

Sudden Cardiac Death Compendium

Circulation Research Compendium on Sudden Cardiac Death

The Spectrum of Epidemiology Underlying Sudden Cardiac Death

Sudden Cardiac Death Risk Stratification

Genetics of Sudden Cardiac Death

Mechanisms of Sudden Cardiac Death: Oxidants and Metabolism

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Gordon Tomaselli, Editor

Genetics of Sudden Cardiac Death

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Abstract: Sudden cardiac death occurs in a broad spectrum of cardiac pathologies and is an important cause of mortality in the general population. Genetic studies conducted during the past 20 years have markedly illuminated the genetic basis of the inherited cardiac disorders associated with sudden cardiac death. Here, we review the genetic basis of sudden cardiac death with a focus on the current knowledge on the genetics of the primary electric disorders caused primarily by mutations in genes encoding ion channels, and the cardiomyopathies, which have been attributed to mutations in genes encoding a broader category of proteins, including those of the sarcomere, the cytoskeleton, and desmosomes. We discuss the challenges currently faced in unraveling genetic factors that predispose to sudden cardiac death in the setting of sequela of coronary artery disease and present the genome-wide association studies conducted in recent years on electrocardiographic parameters, highlighting their potential in uncovering new biological insights into cardiac electric function. (*Circ Res.* 2015;116:1919–1936. DOI: 10.1161/CIRCRESAHA.116.304030.)

Key Words: arrhythmias, cardiac ■ cardiomyopathies ■ death, sudden, cardiac
■ genetics ■ genome-wide association study

Sudden cardiac death (SCD) accounts for ≈20% of mortality in the general population¹ and is associated with significant morbidity among survivors. It occurs in the setting of a broad spectrum of cardiac pathologies, and although it mostly occurs in adults, it may also strike children.² The identification of genetic factors that predispose to SCD is important because this enables genetic testing that may contribute to

diagnosis and risk stratification. The identification of genetic risk factors also provides molecular leads that trigger an increased understanding of disease pathways underlying SCD and development of new therapies. Since the landmark discoveries of the first genes for hypertrophic cardiomyopathy (HCM) by Seidman and coworkers^{3,4} and for the long-QT syndrome (LQTS) by Keating and coworkers^{5–7} in the early to

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Nonstandard Abbreviations and Acronyms

ACM	arrhythmogenic cardiomyopathy
BrS	Brugada syndrome
CCD	cardiac conduction disease
CPVT	catecholaminergic polymorphic ventricular tachycardia
DCM	dilated cardiomyopathy
ER	early repolarization
GWAS	genome-wide association study
HCM	hypertrophic cardiomyopathy
LQTS	long-QT syndrome
MI	myocardial infarction
SCD	sudden cardiac death
SNP	single-nucleotide polymorphism
SQTS	short-QT syndrome
SR	sarcoplasmic reticulum
VF	ventricular fibrillation
VT	ventricular tachycardia

mid-1990s, much progress has been made in the understanding of inherited predisposition to SCD, yet many challenges remain. Here, we provide a bird's eye view of current knowledge about the genetics of SCD and discuss some likely future directions of research in this field.

Uncommon Inherited Cardiac Disorders

We present the inherited channelopathies and cardiomyopathies with a focus on the genes implicated (Figures 1 and 2). Although we will present information on the role of genetic testing in the diagnosis of the disease, we will not enter clinical considerations because they have recently been discussed elsewhere.⁸

Although the major cause of SCD after the age of 45 years is coronary artery disease, in the pediatric population and in young adults, SCD typically occurs in the setting of rare inherited cardiac disorders that are categorized into 2 broad classes, namely the cardiomyopathies,⁹ where

the arrhythmogenic substrate is thought to involve primarily the abnormal structure of the myocardium, and the primary electric disorders,¹⁰ where the heart is structurally normal and arrhythmias arise from abnormalities in the electric function of the heart. It is commonly held that the understanding of the genetic factors underlying these cardiac pathologies will enlighten genetic risk factors of SCD in these disorders. The primary electric disorders and the cardiomyopathies have been classically considered Mendelian disorders with clear familial inheritance across the generations and wherein a potent monogenic component contributes substantially to risk. In line with this, classical linkage analysis in affected families has been successful in identifying disease genes for many of these disorders.^{3–7} Without exception the various cardiomyopathies and primary electric disorders are genetically heterogeneous, that is mutations in different genes can lead to the same clinical disease manifestation. Furthermore, considerable allelic heterogeneity exists in that many different mutations within each gene cause the disease. Most mutations are private (ie, are unique to the family) or are implicated in the disease in only a few families. Exceptions, however, exist in some countries, such as the Netherlands, Finland, and South Africa, where founder mutations have been described^{11–13}; such mutations descend from a shared ancestor who lived generations ago and they account for the disorder in multiple families.

These gene discoveries have had a relevant impact on patient care, enabling diagnosis and guiding to varying degrees prognosis and therapeutic decisions.¹⁴ The major challenge the field currently faces resides in the limited ability to integrate genetic information into schemes for risk stratification and quantification of the risk of SCD. At present, genetic information has an established role in the quantification of risk of SCD only in the LQTS. It is likely that this limitation stems from our current incomplete understanding of the causative genes and modulatory genetic factors in the various disorders. We anticipate that as insight into these factors evolves, genetics will have a stronger role in the assessment of the arrhythmogenic risk. Yield of molecular genetic testing varies across

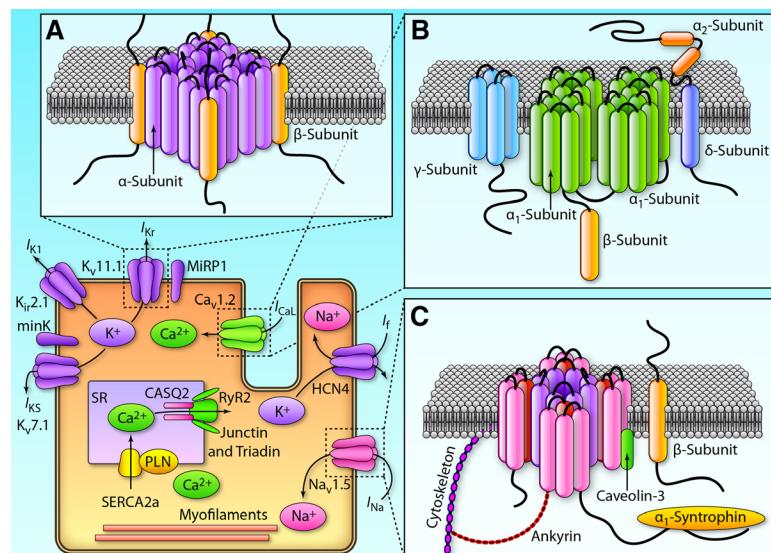


Figure 1. Schematic representation of a cardiomyocyte displaying the proteins involved in the pathogenesis of the primary electric disorders. **A**, potassium (I_{Kr}); **B**, calcium (I_{CaL}); and **(C)** sodium (I_{Na}) channel structures and subunits are shown. CASQ2 indicates calsequestrin-2; PLN, cardiac phospholamban; RyR2, ryanodine receptor 2; SERCA2a, sarcoplasmic/endoplasmic reticulum calcium ATPase 2a; and SR, sarcoplasmic reticulum (Illustration credit: Ben Smith).

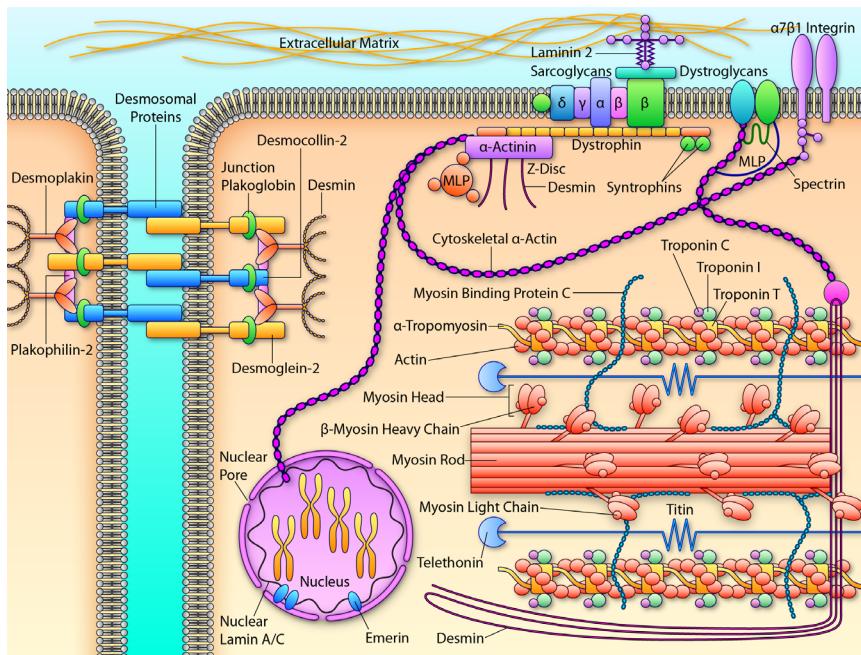


Figure 2. Schematic representation of a cardiomyocyte exhibiting proteins involved in the pathogenesis of the inherited cardiomyopathies, including sarcomeric, cytoskeletal, desmosomal proteins, and nuclear envelope proteins. MLP indicates cysteine and glycine-rich protein 3 (also known as muscle LIM [Lin-11, Islet-1, Mec-3] protein) (Illustration credit: Ben Smith).

the disorders, but it is generally higher in familial cases than in isolated patients.¹⁵ Although in this review we have tried to be as comprehensive as possible in mentioning all genes that have been implicated in these disorders to date, one must keep in mind, however, that the evidence of causality for the different genes in the respective disorders varies widely. For some genes strong evidence has been provided through demonstration of linkage, the consistent involvement of a given gene in multiple cases with the same disease, or through robust functional studies. However, for other genes, which have largely been identified in candidate gene studies, evidence is not always robust.

