugada syndrome
KCNJ2	AND/ LQT7	17q23.1–q24.2	AD	I_{K1} potassium channel (Kir2.1)	Loss of function	Long QT, potassium-sensitive periodic paralysis, dysmorphic features
	SQT3		AD		Gain of function	Short QT
KCNE1	LQT5	21q22.1–q22.2	AD	I_{Ks} potassium channel β-subunit (MinK)	Loss of function	Long QT
	JLN2		AR		Loss of function	Long QT, deafness
KCNE2	LQT6	21q22.1–q22.2	AD	I_{Kr} potassium channel β-subunit (MiRP)	Loss of function	Long QT
ANK2	LQT4	4q25–q27	AD	Ankyrin B, anchoring protein	Loss of function	Long QT
CACNA1c	TS/LQT8	12p13.3	AD/			
mosaicism	Calcium channel	α-subunit	Gain of function	Timothy syndrome: long QT, syndactyly, septal defect, patent foramen ovale		
	BrS3		AD		Loss of function	Brugada syndrome with short QT
CACNB2b	BrS4	10p12	AD	Calcium channel α-subunit	Loss of function	Brugada syndrome with short QT
Cav3	LQT9	3p24	AD	Caveolin	Gain of function of Na current	Long QT
SCN4b	LQT10	11q23.3	AD	Sodium channel β-4 subunit	Gain of function of Na current	Long QT
AKAP9 (Yotiao)	LQT11	7q21–q22	AD	A-kinase-anchoring protein	Reduced I_{Ks} current due to loss of cAMP sensitivity	Long QT
SNTA1	LQT12	20q11.2	AD	α1-syntrophin	Increased sodium current due to S-nitrosylation of SCN5A	Long QT
KCNJ5	LQT13	11q23.3–24.3.	AD	Kir3.4 subunit of I_{KAch} channel	Loss of function	Long QT
GPD1-L	BrS2	3p22.3	AD	Glycerol-3-phosphate dehydrogenase 1 like	Reduced sodium current	Brugada syndrome
SCN1b	BrS5	19q13.1	AD	Sodium channel β-1 subunit	Loss of function	Brugada syndrome
KCNE3	Brs6	11q13–q14	AD	I_{Ks} and I_{to} channels β-subunit	Gain of function	Brugada syndrome
SCN3B	BrS7	11q23.3	AD	Sodium channel β-3 subunit	Loss of function	Brugada syndrome
RyR2	CPVT1	1q42–43	AD	Cardiac Ryanodine receptor	Diastolic calcium release	CPVT
CASQ2	CPVT2	1p13.3–p11	AR	Cardiac calsequestrin	Diastolic calcium release	CPVT

Genetic loci associated with inherited channelopathies. AD, autosomal dominant; AR, autosomal recessive; AND/LQT7, Andersen syndrome/LQT7; TS/LQT8, Timothy syndrome/LQT8.

The long QT syndrome: clinical and genetic features

Long QT syndrome is characterized by an excessive prolongation of cardiac repolarization that leads to ventricular arrhythmias. Symptomatic individuals often manifest with stress-induced syncopal episodes or cardiac arrest. On the surface electrocardiogram (ECG), it is possible to appreciate the QTc interval duration exceeding the normal values (i.e. QT intervals >440 ms in men

