13 Genetic Testing for Inheritable Cardiac Channelopathies

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Abstract

Inheritable cardiac channelopathies (ICC) are defined as primary electrical disorders without identifiable cardiac structural abnormalities and are mostly encountered in young adults (under 40 years). Diagnosis of ICC is often established after the first symptoms such as recurrent palpitations and syncope or more dramatically after unexplained sudden cardiac death (SCD). In this context, familial clinical screening coupled with genetic testing are required to prevent additional (fatal) arrhythmia events in relatives. This review presents an update of the ICC-associated genes and proposes a screening hierarchy according to the phenotype. The impact of the new sequencing technologies on the genetic testing as well as on the patient management will be also discussed.

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13.1 Genetic Testing and Cardiac Channelopathies

Genetic diagnostics were proposed 20 years ago when the first associated channelopathy genes were discovered (Wilde and Behr 2013). Today, genetic information is used to confirm a clinical diagnosis and as a powerful preventive tool to identify family members at risk of developing electrical disorders despite having a normal ECG. Genetic diagnostics are currently performed on six highly phenotypically characterized channelopathies such as Brugada syndrome (BrS), cardiac conduction defects (CCD), long QT syndrome (LQTS), short QT syndrome (SQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular cardiomyopathy (ARVC). These channelopathies present specific ECG indices at baseline or are unmasked by provocative tests that are often coupled with a singular circumstance of an arrhythmic event outcome. Such clinical information guides genetic testing towards candidate genes harbouring the highest rate of causal mutations. Therefore, the added value of molecular diagnostics could be inherent in the preventive screening of relatives who are either negative or borderline after clinical evaluation. Furthermore, according to the gene identified as being associated with the channelopathy, therapy and care can be adapted accordingly (Imbrici et al. 2016). However, in certain cases, such as young patients presenting idiopathic ventricular fibrillation or (resuscitated) sudden cardiac death, neither ECG indices nor relatives can be identified. In this context, genetic testing could uncover variant(s)/gene(s) and guide clinicians to a diagnosis. However, extreme caution must be taken vis-à-vis our ability to interpret rare genetic variants without supporting clinical and functional or familial segregation data (Wijeyeratne and Behr 2017).

13.2 Present Context of Genetic Testing

In the last few years, the number of genes associated with arrhythmias has considerably increased. However, the major genes that account for a large fraction of the cases remain limited. “Minor” genes are usually poorly characterized in terms of function and pathophysiological role. As a consequence, the identification of variations in these genes often leads to results that are difficult to interpret. Genetic testing interpretation is also particularly challenging owing to the overlapping, variable and incompletely penetrant nature of the clinical presentations of the channelopathies.

Next-generation sequencing (NGS) technologies rapidly developed in diagnostic laboratories and have become a routine approach. However, the sequencing scale is a matter of debate. Indeed, the technologies allow to focus the screening on panels of 20–200 genes, explore the whole coding region of the genome (whole exome sequencing (WES)) or even give access to the whole genome of a patient. Target resequencing panels (TRP) is commonly employed for diagnostic purposes given the reasonable cost, high sequencing coverage and limited interpretation and ethical issues. Different TRP strategies can be applied from a cardiac panel with 60–180

genes covering "all" (channelopathies and cardiomyopathies) known genes or an arrhythmia panel focused on 20–60 genes directly associated with genetic electrical defects or even a restricted panel containing only the "key" genes corresponding to the most prevalent cardiac arrhythmia genes. The use of small panel focusing on well-characterized genes aims to reduce the number of genes tested and then limit the identification of variant with uncertain interpretation. The pros and cons related to the different approaches using NGS technologies are discussed further.

13.3 Genetic Testing for Inheritable Cardiac Channelopathies: Daily Practice

13.3.1 Brugada Syndrome (BrS)

13.3.1.1 Clinical Description

Brugada syndrome (BrS) was first described in 1992 (Brugada and Brugada 1992) and was based on a familial form of ventricular fibrillation associated with a singular ECG pattern. The latest guidelines define a diagnosis of BrS diagnostic as follows: "patients with ST-segment elevation with type 1 morphology $\geq$2 mm in one or more leads among the right precordial leads V1 and/or V2 positioned in the second, third, or fourth intercostal space, occurring either spontaneously or after provocative drug test with intravenous administration of sodium channel blockers" (Priori et al. 2015). The prevalence of BrS is estimated at approximately 0.05% in Europe and is higher in Asia (0.12%)—particularly in Thailand, the Philippines and Japan. The mean age on diagnosis is 40. BrS predominantly affects (80%) males after puberty (Andorin et al. 2016) and is associated with sudden cardiac death occurring mostly at rest (Tomaselli and Barth 2016).

13.3.1.2 Genetic Testing

The first gene identified was the *SCN5A* gene encoding for the major cardiac sodium channel NaV1.5 (Chen et al. 1998). The prevalence of mutations in *SCN5A* is from 20 to 25% of the cases and remains the major gene in BrS (Crotti et al. 2012; Le Scouarnec et al. 2015). So far, 24 genes have been described as harbouring rare genetic variations in BrS patients (Table 13.1). They can be categorized in three main groups according to whether they affect the sodium (I_{Na}; *SCN5A, GPD1L, SCN1B, SCN3B, RANGRF, SCN2B, PKP2, SLMAP, SCN10A* and *FGF12*), potassium (I_K; *KCNJ8, KCNH2, KCNE3, KCND3, KCNE5, KCND2, SEMA3A, ABCC9* and *KCNAB2*) or L-type calcium ($I_{Ca\text{-}L}$; *CACNA1C, CACNB2B* and *CACNA2D1*) currents. The last two associated genes affect non-selective channels (*HCN4* and *TRPM4*). It should be noted that among the BrS-associated genes, only two (*GPD1L* and *KCNAB2*) have been uncovered by the powerful and hypothesis-free familial approach. Moreover, apart from the *SCN5A* gene, the contribution of other genes remains extremely low or uncertain for some of them (Fig. 13.1) (Le Scouarnec et al. 2015).

Table 13.1 Genes identified in Brugada syndrome (BrS)

Type	Locus	Gene	Protéine, fonction/courant	Effet des mutations	Transmission	OMIM
BrS1	3p21	*SCN5A*	$Na_v1.5$ (alpha subunit sodium channel), depolarization/I_{Na}	$\downarrow I_{Na}$	AD	600163
BrS2	3p22.3	*GPD1L*	Glycerol-3-phosphate deshydrogenase 1-like	$\downarrow I_{Na}$	AD	611778
BrS3	12p13.3	*CACNA1C*	$Ca_v1.2$ (alpha1C subunit calcium channel), depolarization/$I_{Ca\text{-}L}$	$\downarrow I_{Ca\text{-}L}$	AD	114205
BrS4	10p12	*CACNB2*	Ca_{vB2b} (beta2b subunit calcium channel), depolarization/$I_{Ca\text{-}L}$	$\downarrow I_{Ca\text{-}L}$	AD	600003
BrS5	19q13.1	*SCN1B*	$Na_v\beta1$ (beta1 subunit sodium channel), depolarization/I_{Na}	$\downarrow I_{Na}$	AD	600235
BrS6	11q13-q14	*KCNE3*	MiRP2 (beta subunit potassium channel), repolarization/I_{to}	$I_{to/IKs}$	AD	604433
BrS7	11q23.3	*SCN3B*	$Na_v\beta3$ (beta3 subunit sodium channel), depolarization/I_{Na}	$\downarrow I_{Na}$	AD	608214
BrS8	15q24-q25	*HCN4*	Hyperpolarization-activated cyclic nucleotide-gated potassium channel 4/if current	Courant de fuite	AD	605206
BrS9	1p13.3	*KCND3*	Kv4.3 (alpha subunit potassium channel), repolarization/I_{to}	I_{to}	AD	605411
BrS10	12p11.23	*KCNJ8*	Kir6.1 (ATP potassium channel)/I_{KATP}	I_{KATP}	AD	600935
BrS11	7q21-q22	*CACNA2D1*	$Ca_{v2\delta1}$ (alpha2d1 subunit calcium channel), depolarization/$I_{Ca\text{-}L}$	$\downarrow I_{Ca\text{-}L}$	AD	114204
BrS12	Xq22.3	*KCNE5*	KCNE1-L (beta subunit potassium channel), repolarization/I_{to}	I_{to}/I_{Ks}	lié à l'X	300328
BrS13	17p13.1	*RANGRF/ MOG1*	Ran guanine nucleotide release factor/multicopy suppressor of Gsp1	$\downarrow I_{Na}$	AD	607954
BrS14	3p14.3	*SLMAP*	Sarcolemma-associated protein	$\downarrow I_{Na}$	AD	602701
BrS15	19q13.33	*TRPM4*	Transient receptor potential cation channel subfamily M member 4	↓ et NSCCa	AD	606936
BrS16	11q23	*SCN2B*	$Na_v\beta2$ (beta2 subunit sodium channel), depolarization/I_{Na}	$\downarrow I_{Na}$	AD	601327
BrS17	3q28-q29	*FGF12*	Fibroblast growth factor 12	$\downarrow I_{Na}$	AD	601513
BrS18	12p11	*PKP2*	Plakophilin-2	$\downarrow I_{Na}$	AD	602861