The observation of variable disease severity (reduced penetrance and variable expressivity) among carriers of the same causal mutation within families has brought with it the realization that ultimate disease severity, including SCD risk, in the individual patient also depends on other unknown factors.¹⁶⁻¹⁸ Although factors such as age (age-dependent penetrance),¹⁹ sex,²⁰ environment (eg, medication use²¹), and possibly exercise²² are known to contribute to disease variability in certain disorders, the inheritance of additional modulatory genetic factors is also thought to play a role. The inheritance of >1 mutation in ≥1 genes has in some cases been shown to account for the greater disease severity.^{23,24} In the past years, a flurry of genome-wide association studies (GWAS) conducted in large samples of the general population have uncovered robust associations between single-nucleotide polymorphisms (SNPs; that tag common haplotypes) and several cardiac electric, structural, and functional traits.²⁵⁻³¹ Because these traits are thought to represent relevant intermediate phenotypes for cardiac disease and SCD,^{35,36} although they likely carry modest effects, these SNPs are prime candidates as modulators of clinical disease manifestations in the rare cardiac disorders. Although progress is still slow, this is starting to be

explored.³⁷⁻³⁹ Low-frequency variants that are presumed to be associated with intermediate effect sizes are also expected to contribute. However, these are more challenging to study because of their low prevalence.

For some of the rare cardiac disorders, the notion that they are Mendelian is now being questioned.^{40,41} For these disorders, a somewhat more complex genetic inheritance (oligogenic model) is now suspected; here, in contrast to the

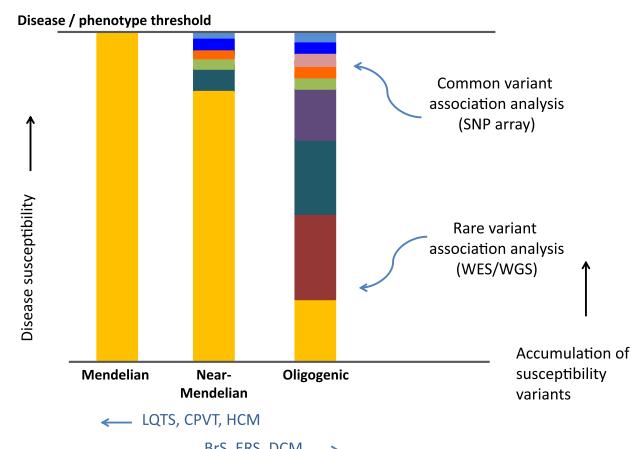


Figure 3. The likely continuum of complexity of genetic architecture in the rare inherited cardiac disorders. Although some disorders are Mendelian or near-Mendelian, where a strong monogenic component contributes substantially to disease susceptibility, genetic susceptibility for others disorders may be determined by the cumulative effect of multiple genetic variants. BrS indicates Brugada syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; ERS, early repolarization syndrome; HCM, hypertrophic cardiomyopathy; LQTS, long-QT syndrome; SNP, single-nucleotide polymorphism; WES, whole exome sequencing; and WGS, whole genome sequencing.

monogenic paradigm, the coinheritance of many genetic risk variants are thought to conspire to cause the disease (Figure 3). Such genetic factors may occur at different frequencies in the general population and are likely to carry different magnitudes of effect on disease susceptibility. Their identification necessitates approaches that are fundamentally different from the family-based approaches applied to date (discussed later in this review).⁴⁰ With the exception of some clearly Mendelian forms, the precise genetic architecture of the various rare inherited cardiac disorders is essentially unknown and will need to be determined empirically; it is highly likely that a continuum of complexity of genetic architecture exists whereby some disorders will be Mendelian or near-Mendelian (where a strong genetic factor is modulated by a few additional genetic factors, accounting for reduced penetrance), whereas others will be oligogenic (Figure 3).

Primary Electric Disorders

The primary electric disorders, which among others include LQTS, short-QT syndrome (SQTS), Brugada syndrome (BrS), and catecholaminergic polymorphic ventricular tachycardia (CPVT), are often characterized by specific ECG abnormalities either at baseline or during particular conditions, such as exercise (eg, CPVT and LQTS), fever (eg, BrS), or pharmacological challenge (eg, BrS).⁸ The list of familial arrhythmia syndromes has been in recent years expanded by the recognition of 2 other disorders, namely early repolarization syndrome and idiopathic ventricular fibrillation (VF).^{42,43} The great majority of genes identified to date for these disorders encode cardiac ion channel subunits or proteins that interact with, and regulate, ion channels (Figure 1).

Long-QT Syndrome

The LQTS, which frequently presents in childhood, is characterized by QT interval prolongation in association with syncope and SCD caused by torsades des pointes: a polymorphic ventricular tachycardia (VT) that easily degenerates into VF.⁴⁴ It is most commonly inherited in an autosomal dominant fashion (previously described as the Romano-Ward syndrome) and has been associated with mutations in 15 genes. LQT1 is the most common form of LQTS, accounting for ≈35% of cases. It arises from loss-of-function mutations in the *KCNQ1* gene, which encodes the slowly activating delayed rectifier current (I_{Ks}).⁶ LQT2, accounting for 30% of cases, arises from loss-of-function mutations in *KNCH2* (also known as *HERG*), encoding the rapidly activating delayed rectifier current (I_{Kr}).⁷ Gain-of-function mutations in *SCN5A* that lead to an increase of the late sodium current (I_{Na}) underlie LQT3 and are found in ≈10% of probands.⁴⁵ Between them, these 3 genes account for ≈90% of genotype-positive LQTS patients.^{14,46} Several additional genes encoding either ion channels subunits (*KCNJ5*, *KCNE1*, *KCNE2*, and *SCN4B*) or for proteins that regulate ion channels function (*AKAP9*, *CAV3*, *ANKB*, *SNT1*, *CALM1*, and *CALM2*) have been associated with LQTS; however, most of them are only rarely implicated (<1%). Interpretation of results of genetic testing in the setting of the latter LQTS genotypic variants is complex because often the DNA changes identified have a low minor allele frequency in the general population and they have not been identified before in patients. Their

pathogenic role is uncertain, and therefore value in managing family members is minimal.

Three clinical variants of LQTS manifest with extracardiac phenotypes in the so-called systemic manifestations of LQTS. The Jervell and Lange-Nielsen syndrome is a rare autosomal recessive form of the LQTS caused by homozygous or compound heterozygous mutations in *KCNQ1*⁴⁷ or *KCNE1*⁴⁸ and is characterized by severely prolonged QTc-interval, life-threatening arrhythmias, and sensorineural deafness. Yet, homozygous or compound heterozygous mutations in the absence of deafness have also been reported and are referred to as autosomal recessive LQTS.^{23,49} The Anderson-Tawil syndrome (LQT7) presents with a triad of clinical features, including QT-interval prolongation, facial dysmorphism, and hypokalemic periodic paralysis. It is caused by loss-of-function mutations in the potassium channel gene *KCNJ2*.⁵⁰ Timothy syndrome (LQT8) is the most severe variant of LQTS because it has a high mortality rate and is the only form of LQTS in which death is caused by extra cardiac phenotypes. Besides a marked QT-interval prolongation and severe ventricular arrhythmia, LQT8 presents with congenital heart defects, AV block, syndactyly, autism, malignant hypoglycemia, and an abnormal immune system. It is primarily caused by the heterozygous G406R mutation in *CACNA1C*.⁵¹ Given the high lethality of the disease, few patients reach reproductive age and therefore in most of the familial forms the transmission to multiple children derives from mosaicism of germinal cells.