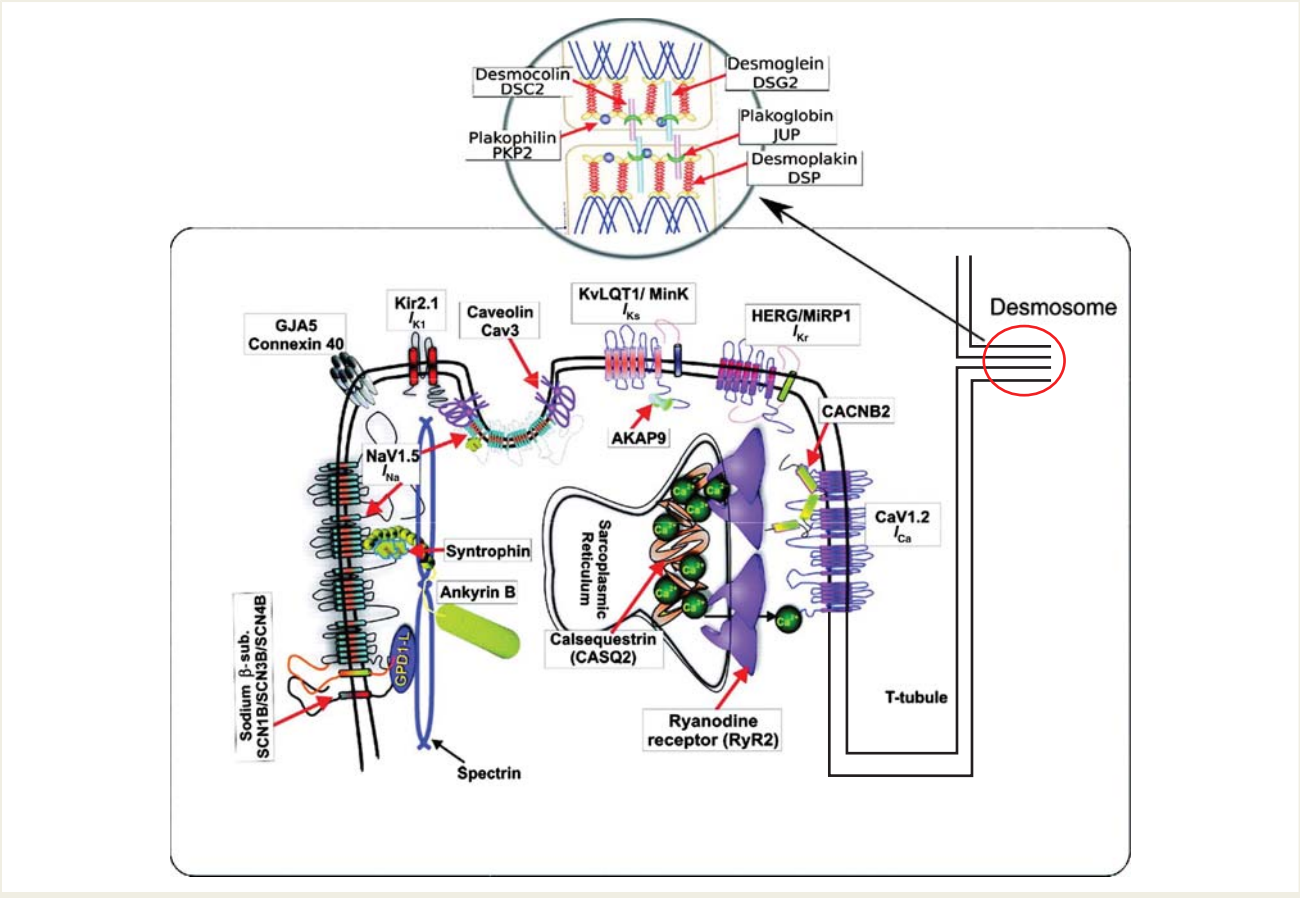


Figure 1 Proteins involved in inherited arrhythmogenic diseases (from Priori[9] with permission).

and >460 ms in women). The spectrum of the ECG abnormalities observed in LQTS patients is broad, encompassing not only QT prolongation but also abnormal T-wave morphologies. Interestingly, the disease may show incomplete penetrance, and therefore, genetically affected individuals may have a normal QTc duration.[10]

The first three LQTS genes identified by the group of Keating were: *KCNQ1*, encoding for the protein that conducts the potassium current I_{Ks}; *KCNH2*, encoding for the channel for the repolarizing potassium current I_{Kr}; and *SCN5A*, encoding for the α-subunit of the sodium channel that conducts the depolarizing sodium current I_{Na}. Mutations affecting I_{Ks} or I_{Kr} cause a decrease in the current, prolonging repolarization, and thus the QT interval duration on the ECG. Homozygous mutations in the *KCNQ1* gene cause a malignant form of LQTS characterized by QT prolongation, life-threatening arrhythmias, and neurosensorial deafness, called the Jervell and Lange-Nielsen syndrome. Mutations in the *SCN5A* gene cause a gain of function of the sodium channel and, by increasing I_{Na}, prolong cardiac repolarization.[10] The first three variants of LQTS corresponding to mutations in *KCNQ1*, *KCNH2*, and *SCN5A* were called LQT1, LQT2, and LQT3.

In the last 15 years, mutations in other genes encoding for subunits of a variety of ion channels as well as mutations in genes encoding ion channels' regulatory proteins were discovered in LQTS patients. At present, 13 different variants of LQTS have been published (*Figure 1* and *Table 1*). Although most of the genotyped patients belong to the first three variants (LQT1, LQT2, and LQT3), the description of the rare forms pointed the attention of researchers beyond genes encoding subunits of ion channels, leading to the identification of LQTS genetic variants caused by mutations in genes encoding a variety of regulatory proteins that becoming dysfunctional prolong cardiac repolarization.[9,11] In the following paragraphs, we will provide an outline of these rare variants of LQTS.

Loss-of-function mutations on the gene encoding for ankyrin B distinguish the LQT4 form.[12] Ankyrins exert a role in membrane protein targeting; specifically, ankyrin B intervenes in the proper localization of Na/Ca exchanger, Na/K ATPase, and the IP3 receptor. The discovery of LQT4 pressed scientists to investigate the effects of its loss in the heart, revealing that it can cause an increase in intracellular Na^+ facilitating afterdepolarizations and triggered arrhythmias under adrenergic stimulation.[11]

LQT5 and LQT6 are caused by mutations on genes encoding β-subunits of channels that conduct I_{Ks} and I_{Kr} currents.[10] LQT7 and LQT8 are distinctive forms of LQTS: their clinical manifestations extend beyond the cardiac phenotype. LQT7[13] (Andersen–Tawil syndrome) presents QT prolongation and large U-waves accompanied by facial dysmorphic features and hypokaliaemic periodic paralysis; it is linked to mutations on the *KCNJ2*

gene encoding for the I_{K1} potassium channel. Finally, LQT8 (Timothy syndrome)[14] is caused by gain-of-function mutations on *CACNA1c* gene that cause an increase in the calcium current. Interestingly, more than 90% of the patients affected by LQT8 carry the same mutations G406R: this is at variance with the other genetic variant of LQTS that is characterized by an extreme heterogeneity of different mutations. Timothy syndrome patients show a marked prolongation of QT interval associated with a complex phenotype that includes syndactyly, atrioventricular block, congenital heart defects, autism, developmental disorders, and reduced immune response.