BrS19	12p12.1	*ABCC9*	ATP-sensitive potassium channels	I_{KATP}	AD	601439
BrS20	7q31.31	*KCND2*	Alpha subunit of the Kv4.2 potassium channel	I_{to}	AD	605410
BrS21	7q36.1	*KCNH2*	Kv11.1 (Alpha subunit potassium channel), repolarization/IKr	I_{Kr}	AD	152427
BrS22	7q21.11	*SEMA3A*	Semaphorin family protein	I_{to}	AD	603961
BrS23	3p22.2	*SCN10A*	Na_v1.8 (alpha subunit sodium channel)	$\downarrow I_{Na}$	AD	604427
BrS24	1p36.31	*KCNAB2*	Potassium voltage-gated channel subfamily A regulatory beta subunit 2	I_{to}	AD	601142

AD autosomal dominant, *AR* autosomal recessive

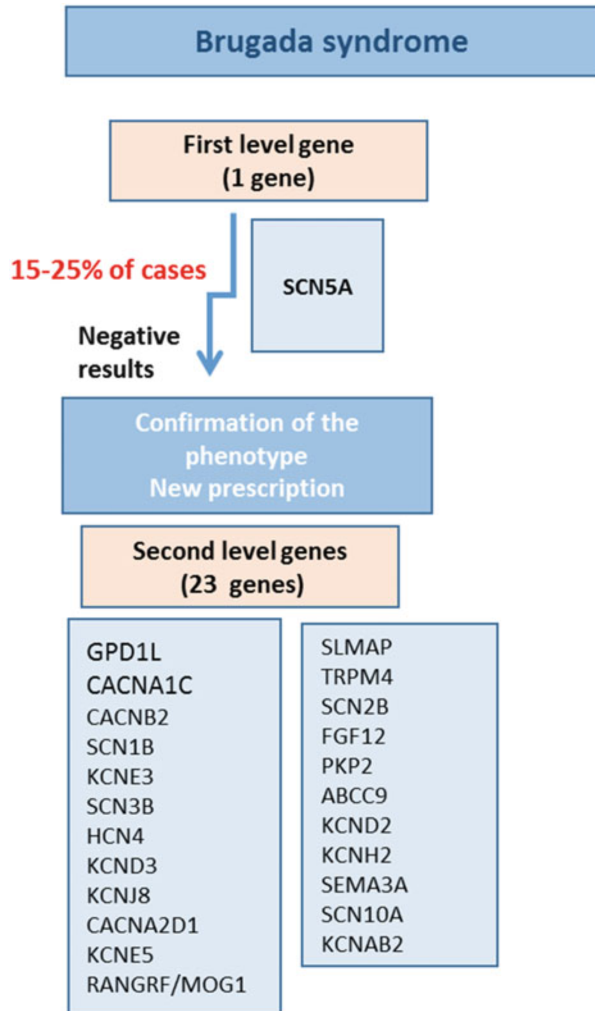

Fig. 13.1 Decision tree model for genetic testing in Brugada syndrome

The autosomal dominant model was the first genetic model proposed. However, the segregation study of *SCN5A* mutations among BrS pedigrees revealed a low penetrance and a variable expressivity of the pathology suggesting modulating factors (Probst et al. 2009). Despite the low predictive value of *SCN5A* status, the genotyping of this sodium channel remains the gold standard for BrS genetic testing since loss of function of the $Na_V1.5$ channel has been clearly implicated in BrS pathophysiology (Tan et al. 2003). Furthermore, clear evidence exists describing the genotype/phenotype relationship between *SCN5A* carriers and conduction defects increasing the risk of arrhythmia (Probst et al. 2010). All in all, approximately 300 distinct *SCN5A* mutations have been described in patients with the disorder.

Of these, two-thirds are missense variations and one-third are nonsense mutations, splice-site mutations and small insertions/deletions that lead to a truncated channel protein (Kapplinger et al. 2010). Other genes can be screened secondarily if *SCN5A* screening remains negative (see decision tree in Fig. 13.1), but extreme caution must be taken for variant interpretation since little evidence has been noted until now (Le Scouarnec et al. 2015).

Genetic testing plays a major role in the presymptomatic screening of BrS relatives. In the context of BrS families presenting a *SCN5A* mutation, genetic screening turns out to be of interest especially when ECG (even after provocative test) fails to diagnose the patient (Probst et al. 2009). Presymptomatic genetic testing appears to be even more pertinent in the context of early childhood when provocative tests using sodium blockers are not systematically performed for questionable relevance. Moreover, episodes of fever are frequent in the young, increase the risk of arrhythmia and emphasize the importance of identifying such individuals. Of note, a recent study on young BrS cases shows that genetic testing can uncover a higher proportion of *SCN5A* mutations (47%) than in adults—supporting the important role of genetic testing.

About 70% of BrS cases remain negative after genetic testing. The first explanation could be the fact that variants within the candidate genes cannot be detected with the sequencing technologies. Indeed, large rearrangements such as large deletion or insertion [also called copy number variation (CNV)] require additional methods for detection such as MLPA (multiplex ligation-dependent probe amplification). This investigation appears to be more and more performed (Selga et al. 2015; García-Molina et al. 2013; Koopmann et al. 2007), but few CNV have been reported so far (Eastaugh et al. 2011). Another hypothesis would be that additional genes could remain uncovered. Furthermore, a recent genome-wide association study suggested that BrS could follow a more complex genetic model than the Mendelian model commonly applied with the combination of common and rare variants of different size effects influencing the risk of developing BrS (Bezzina et al. 2013). Another hypothesis to explain the two-thirds of BrS cases with missing molecular diagnosis could be the presence of acquired Type I BrS ECG in the general population. Drugs have been identified as capable of inducing Type I BrS ECG—especially psychotropic and analgesic-anaesthetic drugs (Konigstein et al. 2016). An up-to-date list of drugs is accessible online to warn BrS patients about increasing their risk of arrhythmia with such drugs (http://www.brugadadrugs.org/) (Postema et al. 2009).

13.3.2 Long QT Syndrome (LQTS)

13.3.2.1 Clinical Description

Congenital long QT syndrome (LQTS) is a group of cardiac “channelopathies” characterized by delayed ventricular repolarization manifesting as QT interval prolongation on the ECG in the setting of a structurally normal heart (Morita et al. 2008). The prevalence of LQTS varies from 1/2000 to 1/5000 (Goldenberg and Moss 2008; Schwartz et al. 2009), with a female predominance (2/3 of the patients)

(Imboden et al. 2006). LQTS also shows variable expressivity and incomplete penetrance (Roden 2008). The first descriptions of the disease were provided by Jervell and Lange-Nielsen in 1957 (Jervell and Lange-Nielsen 1957) and by Romano and Ward (Ward 1964; Romano et al. 1963). Electrocardiographic findings are characterized by the presence of a prolonged heart rate-corrected QT interval on ECG (QTc). LQTS can be diagnosed in the presence of a QTc > 480 ms (Priori et al. 2015). The length of the QT interval is associated with the risk of syncope and sudden death. There is a high risk when QTc > 500 ms and an extremely high risk when QTc > 600 ms (Goldenberg and Moss 2008). The presence of T-wave alternans despite proper therapy is a sign of electrical instability and requires preventive measures. Patients with syncope or cardiac arrest before the age of 7, especially in their first year of life, have a higher risk of arrhythmias and sudden death (Priori et al. 2004; Spazzolini et al. 2009).

13.3.2.2 Genetic Testing

Genetic testing is part of the diagnostic criteria of LQTS (Priori et al. 2013). LQTS is most often inherited in an autosomal dominant manner (Romano-Ward syndrome) (Schwartz et al. 1993) and rarely in an autosomal recessive manner associated with sensorineural deafness (Jervell and Lange-Nielsen syndrome).

To date, more than 1200 pathogenic variations have been identified in 17 different genes (*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, KCNJ5, CALM1, CALM2, TRDN and TECRL*) (Table 13.2) (Tester and Ackerman 2014). A causal mutation is found in these genes in 80–85% of LQTS patients. However, LQTS1 (*KCNQ1*; 35% of the cases), LQTS2 (*KCNH2*; 30% of the cases) and LQTS3 (*SCN5A*; 10% of the cases) comprise 90% of the LQTS mutations (Fig. 13.2) (Tester and Ackerman 2014, 2008). The majority of LQTS-causing mutations are coding-region single-nucleotide substitutions or small insertions/deletions. However, a few large gene rearrangements involving single or multiple exons deletions/duplications have been described (Barc et al. 2011).