Mutation analysis is essential in LQTS, as asymptomatic family members with normal QTc-interval (<440 ms) who carry the causal familial LQTS mutation have a 10-fold increased risk of cardiac events in comparison with noncarriers.^{20,52}

The most powerful predictors of risk in LQTS are cardiac events, including previous syncope, torsades des pointes or aborted SCD, a QTc-interval >500 ms,^{20,53-55} and age (prepubertal men and adult women). In women, particularly the pubertal, postpartum (especially in LQT2), and postmenopausal periods carry a higher risk for cardiac events.^{56,57} With respect to genotype, certain genetic subtypes, namely the recessive Jervell and Lange-Nielsen syndrome⁵⁸ and Timothy syndrome (LQT8),⁵¹ present with a more severe QT-interval prolongation, arrhythmic events at a young age, and a poor response to therapy, compared with other LQTS genetic subtypes. Large LQTS patient databases have enabled extensive genotype–phenotype correlations in the 3 major LQTS genetic subtypes (LQT1–LQT3) and have indicated that genotype, together with the duration of QTc-interval and sex, is a determinant of SCD risk and response to therapy.^{20,59} Genotype–phenotype studies have also uncovered specific features for each of these subtypes.^{60,61} Patients with LQT1 have typically broad-based T waves on their ECG and experience cardiac events during exercise, particularly during swimming and diving. In LQT2, T waves are typically low amplitude or notched and auditory stimuli have been noted as a highly specific trigger of arrhythmia.⁶² LQT3 patients often have a long isoelectric ST-segment and experience cardiac events predominantly during rest or sleep. The natural history of LQT1 is more benign than that of LQT2 and LQT3 patients, and LQT1 patients also have a better response to β blockers.²⁰

The type of mutation and the location of the mutation within the channel subdomain are expected to affect the abundance of the respective ion channel on the sarcolemma or the severity of the biophysical defect, and thereby on the severity of the disease. In support of this, studies conducted in patients with *KCNQ1* mutations have identified mutations at transmembrane regions (versus C-terminal mutations) or mutations with a dominant-negative effect (versus those resulting in haploinsufficiency) as being associated with a higher risk of cardiac events.⁶³ In LQT2, patients with missense mutations in the pore region of the channel seem to be associated with greatest risk of life-threatening arrhythmias.⁶⁴ Accordingly, functional characterization of mutant proteins that to date is performed in research laboratories should be regarded as key information for risk stratification and clinical management.

Variability in disease severity also exists among carriers of the same LQTS-causing mutation in the same family.¹⁶ This is sometimes explained by the inheritance of >1 mutations in ≥ 1 LQTS genes, which has been established to occur in 5% to 10% of probands.²³ These patients have longer QT intervals and are more prone to arrhythmia. A few studies have investigated the role of SNPs in candidate genes for modulatory effects on the QTc-interval and on the occurrence of cardiac events in LQTS patients.^{65–67} When performed in large kindred with the same founder mutation,⁶⁷ such studies benefit from eliminating the confounding effect conferred by the presence of diverse primary genetic defects. Studies are now increasingly focusing on the effects of SNPs that have been identified as modulators of the QT-interval by GWAS conducted in the general population (Figure 4).^{27,68–70} SNPs at *NOS1AP*^{37,38} and

*KCNQ1*³⁹ identified by GWAS have been shown to modulate the QT-interval and the risk of cardiac arrhythmia in patients with LQTS. With respect to SNPs located within or in the vicinity of the gene containing the mutation, allele-specific effects need to be taken into account because genetic variation impacting on gene expression is expected to have different effects depending on whether it is located in *cis* (on the same allele) or in *trans* (opposite allele) to the mutation. Such effects have been shown for polymorphisms in the 3' untranslated region of *KCNQ1* in patients with LQT1.⁶⁶

Arrhythmogenic Mechanisms

In vitro functional studies have shown that mutations in genes that encode for ion channel subunits lead to prolongation of the action potential either by reducing outward potassium currents or by increasing inward sodium or calcium currents. This evidence has supported the classical view that early after depolarizations associated with action potential prolongation lead to torsades des pointes. This view is aligned with clinical data that link more prolonged QT intervals to higher risk of arrhythmias; however, it does not explain how arrhythmias propagate and are maintained in the entire heart. Unfortunately, the lack of knockin animal models that replicate the LQTS phenotype has to date limited the ability to dissect the arrhythmogenic mechanisms further. Data from transgenic rabbits,⁷¹ despite the limitation of the presence of multiple, randomly inserted copies of the mutant gene in the genome of the animals, have recently been instrumental to support a novel view on the arrhythmogenesis in LQTS by Chang et al.⁷² These authors developed an in silico model of

Loci identified in GWAS on ECG parameters

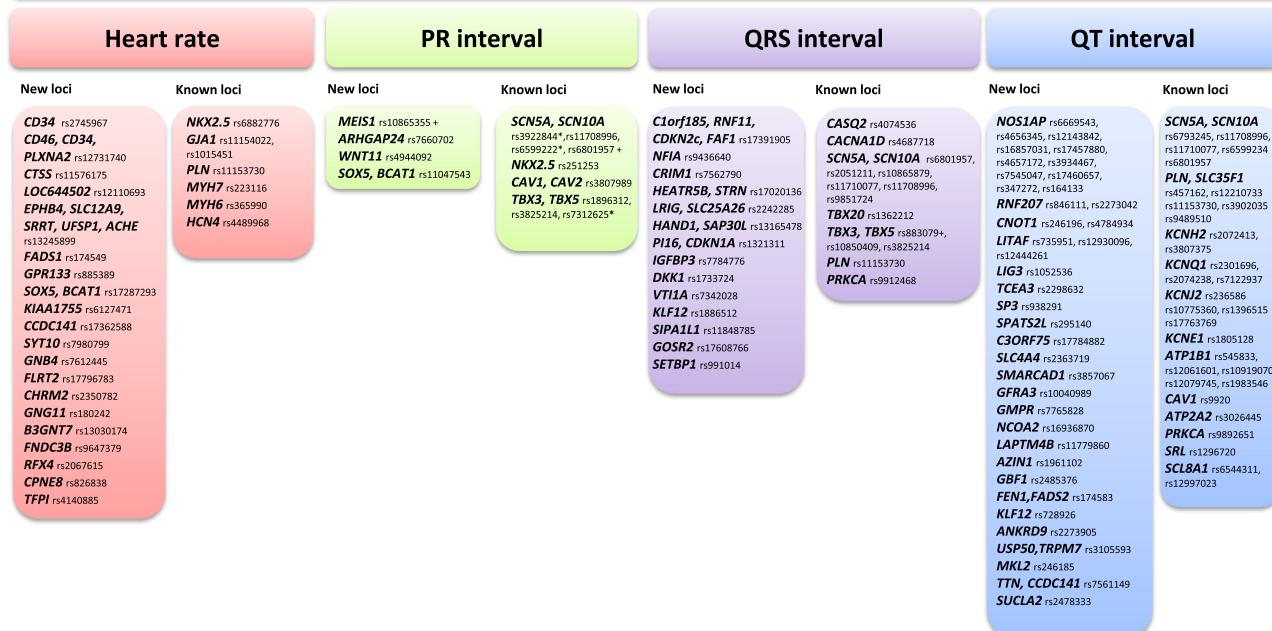


Figure 4. Loci identified by genome-wide association studies (GWAS) as modulators of ECG parameters in the general population. Known loci: For loci with a probable candidate gene in the proximity of the locus, we have listed the respective candidate gene. New loci: In the absence of a probable candidate gene in the proximity of the locus the nearest gene is listed. Where multiple single-nucleotide polymorphisms are listed, $r^2 < 0.1$. Association signals that were identified at $P < 5 \times 10^{-8}$ are listed. +found in both European descent and blacks; *found only in blacks.

prolonged repolarization in 2-dimensional cardiac tissue by increasing I_{Ca} and I_{ks} to induce action potential prolongation and early after depolarizations. In this model, they demonstrated that arrhythmogenesis is caused by 2 types of spiral waves: short cycle rapid I_{Na} generated waves (Figure 5A) and long cycle, slow L-type calcium current ($I_{Ca,L}$) generated spiral waves (Figure 5B).⁷² Interestingly, in this model, the alternation of the 2 types of spiral waves generated a torsades des pointes-like ECG. Subsequently Kim et al⁷¹ investigated arrhythmogenesis in the LQT1 transgenic rabbit models inducing polymorphic VT with isoproterenol and they demonstrated the presence of complex focal excitations occurring in both ventricles and causing oscillations of the QRS complexes consistent with multiple early after depolarizations-generated foci. They also showed a bimodal distribution of the action potential supporting the coexistence of 2 types of excitation that contribute to arrhythmogenesis: I_{Na} -mediated fast conduction and $I_{Ca,L}$ -mediated slow conduction coexist supporting the biexcitability hypothesis by Chang et al.⁷²