Mutations in the *CAV3* gene encoding for caveolin 3 were found in four patients with LQTS, defining the variant LQT9.[15] Caveolins are proteins involved in cellular signalling and endocytosis; in the heart, caveolin 3 interacts with the sodium channel protein, and although the effect of the LQT9 mutations has not been unravelled yet, it has been suggested that they may increase the I_{Na} current.[11]

A mutation in the gene *SCN4B* that encodes for the β-4-subunit of the cardiac sodium channel was described in a single LQTS family and defined as LQT10.[16]

LQT11 is linked to mutations on the *AKAP9* gene[17] that encodes the Yotiao protein. AKAPs are scaffolding proteins that control the protein kinase A pathway. In the heart, the Yotiao has been shown to modulate the response to β-adrenergic stimulation of the potassium current I_{Ks}.[18]

The LQT12 variant of LQTS is associated with defects in the gene *SNTA1* encoding for the cardiac isoform called α1-syntrophin.[19,20] Syntrophins are a family of proteins that link the extracellular matrix to the intracellular cytoskeleton through a macromolecular complex. Until now, few *SNTA1* mutations have been reported associated with the LQTS phenotype.[19,20] Functional characterization showed that mutant *SNTA1* releases inhibition of associated neuronal nitric oxide synthase by the plasma membrane Ca-ATPase (called PMCA4b), leading to an increase in peak and late sodium current via S-nitrosylation of the cardiac sodium channel.

The most recently described variant of LQTS, LQT13, has been identified in a Chinese family carrier of a mutation in the *KNCJ5* gene, encoding for the Kir3.4 subunit of the I_{KAch} potassium current.[21] Functional studies suggested that the mutant protein interferes with the formation of functional I_{KAch} channels.[21]

Influence of genotype on the clinical management of long QT syndrome

Approximately 90% of genotyped LQTS subjects belong to the LQT1, LQT2, or LQT3. The creation of large databases collecting clinical and genetic information on a large number of affected individuals allowed performing genotype/phenotype studies that demonstrated that the three most prevalent genetic variants LQTS have different features, risk indicators, and response to therapy (*Figure 2*). Thanks to these discoveries, genotype has become part of the risk stratification scheme for LQTS patients.

The earliest genotype/phenotype correlations in LQTS pointed out that LQT1 patients experience most of their symptoms during sport activities, especially swimming, whereas LQT3 individuals are at a higher risk of arrhythmias during sleep or at rest[22] and LQT2 experience events in association with loud noise.[22] These findings led to more thorough studies on the differences among LQT1, LQT2, and LQT3. It became evident that genotype, together with gender and QT interval duration, is one of the determinants of the risk of SCD and of the response to therapy.[3,4] As demonstrated in *Figure 2*, LQT2 females and LQT3 men with QT interval >500 ms fall into the higher risk category independently from other factors.[3] Similarly, although LQT1 patients tend to have an optimal response to β-blockers, LQT2 and LQT3 patients experience recurrences despite full-dose β-blockers.[4] On the basis of these data and on the opinion of experts, the ACC AHA ESC 2006 Guidelines for the prevention of SCD[1] state that the presence of a QTc interval of >500 ms in LQT2 and LQT3 opens to the possibility of implanting a prophylactic implantable cardioverter defibrillator (ICD; *Figure 2*). Interestingly, based on the evidence that LQT3 is caused by an excess of inward sodium current, it was suggested that mexiletine may exert an antiarrhythmic effect in LQT3 patients by reducing the QTc interval duration through its sodium channel blocking effect.[23] Recently, *in vitro* characterization of mutant sodium channels in heterologous cells showed that the clinical response to mexiletine is influenced by the biophysical properties of the specific mutation. Interestingly, clinical evidence has supported the role of the individual mutations in determining the response to the drug showing that in selected patients, mexiletine could be either ineffective[24] (*Figure 3*) or even paradoxically harmful.[25]

The Brugada syndrome

The BrS is a channelopathy presenting with a peculiar ECG pattern characterized by ST-segment elevation of the right precordial leads with or without right bundle branch block in the absence of structural heart disease.[26,27] In order to be considered diagnostic for the syndrome, ST-segment elevation in V1, V2, and V3 needs to have a pattern called 'Type 1' and characterized by a coved morphology (*Figure 4B*). This diagnostic ECG pattern maybe intermittent and not always detectable at baseline based on a single ECG recording; quite often the presenting ECG suggests a possible BrS showing saddle-back type ST-segment elevation (*Figure 4A*). A pharmacological challenge with sodium channel blockers (procainamide, flecainide, or ajmaline) may unmask a concealed or non-diagnostic pattern into a coved Type 1 diagnostic pattern[26,27] (*Figure 4*).

The most common clinical manifestations of BrS are syncope or SCD caused by ventricular tachyarrhythmias mostly occurring during sleep or at rest; other arrhythmic triggers could be fever or large meals. Supraventricular arrhythmias such as atrial fibrillation are also present in ~15–20% of the patients with a BrS diagnosis.[28] Interestingly, atrioventricular block and intraventricular conduction delays (right bundle branch block and left anterior hemi-block) are also part of the phenotype of BrS.[29]

The disease is transmitted as an autosomal dominant trait, but the majority of patients with clinical diagnosis are men: it is still unknown how gender modulates the manifestation of the disease.[26,27,30]

The electrophysiological background of arrhythmias in BrS is not fully understood. It has been proposed that the mutations lead to an increase in the transmural dispersion of repolarization across the ventricular wall, thus creating a predisposition to reentry.