LQTS is probably the heritable arrhythmia syndrome for which the genotype-phenotype relationship has been the most understood in terms of clinical manifestations, risk stratification and response to therapy. The phenotype-genotype association could facilitate phenotype-directed genetic testing. Indeed, genotype can be inferred by thorough clinical evaluation: swimming, physical exertion or emotional stress cardiac events strongly indicate mutations in *KCNQ1*. Cardiac events triggered by auditoria stimulation strongly indicate mutations in *KCNH2*, and symptoms at rest or during sleep are generally observed in LQT3 (Schwartz et al. 2001). T-wave morphology could also help to distinguish different LQTS subtypes: LQT1 exhibits a broad-based T-wave, LQT2 exhibits a low-amplitude notched or biphasic T-wave and LQT3 exhibits a late-appearing T-wave. These gene-suggestive ECG patterns could be helpful in guiding genetic testing, but exceptions to these relatively gene-specific T-wave patterns exist. Of note, from 20% to 25% of the patients with a long QT syndrome confirmed by the presence of a mutation could have a normal QTc (Priori et al. 2003; Goldenberg et al. 2011).

Table 13.2 Genes identified in long QT syndrome (LQTS)

Type (OMIM)	Locus	Gene	Protein, function/current	Effect of mutations	Transmission	Frequency	OMIM
LQTS1	11p15.5-p15.4	*KCNQ1*	Kv7.1 (alpha subunit potassium channel), repolarization/I_{Ks}	↓ I_{ks}	Ad+AR	30–35%	607542
LQTS2	7q36.1	*KCNH2*	Kv11.1 (alpha subunit potassium channel), repolarization/IKr	↓ I_{kr}	AD	25–30%	152427
LQTS3	3p21	*SCN5A*	$Na_v1.5$ (alpha subunit sodium channel), depolarization/I_{Na}	I_{Na}	AD	5–10%	600163
LQTS4	4q25-q26	*ANK2*	Ankyrin B (membrane anchoring/adapter protein)	Loss of function	AD	<1%	106410
LQTS5	21q22.12	*KCNE1*	MinK (beta subunit potassium channel), repolarization/IKs	↓ I_{Ks}	Ad+AR	<1%	176261
LQTS6	21q22.11	*KCNE2*	MiRP1 (beta subunit potassium channel), repolarization/I_{Kr}	↓ I_{Kr}	AD	<1%	603796
LQTS7 (ATS1)	17q24.3	KCNJ2	Kir2.1 (alpha subunit potassium channel), repolarization /IK1	↓ IK1	AD	<1%	600681
LQTS8	12p13.33	*CACNA1C*	$Ca_v1.2$ (alpha1C subunit calcium channel), depolarization/$I_{Ca\text{-}L}$	I_{CaL}	AD	<1%	114205
LQTS9	3p25.3	*CAV3*	Caveolin 3 (caveolae coat protein)	I_{Na}	AD	Rare	601253
LQTS10	11q23.3	SCN4B	NaVβ4 (beta1 subunit sodium channel), depolarization/INa	I_{Na}	AD	Rare	608256
LQTS11	7q21.2	AKAP9	A-kinase anchoring protein 9 (adapter protein)	↓ I_{Ks}	AD	Rare	604001
LQTS12	20q11.21	SNTA1	Syntrophin alpha 1 (membrane scaffold)	I_{Na}	AD	Rare	601017
LQTS13	11q24.3	KCNJ5	KIR3.4 (alpha subunit potassium channel)	↓ IK_{Ach}	AD	Rare	600734
LQTS14	14q32.11	*CALM1*	Calmodulin 1	↓ signalization Ca	AD	<1%	114180

(continued)

Table 13.2 (continued)

Type (OMIM)	Locus	Gene	Protein, function/current	Effect of mutations	Transmission	Frequency	OMIM
LQTS15	2p21	*CALM2*	Calmodulin 2	↓ signalization Ca	AD	<1%	114182
	19q13.32	*CALM3*	Calmodulin 3	↓ signalization Ca	AD	<1%	114183
	6q22.31	*TRDN*	Triadin		AR	<1%	603283
	4q13.1	*TECRL*	Trans-2.3-enoyl-CoA reductase-like protein	↓ signalization Ca	AR		617242

AD autosomal dominant, *AR* autosomal recessive

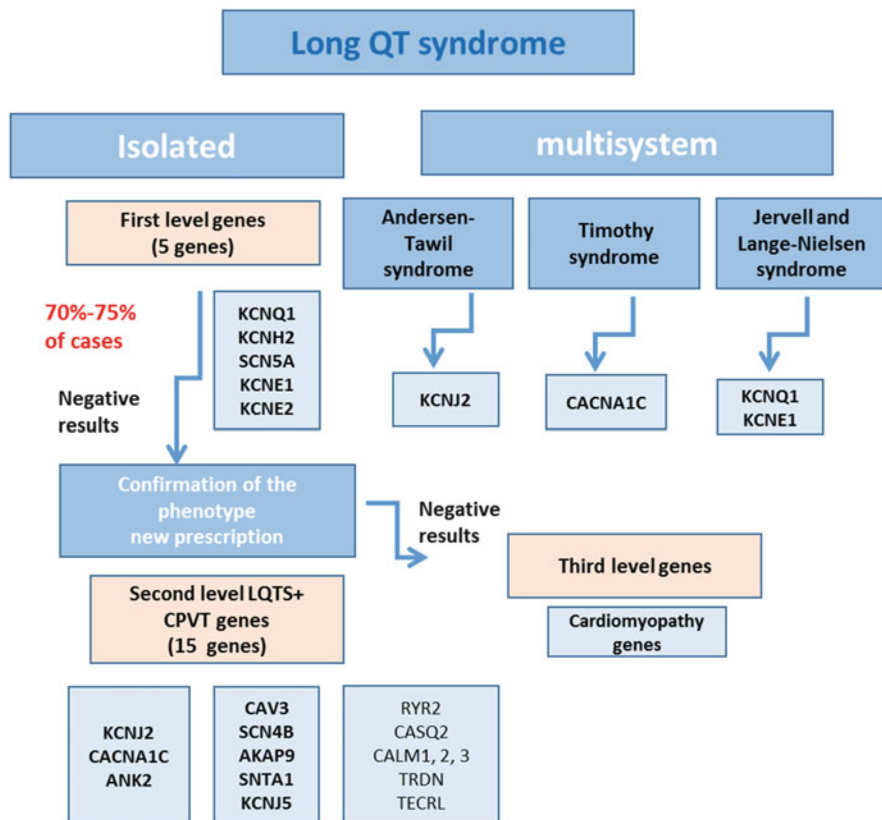

Fig. 13.2 Decision tree model for genetic testing in long QT syndrome

Moreover, the risk of arrhythmia is higher in LQT1 and LQT2 patients with a QTc > 500 ms and LQT3 in men whatever the interval QT duration. Mutation location can also be interpreted: mutations in the cytoplasmic loops of LQT1, mutations with dominant negative ion current effects and mutations in the pore-loop region of LQT2 have been associated with a different risk of cardiac events (Barsheshet et al. 2012; Moss et al. 2002; Shimizu et al. 2009; Migdalovich et al. 2011). In contrast, mutations in the C-terminal region tend to be associated with a milder phenotype (Donger et al. 1997; Crotti et al. 2007).

Genetic testing for the major forms of LQTS (*KCNQ1, KCNH2 and SCN5A)* has a major role in the diagnosis of probands, risk stratification, familial screening and treatment and could be proposed soon after birth. This is applicable only for pathogenic variations. However, many variations are classified as variants of unknown significance (VUS) and remain uninterpretable and therefore have no clinical impact (Steffensen et al. 2015). In contrast, 15–20% of patients have negative genetic tests, but that does not rule out the presence of the disease.

Mutations in other genes (LQT9 to 13) have been identified in a few patients, and their prevalence in LQTS requires evaluation (Schwartz et al. 2012).

Very recently, mutations in calmodulin (*CALM1*, *CALM2*), triadin (*TRDN*) and *TECRL* genes have been associated with highly lethal arrhythmias in the settings of LQTS and catecholaminergic polymorphic ventricular tachycardia (CPVT) (Altmann et al. 2015; Pipilas et al. 2016; Devalla et al. 2016; Nyegaard et al. 2012; Crotti et al. 2013; Marsman et al. 2014).

Syndromic Forms of LQTS

Syndromic forms of LQTS have also been reported as presenting a broader cardiac phenotype than QT interval prolongation or extracardiac abnormalities.

LQT4: Ankyrin-B Syndrome

Ankyrin-B syndrome presents a complex cardiac phenotype including QT interval prolongation, bradycardia, sinus node dysfunction, atrial fibrillation and ventricular arrhythmia (Schott et al. 1995; Le et al. 2008). Of note, the *ANK2* gene encodes for a non-channel protein but is essential for targeting and stabilization of structural proteins and ion channels. Remarkably, most of the mutations are gathered within the death/C-terminal domain of the protein (Hashemi et al. 2009).