Short-QT Syndrome

The SQTS manifests as a short QT-interval on the ECG (<350 ms; although the definition varies in the literature) and a predisposition to supraventricular arrhythmias and SCD.^{73,74} The description of this disorder highlighted the fact that both extremes of the distribution of QT-interval duration (longer and

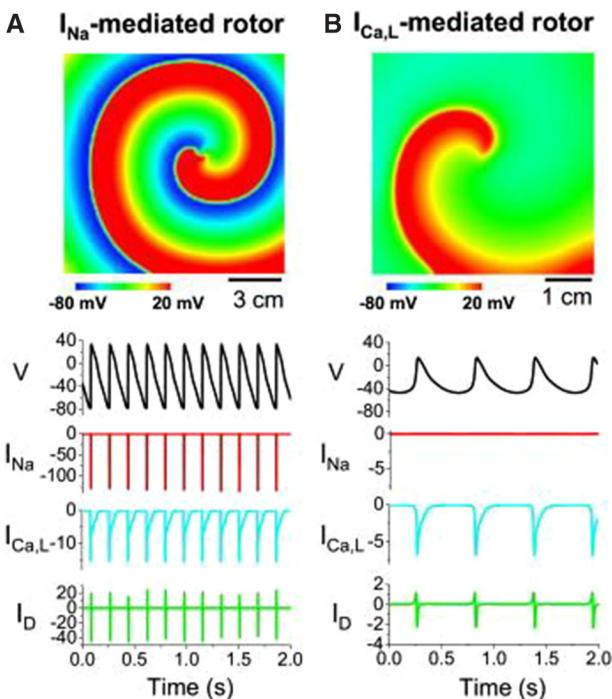


Figure 5. Bi-stable spiral waves in a homogeneous 2-dimensional in silico cardiac tissue model of prolonged repolarization. Arrhythmogenesis is caused by 2 types of spiral waves: (A) An I_{Na} -mediated spiral wave induced by cross-field stimulations (top), with traces of voltage, I_{Na} , and $I_{Ca,L}$ vs time at a representative location (bottom). (B) An $I_{Ca,L}$ -mediated spiral wave (top) induced by a different cross-field stimulation pattern in the same tissue, with traces of voltage, I_{Na} , and $I_{Ca,L}$ versus time from the same site as in A (bottom). Reprinted from Chang et al⁷² with permission of the publisher. Copyright ©2012, Elsevier.

shorter) can be arrhythmogenic. Genetic studies have identified mutations in genes encoding potassium channels that are also causative genes for LQTS (*KCNH2*, *KCNQ1*, and *KCNJ2*).^{75–77} Mutations in genes encoding the CaV1.2 L-type calcium channel subunits⁷⁸ (*CACNA1C* and *CACNB2*) cause either SQTS or an overlapping phenotype that combines an abbreviated QT-interval and a Brugada ECG-phenotype.

SQTS-causing mutations in the potassium channel genes exert a gain-of-function effect on the affected channel and lead to a shortened cardiac repolarization.^{75–77,79,80} Mutations affecting the calcium channel subunits linked to SQTS are expected to cause a loss of channel function, thereby also abbreviating the action potential. These biophysical defects are opposite to those observed for LQTS-causing mutations in the same genes. A recent study conducted in 45 probands with SQTS identified mutations in only 14% of cases, despite familial disease being present in almost half.⁸¹ Data from the same study on 73 patients suggested that the SQTS is highly lethal with SCD often being the first manifestation of the disease.

Arrhythmogenic Mechanisms

Because only a minority of patients with SQTS are successfully genotyped and there are no knockin or transgenic animal models to investigate the pathophysiology of SQTS, the leading hypothesis for an arrhythmogenic mechanisms is based on the vulnerability of the ventricles in the presence of abbreviated repolarization and short refractoriness. Data from cardiac wedge preparations in which a short action potential was pharmacologically induced suggested that transmural dispersion of repolarization caused by large variability of action potential duration is the pivotal arrhythmogenic element in the disease.⁸² This hypothesis from a surrogate *in vitro* model has yet to be confirmed *in vivo*.

Brugada Syndrome

BrS⁸³ is associated with syncope and cardiac arrest resulting from degeneration into VF of episodes of polymorphic VT. Death in BrS patients mostly occurs at rest or during sleep.⁸ The disease has an age-dependent penetrance and a sex-related penetrance and therefore most lethal events occur in men after the fourth decade of life.⁸⁴ According to recent guidelines, BrS is diagnosed in the presence of a type I ECG pattern, namely a coved ST-segment elevation ≥0.2 mV, in >1 precordial lead positioned in the second, third, or fourth intercostal space, occurring spontaneously or after a provocative drug test with intravenous administration of class I antiarrhythmic drugs.⁸ The ECG manifestations of BrS are often dynamic and may be intermittently present. Besides sodium channel blockers, the BrS ECG may be induced by a febrile state.⁸⁴ Mutations in *SCN5A* encoding the α -subunit of the cardiac voltage-gated sodium channel (Nav1.5) were the first-identified genetic cause of BrS.⁸⁵ Mutations in genes encoding the CaV1.2 L-type calcium channel subunits *CACNA1C* or *CACNB2* can cause an overlapping phenotype of BrS and SQTS.⁷⁸ BrS-causing mutations in *SCN5A*, *CACNA1C*, and *CACNB2B* decrease the inward current by impairing trafficking of the channel to the cardiomyocyte membrane or by altering the channel biophysical properties. Among the other uncommonly implicated genes are sodium channel and calcium channel-associated proteins,

such as *SCN1B*, *SCN3B*, and *GPDIL*; it has been suggested that mutations in these genes reduce I_{Na} and I_{Ca} currents.^{86,87} Finally, mutations in genes that encode channels conducting outward potassium currents (*KCND3*, *KCNE3*, and *KCNJ8*) and the pacemaker current gene *HCN4* have been reported in a few BrS patients, but their causative role still requires confirmatory data.⁸⁸ A recent comprehensive mutational analysis of 12 known BrS-susceptibility genes in a large cohort of unrelated BrS patients identified *SCN5A* mutations in 16%, with the other 11 genes accounting for <5% of patients.⁸⁹ The majority (\approx 80%) of BrS, therefore, still remains genetically unresolved. A single study reported that \approx 6% of BrS cases carry mutations in the transient receptor potential melastatin protein number 4 or *TRPM4* gene encoding for a calcium-activated nonselective cation channel, member of a large family of transient receptor potential genes.⁹⁰ These findings, however, still need confirmation. Recently, another study reported that mutations in *SCN10A* account for 16% of patients with typical BrS identified in a cohort of 150 BrS affected individuals.⁹¹ Interestingly, 2 more recent studies did not reproduce the previous findings and the authors conclude that rare variants in *SCN10A* are not enriched in patients with BrS.^{92,93}

The primary role of genetic testing in the BrS is in confirmation of the disorder in the index patient and cascade testing in relatives to distinguish those who need clinical follow-up (for the development of conduction disease or for syncopal episodes) and preventive measures (eg, avoiding specific drugs, prompt management of fever) from those who are both clinically and genetically unaffected.^{8,14} One study suggested that patients carrying an *SCN5A* mutation that leads to a prematurely truncated protein (stop codon or frameshift) were more likely to present with syncope and develop prolonged PR and QRS intervals in comparison with patients harboring missense mutations.⁹⁴

Arrhythmogenic Mechanisms and a Novel Hypothesis About Inheritance of BrS

Arrhythmogenic mechanisms in BrS have been debated in the past decade, with 2 prevailing hypotheses mainly based on the reduction of I_{Na} current that is the final common pathway of most of the functional consequences of mutations in genes implicated in the disease: this debate has been discussed extensively.⁹⁵ Briefly, the so-called repolarization hypothesis proposed by Yan and Antzelevitch⁹⁶ has been developed based on data obtained in a canine wedge preparation, and it considers transmural dispersion of repolarization as the key substrate for reentrant arrhythmias. The depolarization hypothesis supports the view that the conduction abnormalities (right bundle-branch block and atrio-ventricular conduction delay) are the key arrhythmogenic factors. The conduction delay may predispose the heart to reentrant arrhythmias, especially when structural abnormalities in the right ventricle accentuate the inhomogeneity in impulse propagation. This hypothesis views the genetic defect as a substrate that requires a second hit to trigger arrhythmias and will therefore account for the well-known age dependency of the disease that may remain silent for decades before eliciting a life-threatening rhythm. The need for an additional arrhythmogenic element on top of an *SCN5A* mutation to elicit a BrS phenotype is supported by

recent data obtained in a knockin pig model heterozygous carrier of the *SCN5A* nonsense mutation E558X. Interestingly, adult animals developed an overt cardiac conduction defect in vivo and ventricular arrhythmias in the isolated heart, but they failed to show the electrocardiographic pattern of BrS and VF in vivo even when flecainide was administered as a provocative test.⁹⁷