However, why the consequences of the mutations do not affect the entire heart, but they rather predominantly manifest in the right side of the heart, is presently poorly understood. Furthermore, the fact that the ECG Type 1 pattern is intermittently present is also unclear. It is likely that the presence of a mutation that reduces either inward sodium current or cardiac inward current (see below) is required but not sufficient to produce the electrical 'signature' of the disease. In this respect, the presence of structural abnormalities may play an important role.[31,32]

As of today, the genetic component of the syndrome is attributed to mutations on seven different genes (*Table 1*); however, genetic heterogeneity of BrS is likely to be even bigger as mutation screening on the known genes allows identifying a mutation in ~25–30% of clinically affected patients. As a consequence, genetic screening helps confirming the clinical diagnosis and allows identifying silent gene carriers but, at variance with what happens in LQTS, so far there is no evidence that results of genetic testing influence clinical management or risk stratification in BrS.[33]

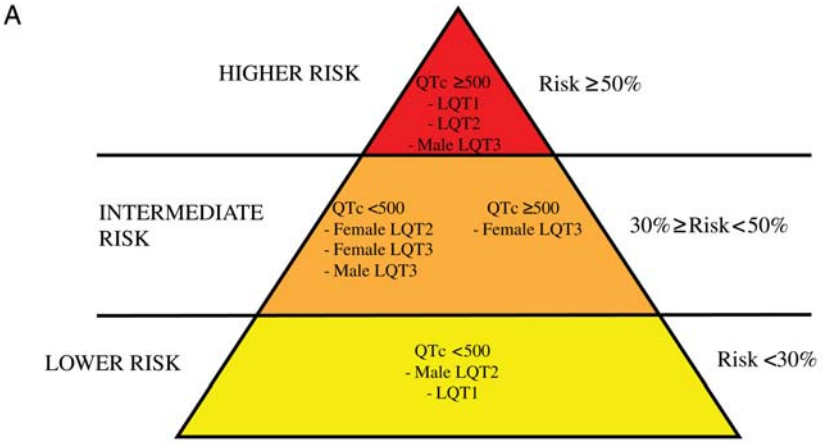
The first gene linked to BrS was discovered in 1998 as the *SCN5A* gene encoding for the cardiac sodium channel; this is the same gene linked to LQT3. As of today, most genotyped patients carry a mutation on this gene.[34]

SCN5A mutations associated with the BrS differ from *SCN5A* mutations identified in LQT3 for their electrophysiological consequences. In both diseases, mutations can occur throughout the open-reading frame of the gene, but what determines the clinical phenotype is whether the mutation increases (LQT3) or decreases (BrS) the inward sodium current. *SCN5A* mutations may cause loss of inward sodium current through different mechanisms. Some studies[35,36] have suggested that increased temperature may worsen the kinetics of the inward sodium current and potentiate the effect of certain mutations, thus providing a potential explanation on the proarrhythmic role of fever in the disease.

The sodium current is definitely an important player in the pathogenesis of BrS as demonstrated by the fact that three additional genes implicated in the disease (*GPD1-L*, *SCN1B*, and *SCN3B*) influence I_{Na}.[37–39] The *SCN1B* and *SCN3B* genes encode, respectively, for the β1 and β3 subunits of the sodium channel; functional characterization of the few mutations so far identified in these genes showed that they determine a reduction in the depolarizing current. Mutations on the *GPD1-L* gene, encoding for the glycerol-3-phosphate-dehydrogenase 1-like protein, dramatically decrease I_{Na} by impairing the membrane targeting of the α-subunit of the sodium channel.[37]

Recently, a BrS family was reported to carry a mutation in the *KCNE3* gene, encoding a β-subunit that co-assembles to form the Kv4.3 channel that conducts the transient outward current (I_{to}).[40] The *KCNE3* β-subunit decreases the I_{to} current conducted by Kv4.3. The *KCNE3* mutation identified in the BrS family reduces the inhibitory effect of *KCNE3* on the Kv4.3 channel, thus resulting in an increase in the I_{to} current. According to the hypothesis that the Brugada phenotype occurs when there is an imbalance between outward and inward currents at the end of Phase 1 of

A



B

Predictors of cardiac events	Relative Risk	*P*-value
1st cardiac event without therapy <7 years old	4.34 (2.35–8.03)	<0.001
QTc >500 ms (in therapy)	2.01 (1.16–3.51)	0.01
Genetic Locus:		
LQT2 vs. LQT1	2.81(1.50–5.27)	0.001
LQT3 vs. LQT1	4 (2.45–8.03)	<0.001

Figure 2 (*A*) Risk stratification in long QT syndrome, according to the QTc interval duration, genotype, and gender. The risk refers to the probability of a first cardiac event (including syncope) before age 40 and before therapy. (*B*) Risk indicators of failure of β-blocker therapy according to the genotype in long QT syndrome (from Priori *et al.*[3,4] with permission).