Lange-Nielsen Syndrome

A recessive form of LQTS, the Jervell and Lange-Nielsen syndrome is caused by homozygous or compound heterozygous mutations in *KCNQ1* or its auxiliary subunit *KCNE1*. It is characterized by a congenital deafness, a prolonged QTc on ECG (>500 ms) and ventricular tachyarrhythmias. This syndrome is very rare (1/200,000 to 1/1,000,000) but virulent, and in 50% of the cases, the pathology manifests before the age of 3 (Pagon et al. 1993).

Andersen-Tawil Syndrome (ATS)

The Andersen-Tawil syndrome is characterized by periodic paralysis, a prominent U wave, a long QT interval with ventricular arrhythmias predisposing to sudden cardiac death and dysmorphic features: short stature, scoliosis, low-set ears, widely spaced eyes, small mandible, clinodactyly, brachydactyly and syndactyly. ATS is inherited in an autosomal dominant manner with variable penetrance. Mutations in the *KCNJ2* gene, encoding the alpha subunit of the Kir2.1 potassium channel, are implicated in 60% of the cases. A mutation in the *KCNJ5* gene, encoding the alpha subunit of the Kir3.4 potassium channel, has also been described (Kokunai et al. 2014).

Timothy Syndrome (TS)

The Timothy syndrome is caused by mutations in the *CACNA1C* gene (LQT8) encoding the alpha subunit of the CaV1.2 calcium channel. The syndrome often results from de novo mutation or from germinal mosaicism in one of the parents. Genetic testing of this syndrome is singular owing to its genetic homogeneity since the cases described so far have been carrying the p.G406R or p.G402R gain-of-

function mutations (Tester and Ackerman 2014). This is a multisystem disease characterized by cardiac, hand/foot, facial and neurodevelopmental features. Typical cardiac findings include a rate-corrected QT interval >480 ms, functional 2:1 AV block with bradycardia, tachyarrhythmias and congenital heart defects. The diagnosis of Timothy syndrome is generally made within the first few days of life although it may be suspected prenatally by identifying 2:1 AV block or bradycardia in the foetus (Pagon et al. 1993).

Thus, based on the LQT clinical presentation and potential associated cardiac or not cardiac symptoms, genetic testing is proposed to be performed according to the decision tree presented in Fig. 13.2.

13.3.3 Short QT Syndrome (SQTS)

13.3.3.1 Clinical Description

SQTS was first described in 2000 and remains one of the rarest channelopathies (Gussak et al. 2000). Prevalence is difficult to estimate because of the limited number of patients (Gollob et al. 2011). SQTS leads to palpitation, syncope and sudden cardiac death, typically during childhood. It is characterized by a short QT interval on the ECG with peaked T-wave and a high risk of fatal arrhythmias (Patel et al. 2010).

A complete consensus of the cut-off value of QT interval does not exist yet. Diagnostic criteria have been proposed: for QTc <330 ms or <360 ms (350 ms in men and 365 ms in women) if associated with a pathogenic mutation, a family history of SQTS, VT/VF with normal structural heart or of sudden death before the age of 40 (Gollob et al. 2011; Bjerregaard 2011; Veltmann and Borggrefe 2011). Of note, patients with SQTS present a fairly similar QT interval whatever the heart rate variations requiring no or very little heart rate correction. Patients with the shortest QT duration present the highest risk of rhythmic events (Giustetto et al. 2011).

An implantable defibrillator is indicated in high-risk SQTS patients who have experienced VT/VF or resuscitated sudden death and in patients with a familial story of cardiac sudden death. Treatment with quinidine could be an alternative because of its prolongation of QT duration (Priori et al. 2015; Giustetto et al. 2011; Gaita et al. 2004).

13.3.3.2 Genetic Testing

Pathogenic variations have been described in 6 genes (*KCNQ1, KCNH2, KCNJ2, CACNA1C, CACNB2B and CACNA2D1*) harbouring mutations that mirror the functional effect of those encountered in LQTS (Brugada et al. 2004; Bellocq et al. 2004; Priori et al. 2005; Antzelevitch et al. 2007). Mutations in potassium channel genes produce a gain of function, and in contrast, mutations in the calcium genes produce a loss of function resulting in shorter AP durations. Mutations in these genes account for 50% of SQTS cases according to the literature, but in clinical practice, the diagnostic yield of genetic testing is very modest in these genes, which

supposes that there are other causal genes and that a negative genetic cannot exclude the diagnosis.

13.3.4 Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

13.3.4.1 Clinical Description

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a hereditary arrhythmogenic disease that is responsible for familial forms of sudden death in infants and young adults. The prevalence for CPVT in the general population is estimated to be 1/10,000 (Van der Werf and Wilde 2013). Arrhythmias occur as recurrent syncope triggered by adrenergic stimulation such as emotional or exercise stress, while baseline ECG is often normal and cardiac imaging reveals no structural abnormality (George et al. 2007). Approximately, 30% of patients with CPVT develop symptoms before 10 years of age and 60% before 40 years (Liu et al. 2008; Ackerman et al. 2011). A family history of exercise-related syncope, seizure or sudden death is reported in 30% of the patients. As with LQTS1, swimming is a potentially lethal arrhythmia precipitating CPVT, and the symptoms are often attributed to alternative diagnoses, most commonly epilepsy. Drug challenge with epinephrine or isoproterenol may elicit arrhythmias. In the absence of beta-blocker treatment, VT/VF and syncope are associated with a high rate of recurrence with 30–50% mortality before the age of 40 years (Hayashi et al. 2009). Beta blockers, combined with restriction of exercise and avoidance of stressful situations, are the first-line therapy for CPVT. All patients and family members who harbour a disease-causing mutation, irrespective of symptoms, should be treated with beta blockers. Left cardiac sympathetic denervation or ICD implantation could be effective to prevent recurrent VT/VF and SCD in patients intolerant to beta blockers or for those with persistent symptoms or arrhythmias despite treatment (Wilde et al. 2008). However, CPVT patients appear to be very sensitive to catecholamines, and the subsequent ICD shock has a high probability of initiating a vicious cycle with multiple shocks caused by recurrent ventricular tachycardia or VF (Wilde et al. 2008).

13.3.4.2 Genetic Testing

The presence of a pathogenic mutation is of diagnostic value. Two genetic models of CPVT have been identified: an autosomal dominant form due to mutations in the cardiac ryanodine receptor 2 (*RYR2*) gene and less common autosomal recessive forms due to mutations in the *CASQ2*, *TRDN* and more recently *TECRL* genes (Table 13.3) (Devalla et al. 2016). Mutations in *RYR2* are found in 50%–60% of CPVT cases, whereas mutations in *CASQ2* and *TRDN* are found in less than 10% of CPVT patients (Fig. 13.3) (Roux-Buisson et al. 2012; Rooryck et al. 2015). Rarely, mutations are found in calmodulin genes (*CALM1*, *CALM2* and *CALM3*), the *KCNJ2* gene (Andersen-Tawil syndrome also known as LQT7) and the *ANK2* gene (also known as LQT4) (Nyegaard et al. 2012; Mohler et al. 2004; Tully et al.

Table 13.3 Genes identified in catecholaminergic polymorphic ventricular tachycardia (CPVT)

Type (OMIM)	Locus	Gene	Protein	Transmission	Frequency	OMIM
CPVT1	1q43	*RYR2*	Ryanodine receptor 2	AD	40–60%	180902
CPVT2	1p13.1	*CASQ2*	Calsequestrin 2	AR	1–2%	114251
	17q24.3	*KCNJ2*	Potassium voltage-gated channel subfamily J member 2	AD	Rare	600681
CPVT5	6q22.31	*TRDN*	Triadin	AR	Rare	603283
	4q25-q26	*ANK2*	Ankyrin 2	AD	Rare	106410
CPVT4	14q32.11	*CALM1*	Calmodulin 1	AD	Rare	114180
	2p21	*CALM2*	Calmodulin 2	AD	Rare	114182
	19q13.32	*CALM3*	Calmodulin 3	AD	Rare	114183
	4q13.1	*TECRL*	Trans-2.3-enoyl-CoA reductase-like protein	AR	Rare	617242

AD autosomal dominant, *AR* autosomal recessive

2015). A locus associated with an early-onset lethal form of recessive CPVT was found on chromosome 7p14-p22, but so far no gene has been identified (Bhuiyan et al. 2007).

Interestingly, *RYR2* mutations cluster in both N- and C-terminal domains of the protein and within transmembrane domains (Priori and Napolitano 2005). Forty percent of all *RYR2* mutations are estimated to be de novo mutations. Only rare cases of rearrangements have been described and remarkably all concern *RYR2* exon3 (Bhuiyan et al. 2007; Campbell et al. 2015). Since SCD can be the first manifestation of CPVT, genetic testing is clinically relevant, especially for the care management of family members.