Recently, interesting observations have questioned the view that BrS is a Mendelian disorder and have suggested a more complex inheritance (Figure 3). This includes the observation of low disease penetrance in families harboring *SCN5A* mutations¹⁷ and, in some instances, absence of the familial *SCN5A* mutation in affected family members.⁹⁸ Also, many cases are sporadic,⁹⁹ and familial linkage analyses have largely been unsuccessful in uncovering new disease-causing genes. A recent international collaborative study used the GWAS approach, comparing patients with BrS with general population controls, to identify common genetic variants contributing to BrS susceptibility. This identified 3 loci: rs10428132 and rs11708996, both at *SCN5A/SCN10A*, and rs9388451 near *HEY2*.⁴⁰ Of note, when the 3 loci were considered in aggregate, disease susceptibility increased consistently with increasing numbers of risk alleles, with an unexpectedly high odds ratio of 21.5 in the presence \geq 5 risk alleles compared with the presence of \leq 1 risk alleles. This provided strong evidence that the genetic architecture of at least some of the rare rhythm disorders could be different from that assumed to date. The associations at *SCN5A/SCN10A* with BrS demonstrated that SNPs identified by GWAS as modifiers of ECG parameters in the general population (Figure 4) can also influence susceptibility to a rare primary electric disorder. About the *HEY2* locus, studies conducted in mice haploinsufficient for *HEY2*, which encodes the transcriptional repressor hairy/enhancer-of-split related with YRPW motif protein, provided evidence for a role of this transcriptional repressor in regulation of *SCN5A* expression and conduction velocity in heart. In these mice, differences in conduction preferentially involved the right ventricular outflow tract, which is in line with ECG manifestations in right precordial leads and concurs with the observation that the right ventricular outflow tract is a common site of origin of ventricular arrhythmias in individuals with BrS.

Catecholaminergic Polymorphic VT

CPVT is a highly malignant heritable arrhythmia syndrome characterized by bidirectional or polymorphic VT during physical or emotional stress.¹⁰⁰ The baseline ECG is often normal, and diagnosis is therefore mainly based on the occurrence of arrhythmia during exercise stress-testing or Holter recording. When left untreated, CPVT leads to significant mortality rate (30% SCD before the age of 40 years).^{101,102} In the presence of a clear diagnosis, a *RYR2* mutation, with autosomal dominant inheritance, is found in \approx 60% of patients with CPVT.^{103–105} These mutations cluster predominantly around 3 specific regions of the ryanodine receptor 2 (RyR2) protein (Figure 6); however, \approx 10% to 15% of mutations still occur outside these clusters.^{105,106} Mutations in *CASQ2* cause a less common but more severe autosomal recessive form of CPVT.¹⁰⁷ In families, individuals carrying homozygous or compound heterozygous

CASQ2 mutations are affected by a severe form of the disease, whereas the heterozygous family members are asymptomatic or mildly affected.^{108–110}

The *RYR2* gene encodes the sarcoplasmic reticulum (SR) Ca^{2+} channel called RyR because of its affinity for binding the alkaloid ryanodine. It is a large 560-kDa protein that localizes in the membrane of the SR in areas facing the T-tubules where the L-type calcium channels are located. The *CASQ2* gene encodes calsequestrin-2, a protein that binds free calcium inside the SR. Recent data have demonstrated that cardiac calsequestrin modulates the function of RyR2 by acting as a luminal calcium sensor.¹¹¹ Mutations in *RYR2* and *CASQ2* result in diastolic calcium release from the SR and the development of triggered arrhythmias. Recently, 2 other genes involved in calcium homeostasis, namely *CALM1* and *TRDN*, have been implicated as a cause for CPVT, respectively, in a linkage and candidate gene approach^{112,113}; these data await confirmation in larger series to define whether *CALM1* and *TRDN* should be regarded as causative for new dominant and recessive CPVT variants. Mutations in *ANKB* and *KCNJ2* that also cause, respectively, type 4 LQTS and the Andersen–Tawil syndrome have been described in a few patients with normal QTc-interval and a CPVT phenotype^{114,115}; the issue whether they cause CPVT or they are CPVT phenocopies has not yet been resolved.

Genotype–phenotype correlations in CPVT are scanty. One study suggested that patients with CPVT carrying a *RYR2* mutation in the C-terminal portion of the protein are at higher risk of developing nonsustained VT compared with N-terminal domain mutation carriers.¹¹⁶ The data, however, have not yet been implicated in recommendations for clinical practice and await confirmation.

Considering the severity of the CPVT phenotype and the evidence that cardiac arrest at young age may be the first manifestation of the disease,¹⁰² genetic testing has a major role

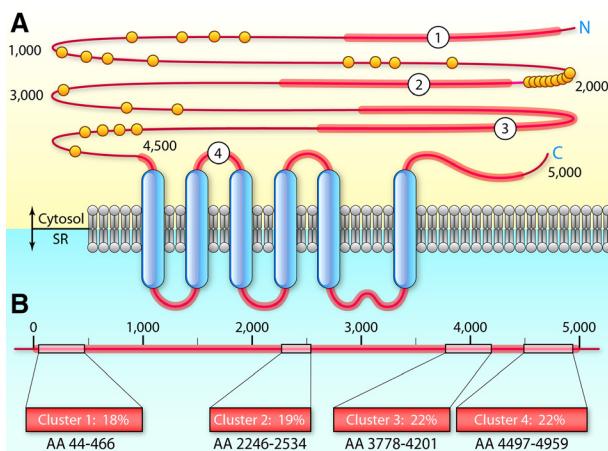


Figure 6. Structure and mutational clusters of RyR2. **A,** Clusters with frequent mutations are depicted with their location along the protein. Clusters are represented by numbered red lines. Individual mutations outside the canonical clusters are depicted as yellow dots. **B,** Clusters are represented by boxes, with the amino acid (AA) range of the clusters and the percentage of published mutations for each cluster (AA numbering refers to the human RyR2 protein sequence). The numbers in **A** correspond to the clusters shown in **B**. SR indicates sarcoplasmic reticulum (Illustration credit: Ben Smith).

in preventing sudden death. Accordingly, after detection of a causal mutation in the proband, first-degree relatives should be offered genetic testing allowing presymptomatic diagnosis and the initiation of preventive measures (eg, β -blocker therapy).¹⁴

Arrhythmogenic Mechanisms

CPVT is a disease caused by abnormal release of calcium from the SR. In the normal heart, Ca^{2+} transients develop after the onset of an action potential when calcium entering through the L-type calcium channels promotes release of calcium from the SR. On the contrary, in CPVT, the presence of mutations in the causative genes modifies the functional properties of the encoded proteins leading to spontaneous Ca^{2+} transients. Thanks to the presence of several mouse models of CPVT that recapitulate the clinical phenotype of the disease, the understanding of arrhythmogenic mechanisms has advanced rapidly.¹¹⁷ We now know that mutations in the *RYR2* and *CASQ2* genes lead to spontaneous diastolic Ca^{2+} release that by increasing Ca^{2+} levels in the cytosol activates the $\text{Na}^+/\text{Ca}^{2+}$ antiporter. In the attempt to normalize Ca^{2+} levels, the $\text{Na}^+/\text{Ca}^{2+}$ antiporter extrudes Ca^{2+} ; the extrusion of a Ca^{2+} ion is accompanied by the influx of 3 Na^+ ions into the cell by the antiporter, generating a transient inward current (I_{ti}) that depolarizes the myocytes generating delayed after depolarizations.¹¹⁷ Interestingly, the availability of animal models and of mechanistic understanding of the disease have allowed the advancement of therapeutic strategies. Watanabe et al¹¹⁸ reported that flecainide is able to specifically attenuate calcium release by binding the RyR and by doing so can inhibit triggered activity. Liu et al,¹¹⁹ on the contrary, demonstrated that flecainide does not inhibit development of delayed after depolarizations, but it reduces the probability of delayed after depolarizations to trigger an action potential by blocking I_{Na} . Collectively these studies supported the therapeutic value of flecainide therapy in the management of CPVT, a concept that has been confirmed in the clinic.¹²⁰ An interesting advancement in the management of this disease comes from studies performed in *Casq2* knockin mice in which gene therapy has been attempted. The authors have used an adenoassociated viral vector to replace wild-type *Casq2* leading to the abolishment of life-threatening arrhythmias and on several disease markers including ultrastructural abnormalities.¹²¹ As we will also see in the section dedicated to hypertrophic cardiomyopathies, the *in vivo* modification of the genetic defect for therapeutic purposes is a novel and fascinating development in the field.