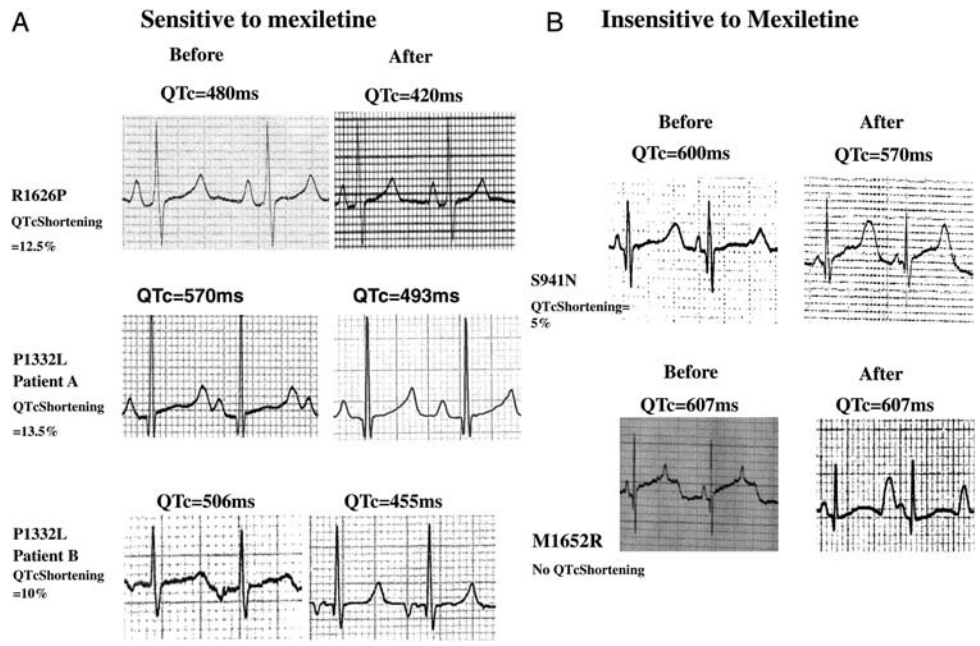


Figure 3 Response to mexiletine therapy in patients affected by LQT3, but carriers of different mutations on the gene *SCN5A* (from Ruan *et al.*[24] with permission). (*A*) LQT3 mutations sensitive to mexiletine and corresponding QTc shortening. (*B*) LQT3 mutations insensitive to mexiletine, in which QTc shortening did not occur.

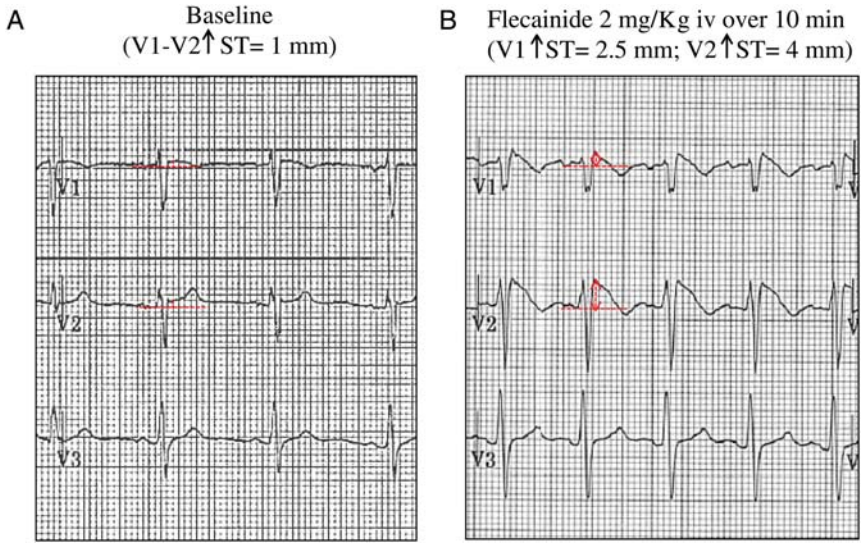


Figure 4 Results of flecainide test in a patient with concealed Brugada syndrome. (*A*) Baseline electrocardiogram. (*B*) Coved-type diagnostic pattern induced by flecainide challenge.

the epicardial ventricular action potential, an increase in I_{to} is expected to trigger the ECG phenotype of BrS.[30] The last two genes associated with the BrS phenotype are *CACNA1c* and *CACNB2*, encoding for the α- and β-subunits of the cardiac calcium channel. Loss-of-function mutations in these genes have been related to the disease.[41] As previously discussed, gain-of-function mutations in *CACNA1c* are responsible for the Timothy syndrome (LQT8).[14] The first BrS patients carriers of

mutations in the *CACNA1c* and *CACNB2* genes described in the literature presented with a distinctive phenotype combining short QT interval and type I BrS ECG pattern: this observation raised the hypothesis that mutations in the calcium channel could lead to a 'mixed phenotype' combining the clinical features of SQTS and those of BrS. A recent analysis showed that around 10% of 152 BrS individuals without short QT interval had mutations on either of these genes.[42]

So far, no medical therapy resulted effective in preventing arrhythmias and sudden death in BrS and the implant of an ICD is the only available treatment.[33] Isoproterenol infusion is useful in acutely controlling arrhythmic storms.[43] The only drug so far that has been used with some success is quinidine;[43,44] on the basis of available data so far, it may be considered as an adjunctive therapy in cases of recurrent arrhythmias and frequent ICD discharges. It is also important to consider that long-term quinidine treatment is poorly tolerated, due to the high incidence of sever gastrointestinal side effects. Clinical trials are ongoing to test the effect of quinidine in BrS (http://clinicaltrials.gov: NCT00789165 and NCT00927732).

In this scenario, risk stratification to select those high-risk patients who may really benefit from an ICD in primary prevention becomes crucial. General agreement exists that a history of syncope in the presence of spontaneous Type 1 ECG pattern identifies subjects at a higher risk of SCD.[33] When Type 1 ECG is elicited through pharmacological challenge but is never spontaneously present, the arrhythmic risk is lower.[33]

Unfortunately, a high percentage of the patients with a BrS diagnosis fall into the category at intermediate risk, because they are asymptomatic and may present a Type 1 spontaneous pattern. Therefore, clinicians and scientists are facing the challenge of identifying new markers to further stratify the arrhythmic risk in this group, thus selecting who should receive an ICD. The value of programmed electrical stimulation is highly debated and no conclusive and prospective data are yet available;[33,45,46] a family history for sudden death and/or the presence of a genetic mutation does not influence the arrhythmic risk. Recent data are suggesting that the presence of QRS fragmentation (recorded with the non-standard ECG filter setting of 0–150 Hz) may correlate with worse prognosis, but data in larger groups are needed to confirm this observation.[47]

The short QT syndrome

In 2000, Gussak *et al.*[48] identified a new IAD characterized by shorter than the normal QT interval (<350 ms) (*Figure 5*), ventricular and atrial arrhythmias, and SCD. Recognizing that only a limited number of SQTS patients have been reported, it seems that the occurrence of SCD as first manifestation is not infrequent. At present, there is no pharmacological therapy of proven efficacy to prevent life-threatening arrhythmias in the disease so, just like in the BrS, the implant of an ICD is the only alternative for high-risk patients and for those who survived a cardiac arrest.