At present, there is no genotype-based risk stratification or therapeutic approach in CPVT. Nearly one-third of possible or atypical LQTS cases with exertion-induced syncope have also been identified as positive for an RYR2 mutation, and accordingly, a clinical presentation of exercise-induced syncope and a QTc <460 ms should always first point to the consideration of CPVT rather than the so-called concealed or normal QT interval LQT1 (Medeiros-Domingo et al. 2009).

Since clinical overlap have been described between CPVT and cardiomyopathy cases (OMIM#604772), cardiomyopathy-related genes may be considered in case of negative diagnostic from the screening of the first and second gene panels (Fig. 13.3).

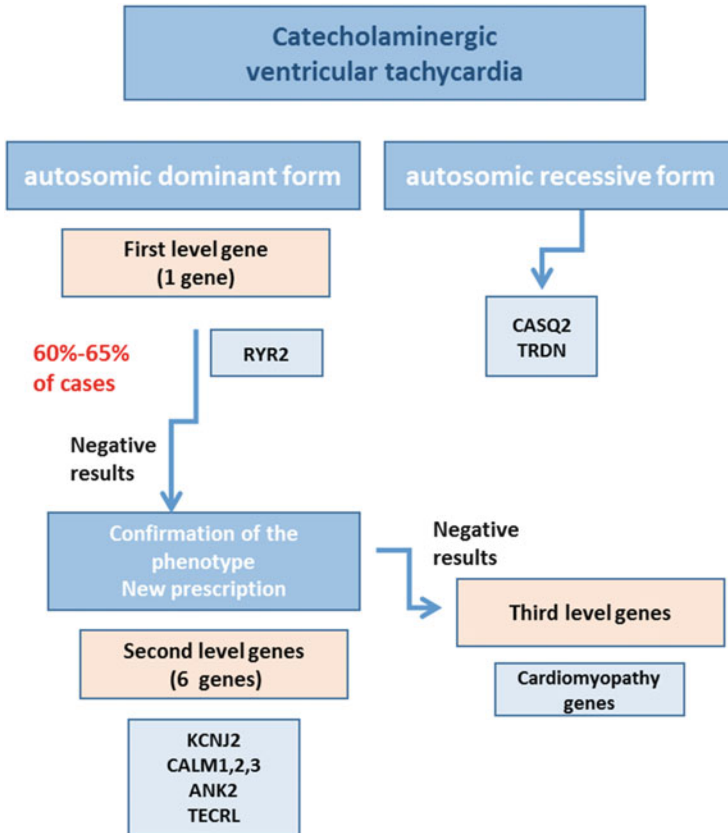

Fig. 13.3 Decision tree model for genetic testing in catecholaminergic polymorphic ventricular tachycardia

13.3.5 Cardiac Conduction Defect (CCD)

13.3.5.1 Clinical Description

Cardiac conduction defect (CCD) is a rare disorder associated with a risk of sudden death in the absence of pacing. Progressive cardiac conduction defect (PCCD), also called Lenegre-Lev disease, is the most frequent form, affecting the His-Purkinje pathway, and may progress to complete atrioventricular block (AVB) (Lev 1964; Lenegre 1964). Diagnosis relies on echocardiogram (ECG) findings showing an advanced conduction defect. Congenital structural CCD can be part of a syndrome and be associated with abnormalities in other organ systems or cardiac malformations including atrial septal defects, ventricular septal defects and tetralogy of Fallot (Baruteau et al. 2015).

Patients who receive pacemaker implantation appear to present an excellent prognosis except in those with *LMNA* mutations (see also below) which can lead

to ventricular tachycardia and sudden cardiac death. In this population, ICD implantation is recommended in case of severe cardiac conduction defect (Anselme et al. 2013).

13.3.5.2 Genetic Testing

Transmission is usually autosomal dominant with incomplete penetrance and variable expressivity. Inherited forms of CCD are attributed to mutations in a wide variety of genes impacting the process of action potential generation and propagation. These electrical disturbances concern mainly isolated form of conduction defects and involve cardiac ion channel genes (*SCN5A, HCN4, TRPM4, SCN1B*) and cytoskeletal and inner nuclear membrane genes (*LMNA*). Mutations appear more frequently in *SCN5A* (Schott et al. 1999; Probst et al. 2003; Watanabe et al. 2008; Liu et al. 2010; Stallmeyer et al. 2012; Daumy et al. 2016). Point mutations and more rarely rearrangement in the gene encoding lamin A/C (LMNA) can lead to dilated cardiomyopathy associated with cardiac conduction system disease and arrhythmias and also to isolated CCD (Marsman et al. 2011). Genetic testing in suspected *LMNA* patients is of great interest since *LMNA* carriers present a high risk of SCD and a poor prognosis (Wolf and Berul 2006; Wolf et al. 2008). A mutation in the *GJA5* gene encoding the gap junction Cx40 gene was identified in a family with progressive cardiac conduction defect (PCCD) characterized by AV block and wide QRS bundle branch block (Makita et al. 2012). Mutations in PRKAG2, which encodes for a regulatory subunit (γ-2) of adenosine monophosphate-activated protein kinase (AMPK), were found in patients with the Wolff-Parkinson-White (WPW) syndrome, a disease characterized by ventricular pre-excitation, atrial fibrillation and conduction defects such as sinoatrial and atrioventricular block and cardiac hypertrophy (Fig. 13.4; Table 13.4) (Wolf and Berul 2006). In the absence of mutations among the above candidate genes, the screening of the cardiomyopathy-associated genes might be considered (Sisakian 2014). Furthermore, in case of atrial septal defect (ASD), abnormalities in transcription-factor *NKX2–5*, *GATA4* and *TBX5* have been reported in congenital atrioventricular block (Schott et al. 1998; Garg et al. 2003; Basson et al. 1994).

13.3.6 Arrhythmogenic Right Ventricular Cardiomyopathy/ Dysplasia (ARVC/D)

13.3.6.1 Clinical Description

Arrhythmogenic right ventricular cardiomyopathy/dysplasia is a heritable cardiomyopathy characterized by life-threatening ventricular arrhythmias and progressive dystrophy of the ventricular myocardium with fibrofatty replacement. Generally referred to as a right ventricular disease, left or bi-ventricular forms prompted the use of the broader term arrhythmogenic cardiomyopathy (Bhonsale et al. 2015). The estimated prevalence in the general population is 1/2000–1/5000 with an age-related penetrance (usually adolescence-young adulthood) (Corrado et al. 2017). Moreover, ARVC/D more frequently affects males than females (up to 3:1) despite a similar

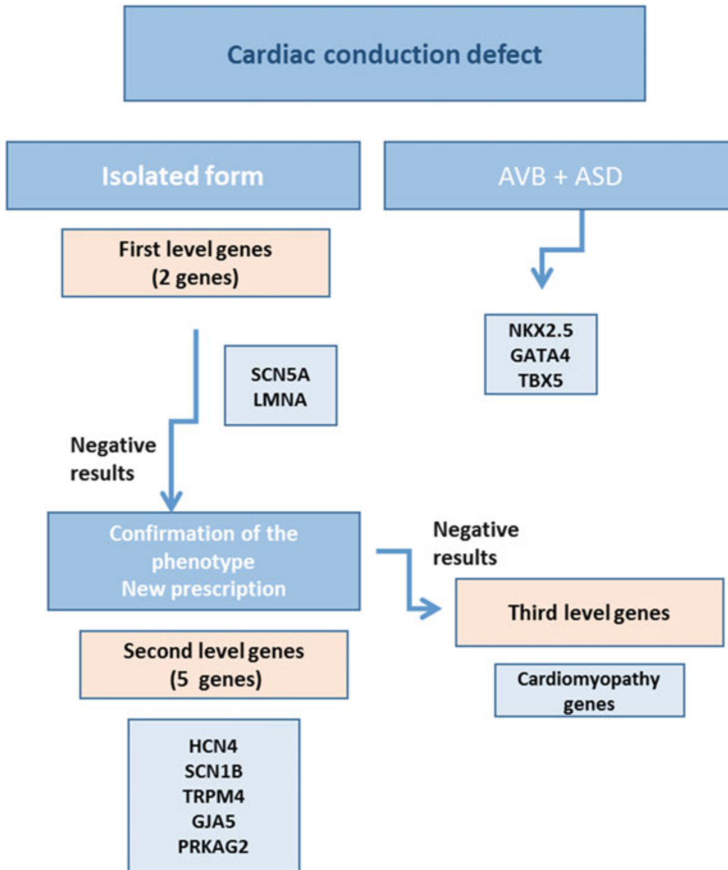

Fig. 13.4 Decision tree model for genetic testing in cardiac conduction defect

prevalence of carrier status between sexes (Bhonsale et al. 2015). ARVC/D could account for up to 20% of cases of aborted cardiac arrest and SCD in the young, especially in athletes (Corrado et al. 2017).