Other Heritable Electric Disorders Associated With SCD

Idiopathic VF is defined as spontaneous occurrence of VF in the absence of known causes that may lead to cardiac arrest and therefore remain unexplained. The term “idiopathic VF” is used in the presence of a negative autopsy in a victim of sudden cardiac death; therefore, the term “IVF” may be used for victims of a variety of causes that cannot be identified postmortem such as electrolyte abnormalities, proarrhythmic effect of drugs, or abuse of recreational drugs, and diseases that are poorly characterized such as short coupled torsades des pointes.¹²² To date, 1 study from the Netherlands revealed

a founder haplotype involving the *DPP6* gene underlying idiopathic VF in several families.⁴³ Testing for this haplotype allows the identification and presymptomatic treatment of family members at risk. The early repolarization (ER) syndrome can be diagnosed in patients with VF or polymorphic VT and an ER pattern on the ECG. However, a conservative approach is recommended because of the high prevalence of this ECG pattern in the general population.⁸ Recently, a study of 4 large French families with SCD and ER syndrome reported that the ER syndrome pattern can be inherited as an autosomal dominant trait associated with the occurrence of SCD.⁴² Rare variants in *KNCJ8*^{123,124} and mutations in L-type calcium channels (*CACNA1C*, *CACNB2B*, and *CACNA2D1*),¹²⁵ as well as *SCN5A*,¹²⁶ have been linked to idiopathic VF and ER in sporadic cases through candidate gene approaches. Cardiac conduction disease (CCD) is characterized by unexplained (progressive) cardiac conduction abnormalities and may lead to syncope and sudden death.¹²⁷ The majority of familial clustering of CCD in the absence of structural heart disease is caused by mutations in *SCN5A*.^{14,127} One study associated mutations in *SCN1B* to CCD and BrS.⁸⁶ Mutations in *TRPM4* have been reported in several families affected by CCD.^{128,129} Sinus node disease has been associated with *SCN5A* and *HCN4* mutations.^{130,131} Recently, 2 simultaneous studies associated loss-of-function mutations in *HCN4* with sinus node disease, arrhythmias, and left ventricular noncompaction, in patients with or without mitral valve prolapse.^{132,133}

Inherited Cardiomyopathies

The inherited cardiomyopathies are classified based on functional and morphological features and include HCM, dilated cardiomyopathy (DCM), and arrhythmogenic cardiomyopathy (ACM, also known as arrhythmogenic right ventricular cardiomyopathy).¹³⁴ The great majority of genes identified, to date, for these disorders encode desmosomal, sarcomeric, cytoskeletal, and nuclear envelope proteins (Figure 2).

Hypertrophic Cardiomyopathy

HCM is characterized by unexplained ventricular hypertrophy often with predominant involvement of the interventricular septum, and by myocyte disarray and fibrosis.¹³⁵ The annual mortality from SCD alone is low ($\leq 1\%$) in most patients. Nevertheless, a small subset of patients have a much higher risk of SCD.¹³⁶ The disorder, which is commonly inherited in an autosomal dominant fashion, has been predominantly linked to mutations in genes encoding components of the sarcomere. A sarcomere gene mutation is identified in 50% to 60% of cases, with the *MYBPC3* or *MYH7* gene being most commonly involved.^{137,138} Albeit with varying evidence for causality, several genes encoding nonsarcomeric proteins have also been reported in patients with HCM, including Z-disk proteins (eg, *ACTN2* and *MYOZ2*) and intracellular calcium modulators (eg, *JPH2*).¹³⁹ Several distinct disease entities, such as Pompe disease (*GAA*), Danon disease (*LAMP2*), left ventricular hypertrophy with Wolff–Parkinson–White syndrome (*PRKAG2*), Fabry disease (*GLA*), and familial amyloidosis (*TTR*), bear similarity to HCM because of presentation of left ventricular hypertrophy, but differ among others in inheritance (some are X-linked or recessive), pathophysiology,

and in extracardiac features. In spite of many efforts in this regard, robust relationships between genotype (affected gene or mutation type and location) and phenotype in HCM are largely lacking. A recent large meta-analysis of 18 different studies (corresponding to 13 distinct cohorts and 2459 patients) uncovered an earlier disease presentation, more severe hypertrophy, and a higher prevalence of family history of the disease and SCD among sarcomere-mutation positive patients versus sarcomere-mutation negative patients.¹⁴⁰ Approximately 5% of HCM cases have double (or compound) mutations in sarcomeric genes, and these individuals are especially prone to worse phenotype and higher risk of SCD, independently of the ventricular wall thickness and other conventional risk factors.¹⁴¹ These observations add to the hypothesis that double (or compound) mutations may confer a gene dose effect in HCM disease severity. An interesting advancement in the study of HCM is represented by the recent report¹⁴² that delivery of adenoassociated virus-9-mediated RNAi was able to preferentially suppress the expression of the R403Q mutant allele of the myosin heavy chain *Myh6* over the wild-type allele in a mouse model of HCM, by directly targeting the mutation or a nearby SNP. Data showed that a mere 30% of reduction of the mutant allele was able to abrogate cardiac hypertrophy. These data opened the concept that, in analogy with what has been described for recessive CPVT,¹²¹ gene therapy may become a promising therapeutic approach for inherited cardiomyopathies.

Dilated Cardiomyopathy

DCM is characterized by left ventricular dilatation, systolic dysfunction, and fibrosis, and besides SCD is associated with heart failure and thromboembolism.¹⁴³ Familial disease is observed in 20% to 35% of cases initially diagnosed as idiopathic DCM.¹⁴⁴ Familial DCM is mainly inherited as an autosomal dominant trait; autosomal recessive, mitochondrial (maternal transmission), or X-linked inheritance are less common.^{139,145} DCM is genetically heterogeneous with >30 disease genes being reported to date. These genes encode a wide range of proteins, with the following 4 genes accounting for the majority of genotype positive cases: titin (*TTN*), lamin A/C (*LMNA*), β -myosin heavy chain (*MYH7*), and cardiac troponin T (*TNNI2*).¹⁴⁵ Mutations are identified in $\approx 30\%$ of patients with familial disease.¹⁴ A screen of the *TTN* gene in a cohort of 312 patients with DCM identified truncating mutations in 18% of sporadic DCM and 25% of familial DCM although 3% of controls also carried a *TTN* truncating variant.¹⁴⁶ A recent screen across a broad panel of 84 genes (including *TTN*) in 639 patients with familial and sporadic DCM identified compound or combined mutations >38% of patients and ≥ 3 mutations in 12.8%.¹⁴⁷ DCM can present together with prominent CCD and arrhythmia. This combined presentation is caused primarily by mutations in *LMNA*, *DES*, or *SCN5A*,^{148–150} and consequently the yield of genetic testing of this particular clinical variant of DCM is particularly high.¹⁴ Detection of an *LMNA* mutation allows for monitoring of carriers for preclinical signs of conduction disease and timely intervention with a pacemaker or an implantable defibrillator because of their higher risk of malignant arrhythmias.¹⁵¹ DCM may also present as a part of the clinical picture of several multisystem syndromic

disorders (eg, Barth syndrome, Emery–Dreifuss Muscular Dystrophy, Duchenne or Becker Muscular Dystrophy, Limb–Girdle Muscular Dystrophy, and Myofibrillar Myopathy).^{14,152}

Arrhythmogenic Cardiomyopathy

ACM is a progressive disease that leads to structural changes involving cardiomyocyte loss and fatty infiltration and is clinically associated with systolic impairment, dilatation, features of heart failure and SCD at young age.¹⁵³ Although it has been classically considered to affect the right ventricle, the increasing awareness of the disease has led to the recognition of forms that present with left ventricular or biventricular involvement: for this reason, the original name of arrhythmogenic right ventricular cardiomyopathy has now been abandoned. ACM is an autosomal dominant disorder, and it has been estimated that ≈30% to 50% of patients harbor a putative mutation in 1 of 5 genes encoding desmosomal proteins: plakophilin (*PKP2*), desmoplakin (*DSP*), plakoglobin (*JUP*), desmoglein-2 (*DSG2*), and desmocollin-2 (*DSC2*), with the most commonly involved being *PKP2*.¹⁵⁴ The yield of genotyping is variable, and the occurrence of founder mutations may increase the yield of genotyping in selected regions. Interpretation of the pathogenic role of missense mutations in genes encoding desmosomal proteins is complicated by the evidence that ≤16% of control individuals harbor missense variants that would meet clinical criteria for a so-called positive genetic test result.¹⁵⁵ Nondesmosomal genes, namely *DES*, *TMEM43*, and *PLN*, have also been implicated in the disorder but whether they are causative of arrhythmogenic right ventricular cardiomyopathy or represent its phenocopies is unknown.