An appealing hypothesis that was successfully explored in the attempt to unravel SQTS genes was that of testing whether LQTS genes that when affected by loss-of-function mutations prolong repolarization could be implicated in the pathogenesis of SQTS. Accordingly, mutations in the *KCNH2* (I_{Kr} current), *KCNQ1* (I_{Ks} current), and *KCNJ2* (I_{K1} current) genes were found in individuals diagnosed with SQTS (*Table 1*).[49–51] When expressed in heterologous systems, SQTS mutations showed an increase in the potassium currents involved, thus resulting in an abnormally short QT interval.

Therefore, LQTS and SQTS are allelic diseases, in analogy with what described for BrS and LQTS. The discovery of SQTS raised awareness on the importance of the delicate balance that regulates cardiac action potential and on how variation in the repolarization phase in both ends (prolonged or abbreviated) correlates with higher arrhythmogenic risk.

Catecholaminergic polymorphic ventricular tachycardia: from membrane to intracellular channels

Among inherited channelopathies, CPVT is one of the most lethal, with a natural history that shows up to 30% of SCD before age 40 in the absence of antiadrenergic therapy.[52,53] The disease manifests with adrenergically mediated life-threatening arrhythmias that cause syncope and/or cardiac arrest with onset during the paediatric age. The surface ECG is unremarkable; therefore, the diagnosis is mostly based on symptoms and on the detection of stress-induced arrhythmias during exercise stress test or Holter recording. Catecholaminergic polymorphic ventricular tachycardia patients also present supraventricular arrhythmias, mainly bursts of supraventricular tachycardia or of atrial fibrillation that overlap with ventricular extrasystoles and ventricular tachycardia (VT). During exercise stress test, very often patients experience progressive increase in the complexity of the arrhythmia until VT develops. Bidirectional VT is almost diagnostic for the disease: it is characterized by beat-to-beat 180° rotation of the QRS complexes (*Figure 6*). Some patients do not present bidirectional VT and develop during exercise polymorphic VT.[52,53] Interestingly, bidirectional VT is the typical arrhythmias that occurs during digitalis intoxication when the drug inhibits the Na^+/K^+ ATPase pump leading to an increase in intracellular sodium that in turn creates an intracellular calcium overload and triggers arrhythmogenic afterdepolarizations.[54] It is therefore reasonable to hypothesize that bidirectional VT in CPVT is elicited by intracellular calcium overload. Investigations to disclose the molecular basis of CPVT led to the identification in affected individuals of mutations on two genes encoding for proteins of the sarcoplasmic reticulum (SR): the ryanodine receptor (*RyR2*) and cardiac calsequestrin (*CASQ2*) that are associated, respectively, to the autosomal dominant and recessive forms of CPVT (*Table 1*).[55,56] This discovery demonstrated that not only proteins that form voltage-dependent channels localized on the membrane of cardiac myocytes are the substrates of inherited channelopathies, but also SR proteins may cause genetic arrhythmias by altering calcium homeostasis.

The discovery of the genetic bases of CPVT accelerated the understanding of the molecular basis of triggered arrhythmias. It has been demonstrated that mutations on *RyR2* and *CASQ2* genes result in an increase in calcium release from the SR and they promote the onset of triggered arrhythmias in isolated cells. Engineered mouse models have been instrumental for the understanding of CPVT, because they phenocopy with impressive