Multiple criteria are required for diagnosis combining repolarization abnormalities, morpho-functional alterations, histopathological features on endomyocardial biopsy, family history and genetics, but diagnosis of ARVC/D is often complicated by its evolution over time (Marcus et al. 2010). Patients typically present with symptomatic ventricular arrhythmias, characterized by premature ventricular contraction (PVC) or VT with LBBB morphology and T-wave inversion in V1–V3 leads on basal electrocardiogram, leading to syncope or cardiac arrest. The first symptoms manifest before the age of 40 and are encountered in the large majority of patients (Orgeron and Calkins 2016).

Table 13.4 Genes identified in cardiac conduction defect (CCD)

Type (omim)	Locus (HGNC)	Gene	Protein	Transmission	OMIM
PFHB-1A	3p22.2	SCN5A	$Na_v1.5$	AD	600163
	1q22	LMNA	Lamin A/C	AD/AR	150330
	19q13.11	SCN1B	Sodium channel, voltage-gated, type i, beta subunit	AD	600235
PFHB-1B	19q13.33	TRPM4	Transient receptor potential cation channel, subfamily M, member 4	AD	606936
		HCN4		AD	
	5q35.1	Nkx2.5	nk2, drosophila, homolog of, e; nkx2e	AD	600584
			Cardiac-specific homeobox 1; csx1		
	1q21.2	GJA5	Gap junction protein, alpha-5	AD	121013
	7q36.1	PRKAG2	Protein kinase, amp-activated, noncatalytic, gamma-2	AD	602743

AD autosomal dominant, *AR,* autosomal recessive*, PFHB* progressive familial heart block

13.3.6.2 Genetic Testing

ARVC/D is inherited predominantly as an autosomal dominant trait with incomplete penetrance and with a variable age-dependent expressivity. Inter- and intra-familial variation in disease severity and expressivity is frequently observed with coexistence of both classic RV and dominant LV pattern in the same family and/or life-threatening ventricular arrhythmias in probands vs. a more favourable prognosis in relatives (Sen-Chowdhry et al. 2007). In a few cases, ARVC/D inheritance follows an autosomal recessive trait (Naxos disease or Carvajal syndrome). These ARVC/D patients present extracardiac symptoms such as a cutaneous phenotype (palmoplantar keratoderma) and woolly hair (Corrado et al. 2017). ARVC/D is widely considered to be a desmosomal disease. Desmosomes are one of the junctional complexes at the intercalated disc that are essential for the structural and functional integrity of cardiac tissue. Desmosomal gene mutations lead to defective desmosomal components and to the development of structural abnormalities. Mutations in five desmosomal genes have been identified: plakophilin-2 (*PKP2*) desmoplakin (*DSP*), desmocollin-2 (*DSC2*), desmoglein-2 (*DSG2*), plakoglobin (*JUP*) and more recently the cadherin 2 (*CDH2*) (Mayosi et al. 2017). Non-desmosomal genes, transmembrane protein 43 (*TMEM43*), desmin (DES), lamin A/C (*LMNA*), titin (*TTN*), phospholamban (*PLN*) and alpha T-catenin (*CTNNA3*), have been associated with atypical forms of ARVC/D. Mutations in the regulatory region of transforming growth factor beta-3 (*TGFB3*) have also been reported, but their pathogenicity is still controversial. Rarely, mutations in the *RYR2* gene and the *SCN5A* gene have been described (Table 13.5) (Te Riele et al. 2017). ARVC/D gene mutations are found in approximately 60% of ARVC/D index cases, of which the majority are desmosomal gene mutations (*PKP2* 10–45%, *DSP*

Table 13.5 Genes identified in arrhythmogenic right ventricular dysplasia (ARVD)

Type (OMIM)	Locus	Gene	Protein	Transmission	Frequency (%)	OMIM
ARVD9	12p11	*PKP2*	Plakophilin-2	AD/AR	30–40%	602861
ARVD8	6p24	DSP	Desmoplakin	AD/AR	10–15%	125647
ARVD10	18q12.1	*DSG2*	Desmoglein-2	AD/AR	3–8%	125671
ARVD11	18q21	*DSC2*	Desmocollin-2	AD/AR	1–5%	125645
ARVD12	17q21	*JUP*	Junction plakoglobin	AD/AR	<1%	173325
ARVD2	1q43	*RYR2*	Ryanodine receptor-2	AD	<1%	180902
ARVD1	14q24	*TGFB3*	Transforming growth factor b3	AD	<1%	190230
ARVD5	3p25.1	*TMEM43*	Transmembrane protein 43	AD	<1%	612048
ARVD13	10q22.3	CTNNA3	Alpha T-catenin	AD	Rare	607667
	2q31	*TTN*	Titin	AD	Rare	188840
	6q22.1	*PLN*	Phospholamban	AD	Rare	172405
	1q22	LMNA	Lamin A/C	AD	Rare	150330
	2q35	DES	Desmin	AD	Rare	125660
	3p22.2	SCN5A	Sodium channel, voltage-gated, type v, alpha subunit	AD	Rare	600163
	18q12.1	CDH2	Cadherin 2	AD	Rare	114020

AD autosomal dominant, *AR* autosomal recessive

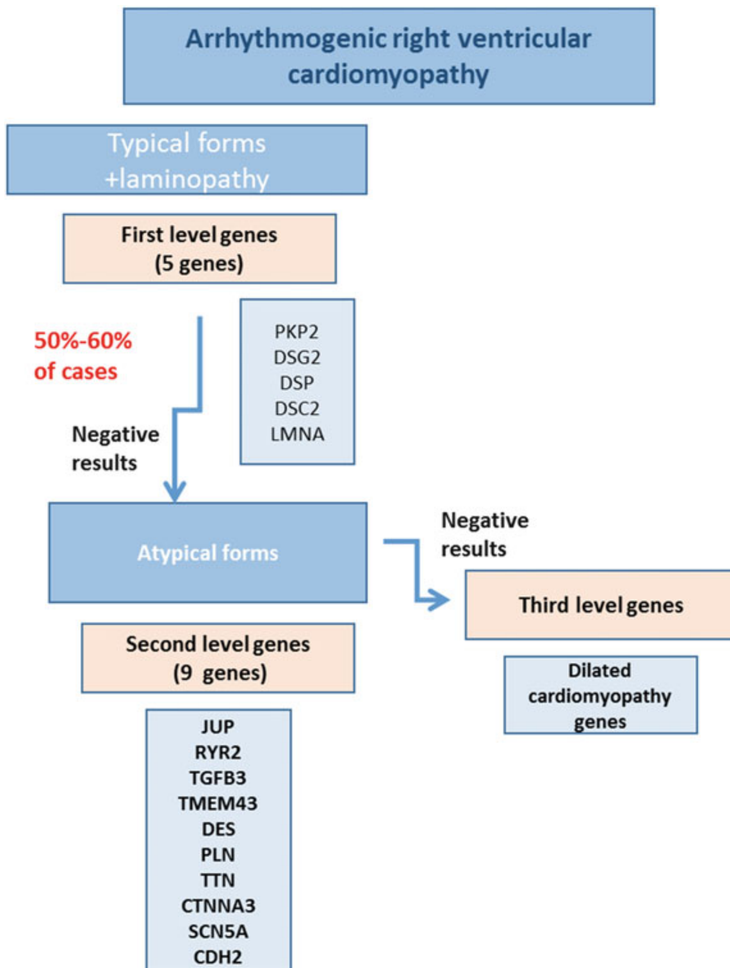

Fig. 13.5 Decision tree model for genetic testing in arrhythmogenic right ventricular cardiomyopathy/dysplasia

10–15%, *DSG2* 7–10%, *DSC2* 1–2%, *JUP* 1–2%) (Fig. 13.5), but the proportion of causal genes differs according to cohort location and ethnicity (Ohno 2016). Compound/digenic mutations may be found in 10–25% of patients and lead to earlier onset of symptoms, and carriers of mutations in *DSP* are more likely to present with left ventricular dysfunction, heart failure and SCD than carriers with mutations in *PKP2* (Bhonsale et al. 2015; Rigato et al. 2013). Among single mutation carriers, premature truncating, splice-site and missense mutations were identified in, respectively, 60%, 23% and 14% of cases, but the outcomes did not differ significantly (Bhonsale et al. 2015). Entire *PKP2* exons or even whole gene deletions have been

recently described in families with a frequency of approximately 2% (Roberts et al. 2013; Li Mura et al. 2013).

While the 2010 Diagnostic Task Force Criteria include identification of a putative ARVC/D susceptibility gene mutation as a new major diagnostic criterion, genetic testing should be used as a confirmatory tool for diagnosis of ARVC/D in index cases (Fig. 13.5). Presymptomatic screening is recommended in family members. If they carry the same mutation, they will then require clinical evaluation and long-term observation. Nevertheless, genetic findings require careful interpretation owing to the large number of genetic variants of uncertain significance (Andreasen et al. 2013).