In analogy with LQTS also ACM presents in the setting of clinical variants with systemic involvement such as the recessively inherited cardiocutaneous syndromes Naxos syndrome¹⁵⁶ and Carvajal syndrome,¹⁵⁷ manifesting in the skin and heart and caused, respectively, by recessive mutations in the *JUP* and *DSP* genes. Mutation-specific testing is recommended when a genetic diagnosis of ACM is made in a family member to determine the possible risk in close relatives.¹⁴ In general, such as HCM and the primary electric disorders, patients carrying >1 mutation usually have a more severe disease phenotype.¹⁵⁸

Arrhythmogenic Mechanisms in the Cardiomyopathies

The abnormal structure of the heart is generally considered to form the substrate for arrhythmia in patients with cardiomyopathies, and mechanistic studies on genes causing the cardiomyopathies have largely focused on pathways involved in the cardiomyopathic process. The fact that some patients have arrhythmia early in the course of the disease or in the absence of gross structural remodeling has, however, prompted some investigators to hypothesize that the gene defect underlying the cardiomyopathy may also affect other arrhythmia susceptibility pathways. For example, studies in mice have implicated changes in calcium handling in predisposition to arrhythmia in troponin T-associated HCM.¹⁵⁹ Similarly, desmosomal gene mutations have been proposed to affect arrhythmia susceptibility through effects on sodium channel function and conduction slowing.^{160,161}

Next-Generation Sequencing for Diagnostics and Gene Discovery

The advent of next-generation sequencing has led to a shift in most DNA diagnostic laboratories, from the screening of single genes or small panels of genes to testing of large, multigene panels consisting of tens of genes, for example, those encompassing all genes implicated in the primary electric diseases or the cardiomyopathies. Although such comprehensive multigene panels may be useful in that, they may increase diagnostic yield particularly in disorders that are genetically and clinically heterogeneous like DCM; their use presents a huge interpretive challenge in that they uncover more variants of unknown significance. This was illustrated clearly in a recent study on DCM that showed that although clinical sensitivity increased from 10% to 37% as gene panel sizes increased from 5 to 46 genes, the number of inconclusive cases (only variants of unknown significance detected) also increased from 4.6% to 51%.¹⁶²

Related to this is the fact that next-generation sequencing has also enabled large-scale surveys of genetic variation in tens of thousands of unrelated individuals sequenced as a part of various disease-specific and population genetic studies (now assembled in the Exome Aggregation Consortium initiative).¹⁶³ The availability of these databases has brought with it the realization that many variants originally thought to be disease-causing based on their absence in modestly sized control cohorts could possibly be rare or low-frequency benign variants.¹⁶⁴ Besides pointing to inaccurate variant-disease associations in the literature, this also possibly points to inaccurate gene-disease associations in some cases, calling for a systematic reassessment, or at least a critical evaluation, of some previously assigned gene-disease associations, particularly those reported in single probands or single small families. Establishing the likely deleteriousness or not of variants identified in the setting of clinical genetic testing would clearly benefit from the sharing of genotypic and (expert-curated) clinical data among clinical centers through centralized depositories.

About gene discovery by next-generation sequencing (exome or whole genome sequencing), a recent article by a working group of experts in genomic research, convened by the US NHGRI, stressed the importance of study design.¹⁶⁵ In keeping with the history of the field of human genetics, they emphasized the critical primacy of robust statistical genetic support. In the absence of large pedigrees that preclude the establishment of statistical significance using linkage data, declaring significance should entail demonstration that the gene concerned shows statistical excess of rare probably damaging variants in cases compared with controls (eg, burden testing).¹⁶⁵ The likely complex genetic architecture of the primary electric diseases and cardiomyopathies that have to date defied genetic elucidation will necessitate the latter approach for robust gene discovery in these disorders. The rarity of the individual disorders will likely require international collaboration to achieve sufficiently large patient sets.

Yet, such an approach may, however, not be possible in all diseases, particularly the rare ones or those that have a high degree of locus heterogeneity. In such cases, suggestive

evidence pointing to a gene's potential implication can nevertheless be valuable in future clinical and research investigations. Genes identified in this way would, however, need to be approached in the light of the robustness of supporting evidence and confidence in causality (eg, based on involvement in multiple patients and functional studies in suitable model systems).¹⁶⁵

SCD in Complex Acquired Cardiac Disease

In the older segment of the population, SCD largely occurs in the setting of sequela of coronary artery disease, namely acute ischemia, acute myocardial infarction (MI), or structural alterations such as scar formation or ventricular dilatation after ischemia or infarction.¹⁶⁶ In these conditions, the cardiac substrate predisposing to SCD is further affected by other comorbidities present in this age group, such as hypertension and diabetes mellitus. All these are in turn considerably influenced by environmental factors (eg, smoking and diet) and thus risk for SCD in these pathologies is likely governed by a large environmental component. Yet several studies have provided convincing evidence for the existence of heritable factors in the determination of SCD risk.¹⁶⁷⁻¹⁷¹ Such evidence was first reported in 2 studies published in the late 1990s.^{167,168} These studies, which included cases of SCD regardless of the underlying cardiac pathology, demonstrated that a family history of SCD was an independent risk factor for SCD. Two subsequent studies provided similar evidence specifically in patients presenting with VF/SCD during first acute MI.^{170,171} Crucially, all these studies distinguished between inherited predisposition to SCD from inherited predisposition to coronary artery disease. Yet, although this rationalized the search for genetic factors, progress in their identification has to date been limited and the genetic architecture of VF/SCD predisposition in this setting remains largely unknown.

Here, the slow progress in uncovering genetic risk factors is essentially rooted in the challenges faced in conducting genetic studies on this phenotype. These include most notably the paucity of VF/SCD patient sets and the varying level of characterization across the available sets that hinder both genetic locus discovery and replication efforts. Accurate phenotyping is essential both at the level of the arrhythmia (not all SCD cases are arrhythmic in origin) and at the level of the cardiac pathology wherein it occurs. The latter is relevant because arrhythmia in different pathologies may stem from different mechanisms, which may have different genetic underpinnings. Several early candidate gene studies implicated common and rare variants in candidate genes in risk (reviewed in Marsman et al³¹), yet for the most part these have not been followed by replication efforts. Two genome-wide association studies have reported association of common genetic variants with VF/SCD at genome-wide statistical significance ($P < 5 \times 10^{-8}$). One study, conducted in the AGNES case-control set, which compared patients with or without VF in the setting of a first ST-segment-elevation MI, identified variation at rs2824292 near the *CXADR* gene as a risk factor for VF.¹⁷² The involvement of *CXADR*, encoding the coxsackie and adenovirus receptor, at this locus is supported by the demonstration that rs2824292 affects *CXADR* transcript abundance in human heart and by data in mice, which uncovered slower cardiac

conduction and arrhythmia vulnerability during ischemia in mice haploinsufficient for coxsackie and adenovirus receptor.¹⁷³ The other study entailed a meta-analysis of GWAS data in SCD cases versus general population controls from 5 community-based cohorts.¹⁷⁴ This study detected an association signal at rs4665058 in *BAZ2B*, which encodes the bromodomain adjacent zinc finger domain 2B. *BAZ2B* and other genes in the vicinity of rs4665058 are expressed in heart, yet the causal gene at this locus and the way it affects arrhythmia susceptibility is unknown. Although these 2 GWAS studies uncovered associations at genome-wide statistical significance, replication efforts, both within these studies and outside, have been limited and findings have been inconsistent.^{172,174,175} Additional studies are needed to explore the possibility that inconsistent findings may stem from SNP effects that are restricted to specific cardiac pathologies. As expected, the common genetic variants that have been identified have a small effect on risk precluding their immediate clinical utility, yet as for *CXADR*, they may highlight new molecular mechanisms underlying susceptibility to VF.¹⁷³ Rare variants that may carry larger effects are also expected to contribute to VF/SCD risk; their study will, however, require larger samples necessitating the continued expansion of VF/SCD patient sets.