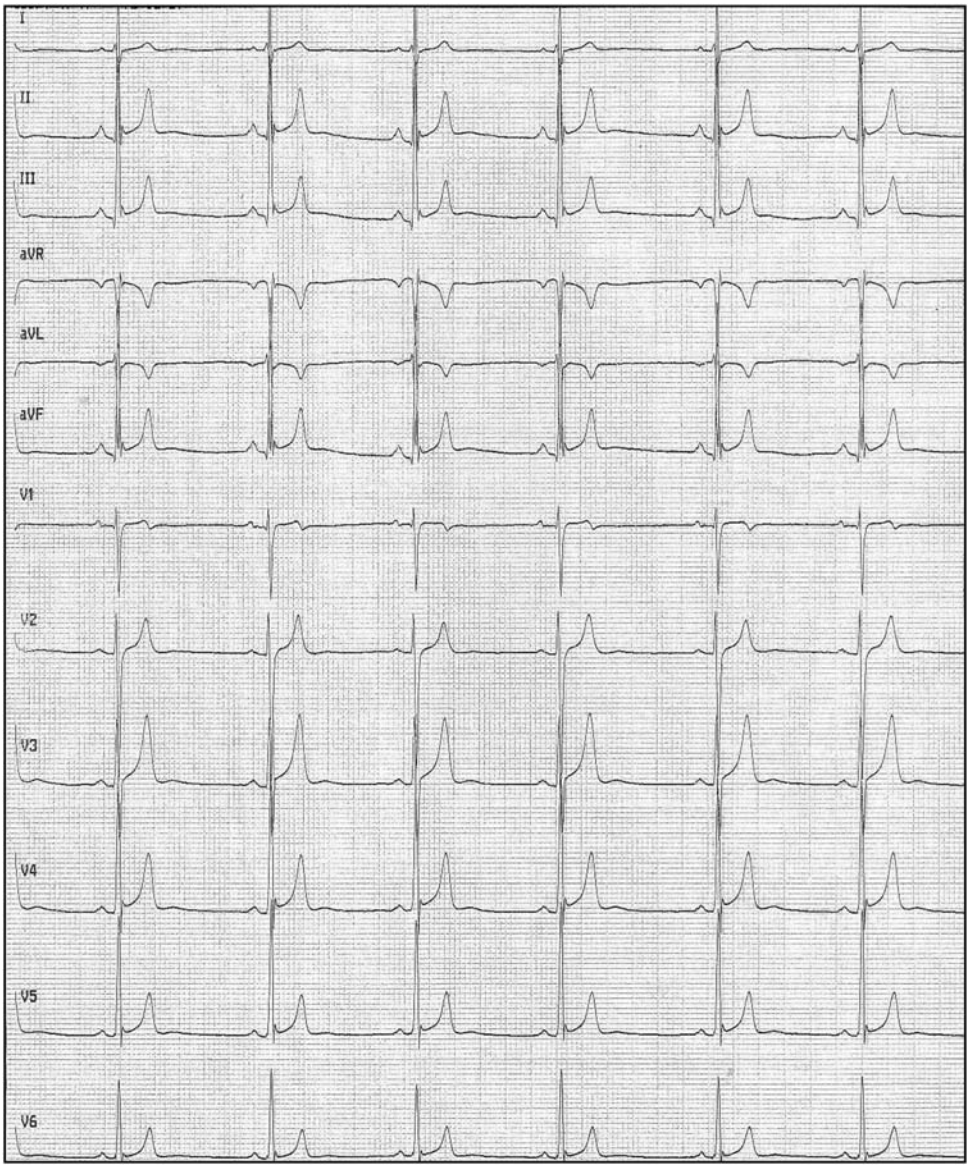


Figure 5 Baseline electrocardiogram in a patient with short QT syndrome (QT/QTc: 340/330 ms in II).

resemblance human arrhythmias[57,58] (*Figure 6*). Animal studies confirmed that delayed afterdepolarization (DAD)-induced triggered activity is at the origin of bidirectional and polymorphic VT in this syndrome.[59–61] Furthermore, these experiments contributed to the identification of new therapeutic options. It has been proposed that flecainide, known for its sodium channel blocker action, may also exert a direct effect on the ryanodine receptor and could inhibit DADs and triggered activity both *in vitro* and *in vivo*.[62] In other experimental model, it has been highlighted that the sodium channel blocking effect of flecainide might be instrumental to prevent DADs to elicit triggered beats.[63] The encouraging experimental observations were rapidly moved from the bench to pilot studies in patients refractory to β-blockers, and a clinical trial is now ongoing to prove the potential beneficial effect of flecainide in CPVT (http://clinicaltrials.gov: NCT01117454).

Genetic predisposition to sudden death in acquired diseases

One of the major achievements derived by the study of the molecular substrate of IADs has been to raise the attention of the role that genetic variations play in determining the risk of SCD.

Some studies in large populations demonstrated that family history of SCD is a significant risk factor for SCD, thus suggesting that it is likely that genetic factors control this predisposition.[64] In spite of the strong epidemiological data, the identification of the genetic substrate influencing SCD risk in the

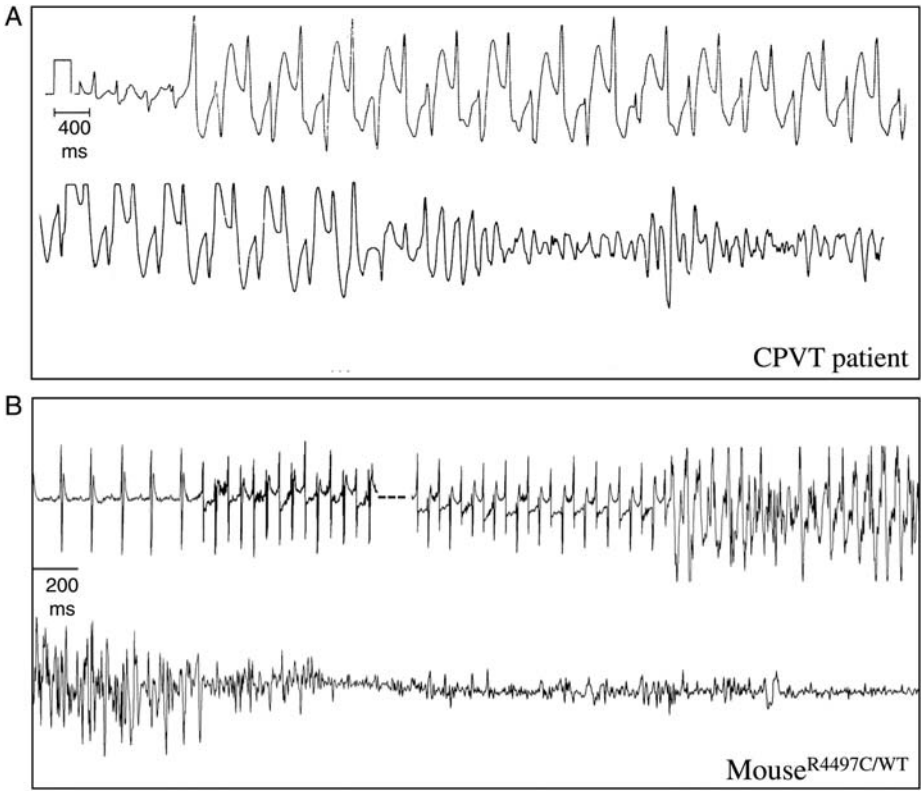


Figure 6 (*A*) Bidirectional ventricular tachycardia degenerating into ventricular fibrillation in a patient affected by catecholaminergic polymorphic ventricular tachycardia, a carrier of one RYR2 mutation. (*B*) Bidirectional and polymorphic ventricular tachycardia degenerating into ventricular fibrillation in a catecholaminergic polymorphic ventricular tachycardia mouse model, showing how the morphology resembles the human one (from Cerrone *et al.*[57] with permission).

general population has proven to be a challenging task. Genome-wide association studies (GWAS) represent the new frontier in epidemiology; through the analysis of the entire human genome on large cohorts, they seek to establish a correlation between common genetic variations and a disease. These studies focus on the detection of single-nucleotide polymorphisms (SNPs), i.e. genetic variations that are common (>1%) in the general population or in some ethnic groups and supposedly non-deleterious, in contrast with a genetic mutation. Although disease-causing mutations are rare but confirm to the carriers a much higher risk of manifesting a phenotype, SNPs are prevalent in the general population but they confer only a small incremental risk of having a disease.