13.3.7 Role of Genetic Testing in Sudden Unexplained Death (SUD)

13.3.7.1 Clinical Description

Sudden cardiac death is responsible for a large proportion of sudden unexpected deaths (SUD) in young individuals. A national study in Denmark estimated that SUD represents 29% of the deaths in persons aged 1–35 years after medicolegal investigation (Winkel et al. 2011). In 5–10% of all cases, SUD is caused by inherited cardiac diseases, especially cardiac channelopathies such as long QT syndrome, short QT syndrome, Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, progressive cardiac conduction disorder or early repolarization syndrome (Hayashi et al. 2009). In these cases, SUD may represent an early malignant arrhythmogenic presentation, preceding the development of the ECG indices. Therefore, when no identifiable ECG abnormalities can be observed or when only a post-mortem blood sample can be collected, genetic testing may provide insight into the causal mechanism leading to (fatal) arrhythmia. Comprehensive clinical investigation of SCD families would identify causes of death in 40% of cases, demonstrating that SCD were probably due to inherited heart disease (Tan et al. 2005; Behr et al. 2008). Next-generation sequencing (NGS), with the ability to screen hundreds of genes simultaneously, could provide confirmation of the clinical investigation or become the only possibility to point clinicians to a clinical diagnosis and identify the relatives at risk of similar severe arrhythmia. Furthermore, when patients present a borderline phenotype, genetic testing could encourage clinicians to further look for specific ECG abnormalities and employ adapted provocative tests.

13.3.7.2 Genetic Testing

The identification of the genes associated with cardiomyopathies and channelopathies has provided insights into disease mechanisms of cardiac arrest. Most genetic studies have focused on cardiac channelopathy-associated genes. Mutation analysis in 5 LQTS genes (*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*) identified pathogenic variations in 11–17% of SCD cases (Chugh et al. 2003; Skinner et al. 2011; Winkel et al. 2012). More recently, post-mortem genetic testing for a large cohort of cases of autopsy-negative sudden unexplained deaths identified putative pathogenic variations in LQTS and CPVT genes in 20% of the cases (Tester

et al. 2012). Another study on 34 genes of channelopathies identified 3 likely pathogenic variants in *KCNH2, SNTA1* and *RYR2* in 3 out of 15 (20%) SUD cases (Hertz et al. 2015). A prevalence of 5–10% of variants in the CPVT-associated gene *RYR2* has been found in sudden infant death syndrome cases (Larsen et al. 2013). Exome analysis of 28 cases of autopsy-negative sudden unexplained death identified 3 rare variants in *KCNQ1* and *KCNH2* genes in 3 SUD cases (10%) and 4 rare variations in cardiomyopathy genes (*DSP, MYBPC3* and *TTN*) (Bagnall et al. 2014). A recent screening of 100 genes in 61 cases of autopsy-negative sudden unexplained death (<50 years) identified likely pathogenic variants in 34% of the SCD cases, 40% associated with inherited cardiomyopathies and 60% associated with channelopathies (Christiansen et al. 2016).

However, the use of genetics as a clinical diagnostic tool must always be considered the context of the clinical case. Therefore, particularly in the context of SCD, genetic testing should complement or guide but never supplant the clinical investigation and segregation analyses. Without a clear clinical picture and a clear correlation between the disease and the genetic variants, causality may be nonconclusive, since several genes have been linked to several phenotypes or SCD could be caused by other unknown genetic causes or factors.

13.4 Genetic Testing and Technological Evolution: Progress and Side Effects

13.4.1 The Next-Generation Sequencing: High Throughput and Low Cost

As described above, the number of channelopathy-associated genes has increased dramatically in the last decades. With capillary sequencing, genetic testing was restricted to the major genes since this molecular analysis was expensive and had limited throughput. Since 2011, next-generation sequencing has revolutionized genetic diagnostics by offering the possibility to screen thousands of genes simultaneously coupled with a dramatic reduction (10,000-fold; www.genome.gov/sequencing costs) in sequencing cost (Kingsmore and Saunders 2011). However, knowing the number of genes to explore is crucial since one must maintain a good ratio in order to guarantee a reasonable cost (number of genes to be sequenced), data that are of sufficient quality to interpret the results (depth of sequencing) and a throughput that is compatible with an analysis of numerous samples. A minimum mean depth of 30X has been considered reasonable to achieve confident variant calls. In the absence of consensus gene design for channelopathies, a rationalized approach could consist in defining a set of genes comprising the channel subunits and partners predominantly implicated in rhythm disorders (see above). In case of negative results, a second panel that includes all genes associated with any primary rhythm disorders or even cardiomyopathies could be employed (Novelli et al. 2016). However, our broad sequencing capability could be moderated by interpretation, ethical and storage issues.

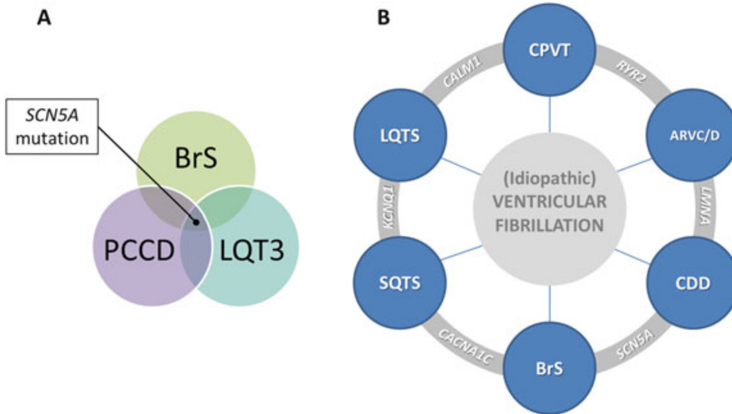

Fig. 13.6 (**a**) *SCN5A* overlap syndromes and (**b**) genetic continuum among the cardiac channelopathies

13.4.2 The More You Sequence, the More Complex Will Be the Interpretation

As illustrated by the genetics of the channelopathies, we observed a large genetic heterogeneity among each clinical entity coupled with gene overlap between them (Fig. 13.6). This gene overlap could also reveal a potential continuum among these channelopathies (Makita 2009). Moreover, several studies have reported channelopathies due to double mutations harboured by different genes (Kapplinger et al. 2010, 2009; Bauce et al. 2010). These observations suggest the need to screen large panels of genes. These panels are now widely employed within the molecular diagnostic centres covering between 50 and 200 candidate genes according technologies and diseases targeted. The continuous reduction of the sequencing cost tends to switch to whole exome (all coding regions of the genome) sequencing (WES), or even whole genome sequencing (WGS), to overcome the limitation of the panel(s). However, reagent cost for capturing approximately 100 genes remains 4 times lower than for WES and 10 times cheaper than for WGS. Aside from the financial point of view, drawbacks need to be anticipated before producing and analysing a large/whole gene scan. First of all, besides the promise that all coding regions and then the "Mendelianome" will be covered by a WES, daily practice reveals that WES actually provides a poorer coverage (% of targeted bases covered at a defined mean depth) than the targeted gene panels (Pua et al. 2016). Coverage for genetic diagnostic is crucial since all candidate DNA base pairs have to be confidently explored. Whole genome sequencing presents the advantage of uniformly covering the genome and facilitating genomic rearrangement identification (Sudmant et al. 2015) but at twice the WES price.

Today, genetic diagnostics face the challenge of variant interpretation uncovered by the large-scale sequencing technologies offering the advantage of simultaneously screening hundreds of candidate genes but at the same time revealing multiple rare genetic variations of unknown significance (Novelli et al. 2016). For example, rare

genetic variations in associated channelopathy genes are also commonly found in the general population (Risgaard et al. 2013; Ghouse et al. 2017; Paludan-Müller et al. 2017). Large databases such as gnomad.broadinstitute.org consisting of >120,000 exomes and >15,000 whole genomes currently constitute a powerful tool to estimate the minor allele frequency (MAF) of a candidate variant. In addition, such databases furnish MAF in different broad categories of ethnicities which could help in the possible absence of demographically matched controls to prevent false-positive mutation. For this reason, numerous countries have developed their own national databases to reflect the specific genetic architecture of their population (Zawistowski et al. 2014). However, the rarity of a variant does not make it necessarily causal, and it can even turn out to be a variant of uncertain/unknown significance (VUS). Indeed, within an exome, between 300 and 600 rare variants will be identified which obviously do not at all cause a pathologic phenotype (Tennessen et al. 2012). Then, considering that 12,958 (64%) out of 20,344 genes in the genome (http://www.proteinatlas.org/) are expressed in the cardiac tissue, we can estimate that between 200 and 400 of them could be of potential interest in a context of a patient presenting a cardiac disease. This estimation puts into perspective the challenge of the number of variants generated by the NGS and our ability to provide a pertinent molecular diagnosis. Misinterpretation of a VUS can lead to inappropriate treatment such as an implantable cardioverter-defibrillator which could have a dramatic impact on the patient's quality of life with possible severe complications and potentially serious psychologic consequences (Ackerman 2015).