GWAS on ECG and Cardiac Structure and Function Parameters

Large consortia have pursued genome-wide association analysis for the systematic identification of common SNPs that govern interindividual variability in ECG parameters in the general population. The concept behind this approach is that these parameters, which clearly have a heritable component and are therefore modified by genetic factors, constitute intermediate phenotypes of arrhythmia.¹⁷⁶ For instance, the QRS-interval reflects ventricular depolarization, and its duration is a function of electrophysiological properties within the His-Purkinje system and the ventricular myocardium. Slowed conduction is an established mediator of arrhythmia¹⁷⁷ and therefore genetic factors that modulate the QRS interval may also affect arrhythmia risk. Similar arguments apply for other ECG parameters, such as the QTc-interval. This notion is also supported by studies in the general population and in patients with specific cardiac diseases, which have shown that prolonged QTc or QRS intervals could be a risk factor for SCD.^{35,178-181} As expected, these GWAS studies have identified loci within or in the vicinity of genes already implicated in cardiac electric function and arrhythmia (Figure 4). However, they have also identified numerous loci that do not harbor known genes. This is an important (although often underappreciated) aspect of the utility of GWAS, in that it has the potential of uncovering new biological insights. This is relevant when one considers the fact that beyond knowledge of the specific ion channels mediating the various ionic currents in the heart, our comprehension of the higher-order regulators of cardiac electric function (such as the regulators of ion channels) remains rudimentary. With few exceptions, GWAS-identified loci that were not previously linked to cardiac electric function are yet untapped for gene identification and mechanistic insight.¹⁸²⁻¹⁸⁴ In conducting functional studies on genes from GWAS loci (Figure 7), many considerations should be taken into account

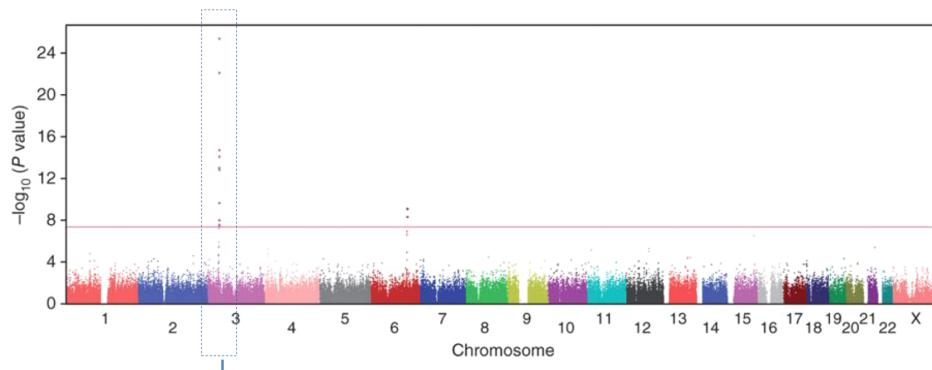
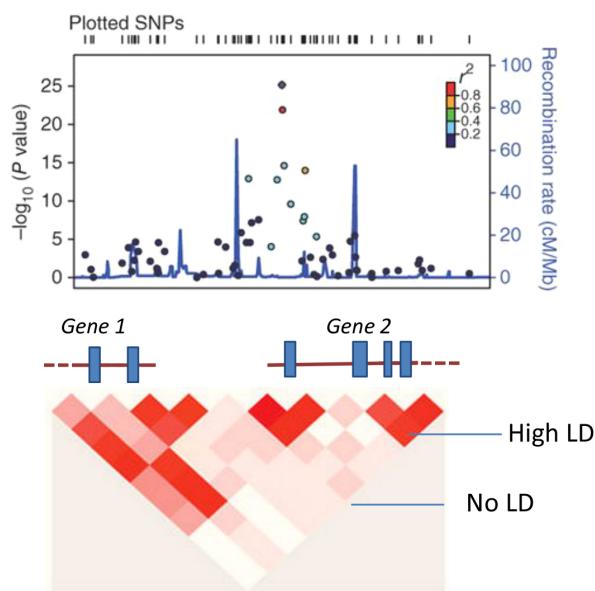
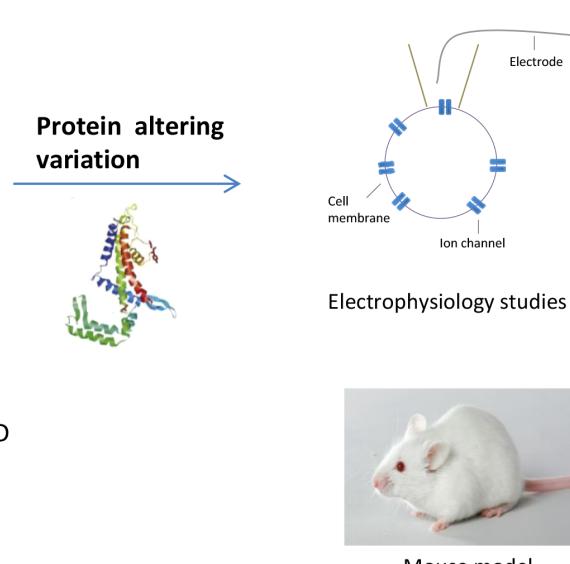
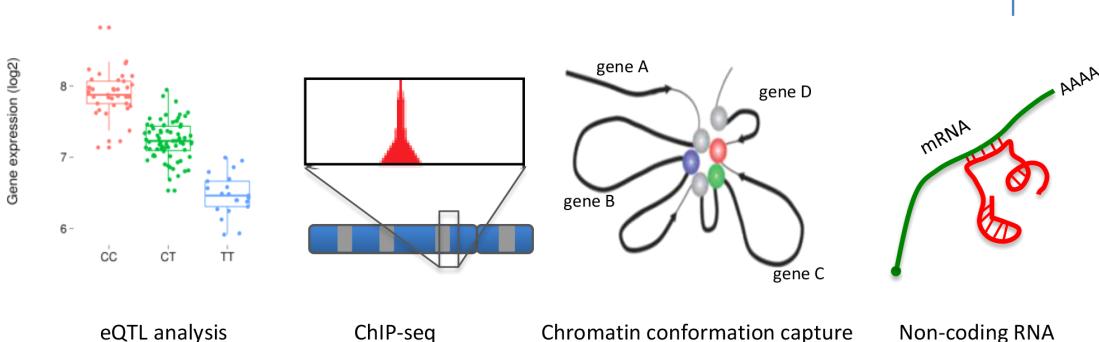
A Manhattan plot showing the association of SNPs with trait**B** Association plot for lead SNP**D** Functional studies**C** Genetic studies for regulatory variation

Figure 7. A and B, Follow-up studies on loci identified in genome-wide association studies. In the absence of a candidate coding region variant that can be tested directly in functional studies (**D**), studies on noncoding putatively regulatory genetic variation may entail experimental approaches (eg, expression quantitative trait loci [eQTL], ChIP-seq, circularized chromosome conformation capture, and noncoding RNA analyses) for elucidation of the genetic mechanism and the regulated gene. LD indicates linkage disequilibrium; and SNP, single-nucleotide polymorphism.

at the outset. Rather than a specific variant, GWAS illuminates a haplotype and any SNP on that haplotype that is in linkage disequilibrium with the lead-SNP from GWAS could

in theory mediate the observed effect (Figure 7B). In some cases, haplotypes harbor SNPs that lead to amino acid altering changes in the encoded protein that can be directly tested in

functional studies. However, as for GWAS in general, most of the SNPs/haplotypes associated with ECG parameters occur in noncoding or intergenic regions and are expected to affect the trait through effects on gene expression.¹⁸⁵ In such cases, linking the SNP/haplotype to variability in the expression of a particular gene in heart could assist the process of prioritization of genes from GWAS loci for functional studies. This will become increasingly possible because the expression quantitative trait loci resources in human heart continue to expand.^{186,187} Furthermore, the rapid developments in functional annotation of noncoding regulatory elements of the genome (eg, enhancers¹⁸⁸ and noncoding RNAs¹⁸⁹) and in related technologies (eg, ChIP-seq, chromosome conformation capture) will continue to increase our ability to understand the genetic mechanisms operative at these loci.¹⁹⁰ An example of how such integrative approaches could facilitate this comes from the recent collaborative work of many groups on the genetic mechanism of rs6801957 located intronically in *SCN10A*.^{184,191} This work provided compelling evidence that this SNP affects the associated traits (PR, QRS, BrS, and MI-induced VF),³¹ at least in part, by reducing *SCN5A* expression as a consequence of altered transcription factor binding to an enhancer element containing this SNP.

Although cardiac structure and function are heritable¹⁹² and are established intermediate phenotypes for cardiovascular disease outcomes including SCD,³⁶ the pace of locus discovery by GWAS for these traits has been slower than observed for ECG traits.^{32–34} This is likely related to obstacles encountered in the acquisition of these phenotypes with technologies used to date.¹⁹³

Conclusions

Although major advancements have been made in uncovering the genetic underpinnings of inherited cardiac disorders associated with SCD, significant challenges remain. Some of the rare disorders have to date defied genetic elucidation, with the known genes only accounting for a small proportion of patients. We envisage that the likely complex genetic architecture of these disorders will necessitate gene discovery approaches that are fundamentally different than those applied to date and that will entail genome-wide association analysis of genetic variants across the entire spectrum of allele frequencies and effect sizes. Similar approaches are likely to uncover genetic factors that modulate disease expression in patients with disorders that are (near)-Mendelian. With respect to genetic studies on SCD susceptibility in the setting of complex cardiac pathologies, such as acute MI and heart failure, considering the clinical heterogeneity in these pathologies and associated comorbidities, this area will likely benefit from the continued construction of large deeply phenotyped cohorts. With respect to the large yield of genetic loci from GWAS on ECG parameters, although these are currently not of imminent clinical utility, they have provided the cardiac research community with multiple leads for research that may provide new insight into cardiac electric function.

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Disclosures

None.

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