The first evidence of the potential role of SNPs in influencing susceptibility to arrhythmias came once more from the field of channelopathies. Early studies demonstrated that certain SNPs could modulate the effect of deleterious mutations in BrS[65] or LQTS.[66] Other scientists investigated the role of SNPs located in the genes implicated in the genesis of inherited arrhythmias in the susceptibility to acquired arrhythmias. A recent study by Albert *et al.*[5] collected SCD cases from six different cohorts and found a correlation between two common intronic variants in the genes *KCNQ1* and *SCN5A* and SCD. Even if these data still require validation in different population and possibly complemented by functional studies, this may represent a starting point in the evaluation of genetic factors predisposing to SCD. Another interesting set of investigations explored the effect of SNPs on ECG parameters. Chambers *et al.*[67] found that one non-synonymous SNP in the gene *SCN10A* could modulate the PR interval duration. This was initially found in an Indian Asian cohort and subsequently replicated in a small European population. Studies aimed to clarify how this SNP could modulate cardiac atrioventricular conduction led to the discovery that *SCN10A* is expressed in the human atria and ventricles. Data from mouse models lacking this gene seem to suggest that *SCN10A* expression may lengthen cardiac conduction and that the specific SNPs identified by the study may exert a gain-of-function effect.[67]

Another gene that has been demonstrated to have a role in influencing ECG parameters and the risk of SCD is the gene *NOS1AP*.[6,7] *NOS1AP* is a gene that encodes for the protein CAPON, a neuronal regulator of the nitric oxide synthase pathway that is expressed in the heart and has been demonstrated to modulate ion channel function in cellular studies.[68] Arking *et al.*[7] were the first to correlate SNPs in this gene with the QT interval duration. Interestingly, another large GWAS study confirmed that common SNPs in the genes encoding cardiac ionic channels involved in the aetiology of congenital LQTS could influence the QTc interval duration also in the general population.[6,69]

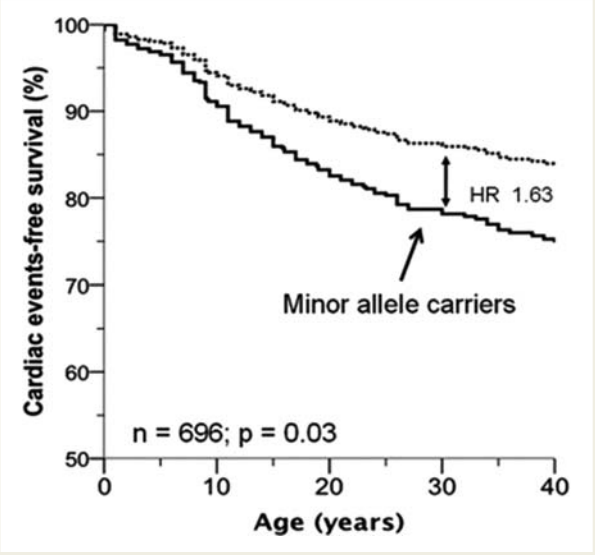


Figure 7 Independent effect of the rs10494366 SNP on *NOS1AP* on the risk of events in genetically affected long QT syndrome patients with QTc <500 ms (from Tomas *et al.*[70] with permission from the publisher).

Stemming from these observations, several studies were able to link common SNPs of *NOS1AP* to increased risk of SCD in different cohorts.[68,69]

The obvious corollary to these findings was to look back at inherited channelopathies to explore whether *NOS1AP* could influence the QTc interval duration clinical outcome also in patients with congenital LQTS.[66,70] Our study on 901 genotyped LQTS patients from the database maintained at the Maugeri Foundation in Pavia showed that some SNPs on *NOS1AP* are able to modulate the QTc interval duration and risk of cardiac events.[70] Further analysis demonstrated that the presence of one of these SNPs may influence the arrhythmic risk of those patients who show a baseline QTc interval of <500 ms[70] (*Figure 7*). Although a QTc of >500 ms identifies individuals at a higher risk for SCD, the possibility of further stratifying the risk of the group at intermediate/low risk may constitute an important advancement for the clinical management of the disease. These are still to be considered preliminary observations that need to be replicated in additional groups; however, they represent an encouraging example of the potential clinical application of the results derived by GWAS.

Conclusions

The past 20 years have witnessed an incredible advancement in the appreciation of the role exerted by genetics in cardiac arrhythmias and SCD. The growing body of information now available opens new challenges, calling for an increasing interaction between the cardiologist and basic scientists. In the field of IADs, once a genetic mutation is discovered, it becomes important to integrate bench and clinical studies to assess its potential deleterious effect and define the best management strategy for mutation carriers. Data from GWAS highlighted how further experimental studies are mandatory to understand if the SNPs singled out really can predispose to acquired diseases and through which mechanisms. Altogether, the newly developed field of cardiovascular genetics is facing future challenges aimed to fill the existing gaps separating current practice from personalized patients' management.

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