This led to re-evaluation of the criteria defining the pathogenicity of a variant and then to modification of genetic test interpretations in order to avoid false positives (Ackerman 2015). Segregation studies among family members and/or functional experiments constitute the best approaches for causal mutation validation but remain rare owing to the time and cost investment required and are not adapted to a large number of variants. This sequencing technology revolution could therefore change the skill profiles required for genetic testing. High-throughput functional screening (in vitro) technologies to cope with massive VUS identification could emerge to characterize the variants isolated by data analysis and interpretations performed by bioinformatics. Indeed, after filtering out common variants by checking their MAF which is available in large public databases, additional tools could guide a geneticist to isolate the causal variant. The type of mutation can be considered (missense, nonsense, frameshift or splice-site variants) especially when loss of function of the protein can be predicted. Another argument of causality can reside into a previous observation of this variation in patients presenting the same phenotype and listed in dedicated databases (HGMD http://www.hgmd.cf.ac.uk/ac/index.php, CLINVAR www.ncbi.nlm.nih.gov/clinvar). When a variant is rare but never found in patients or in dedicated databases and no family members could have been tested, multiple algorithms can be used to indicate the likelihood of variant pathogenicity. These tools can combine information from nucleotide or/and amino acid conservation among species or include parameters such as modification of amino acid polarity or/and 3D protein structure. Others combine several algorithms to provide a consensus score (Table 13.6). Guidelines for interpretation and classification of genetic

Table 13.6 Non-exhaustive list of tools for variant consequence prediction

Name	Methods	REF/PMID
CADD	Combined annotation-dependent depletion (CADD) is a framework that integrates multiple annotations into one metric by contrasting variants that survived natural selection with simulated mutations	24487276
Condel	**Consensus deleteriousness score of** MutationAssessor and FATHMM	21457909
ENTPRISE	A boosted tree regression **machine-learning** approach to predict human disease-associated amino acid variations by utilizing a comprehensive combination of protein sequence and structure features	26982818
FunSeq2	The pipeline has a weighted scoring system combining: Inter- and intraspecies conservation, loss- and gain-of-function events for transcription-factor binding, enhancer-gene linkages and network centrality, and per-element recurrence across samples. We further highlight putative drivers with information specific to a particular sample, such as differential expression	25273974
GWAVA	Based on a wide range of annotations of non-coding elements (largely from ENCODE/ GENCODE), along with genome-wide properties such as evolutionary conservation and GC-content	https://www.sanger.ac.uk/sanger/StatGen_Gwava
iFish	**Gene-specific and family-specific customized classifiers. Bayesian model**	27527004
LoFtool	LoFtool provides a rank of genic intolerance and consequent susceptibility to disease based on the **ratio of loss of function (LoF)** to synonymous mutations for each gene in 60,706 individuals from ExAC, adjusting for the gene de novo mutation rate and evolutionary protein conservation	27563026
Mechismo	Enables simultaneous consideration of thousands of **3D structures** and biomolecular interactions to predict rapidly mechanistic consequences for mutations and modifications. Compares to protein close in structure but not necessarily in sequence	25392414
MutationAssessor	Functional impact score (FIS) for amino acid residue changes using **evolutionary conservation patterns**	21727090
MutationTaster2	**Comprises evolutionary conservation, splice-site changes, loss of proteinfeatures and changes that might affect the amount of mRNA. Bayesian model**	20676075

(continued)

Table 13.6 (continued)

Name	Methods	REF/PMID
PaPi	Classify and score human coding variants by estimating the probability to damage their protein-related function. Consists in using **pseudo amino acid composition (PseAAC)** through which wild and mutated protein sequences are represented in a discrete model. Then **machine learning (random forest)**. Uses the scores of **SIFT** and **PolyPhen** for training	25928477
PolyPhen-2	Compare to homologous protein sequences, then prediction of 3D structure. Compare of structures and merging proteins in cluster. Score calculation on the cluster with the interest protein	12202775
PON-P2	**Random forest algorithm and machine learning.** PON-P2 is a novel tool that employs features of evolutionary sequence conservation, properties of amino acids, GO annotations and functional annotations	25647319
PredictSNP2	**Consensus** on eight established prediction tools: MAPP, nsSNPAnalyzer, PANTHER, PhD-SNP, PolyPhen-1, PolyPhen-2, SIFT and SNAP	27224906
PROVEAN	This **alignment-based score** measures the change in sequence similarity of a query sequence to a protein sequence homolog before and after the introduction of an amino acid variation to the query sequence	25851949
REVEL	Make a **consensus** between: MutPred, FATHMM, VEST, PolyPhen, SIFT, PROVEAN, MutationAssessor, MutationTaster, LRT, GERP, SiPhy, phyloP and phastCons	27666373
SIFT	Compare to homologous protein sequences: The more the position is conserved in evolution, the more mutation is supposed to be damaging	11337480

variations are proposed by the American College of Medical Genetics and Genomics to evaluate the pathogenicity of a variant (Richards et al. 2015).

This unexpected number of rare variants that harbours a genome and the difficulty of their interpretation could reinforce, especially for rare diseases such as channelopathies, the benefit of gathering clinical and genotype information in common databases to improve the knowledge acquired by the number of cases explored.

13.4.3 Genetic Testing and the Impact of Identifying a Mutation for Relatives and Predictive Testing

Because of the complexities involved, genetic testing should only be performed in dedicated specialized centres such as a cardiac genetics clinic or clinical genetics service where appropriate family management and genetic counselling before and after testing can be offered. This should be performed by cardiologists, nurses, clinical geneticists and genetic counsellors with specialized training in cardiovascular genetics.

Identification of a mutation in a family can often explain why the disease has occurred. It also allows for cascade testing of other affected and unaffected family members. Testing of affected family members is performed as a confirmation of their disease status and to exclude the possibility of a "phenocopy" (i.e. an individual who has an acquired rather than genetic cause of the same condition as other members of a family). This enables an accurate risk assessment for their offspring. Asymptomatic family members can be offered a predictive genetic test to clarify whether they are at risk of developing clinical disease and to determine the inheritance risk to their children. There may also be implications for participation in sports and employment.

13.4.4 Genetic Testing and Psychological Impact

The quality of life and psychological stress of cardiac variant carriers are most often not evaluated. A few studies have provided a quantification of the level of anxiety and depression in cardiac mutation carriers and their relatives (Hendriks et al. 2008; Christiaans et al. 2009). Genetic testing can help with anxiety regarding the disease especially when the clinical diagnostic is uncertain. Stress should also be considered in partners since the degree of anxiety is higher in carrier partners than in those of noncarriers. In this context, genetic counselling is essential to answer the questions generated by the clinical and genetic diagnosis to prevent anxiety. The uncertainty of the functional effect of VUS and, more generally, the large number of rare variants present in all individuals could also give rise to anxiety in genetic counsellors (Spoonamore and Ware 2016).

13.4.5 Large-Scale Sequencing and Incidental Findings

Aside from the complex VUS interpretation, known causal mutation may be uncovered and sometimes aside from the initial indication of cardiac diseases. These incidental findings are a matter of clinical and ethical debate since it is difficult to assess a clear benefit for a patient and/or a clinician to be informed of such mutations. The American College of Medical Genetics and Genomics (ACMG) recommends reporting variants belonging to a defined list of genes to an appropriate clinician for re-evaluation and surveillance of the patient and his family. The ACMG proposes a list of 57 genes associated with 24 syndromes, cancer, endocrinal or cardiac diseases.

Of note, this recommendation concerns 9 channelopathy-associated genes and 11 cardiomyopathy genes (Green et al. 2013; Kalia et al. 2017). This risk should be well explained to the patient before performing a whole exome scan.

13.4.6 Large-Scale Sequencing Data, Storage and Privacy

The rapid development of sequencing throughput has encouraged us to screen increasingly larger numbers of genes and the entire genome in the very short term. Thus, there have been problems in bioinformatics to handle and characterize the great number of rare variants contained within the genome. Moreover, large-scale sequencing, in particular whole genome screening, also implies anticipating storage capability since data associated with the sequencing of a whole genome represents approximately 120GB. A 3-day run of a last-generation sequencer (i.e. Illumina X Ten solution) produces 16 whole genomes and 1.8 TB of data. For example, a centre such as UPPMAX accumulated 2200 TB of data from 2011 to the end of 2015 (Spjuth et al. 2016). In addition, whole genome data requires secure storage since they contain sensitive and private information. Aside from storage, privacy must be maintained during the phases of alignment, calling or comparison with public databases when they are not performed on a local level (Akgün et al. 2015). These large genome scans shake our habits and require thought before rushing into data production.

13.5 Conclusion

The last decade saw the new high-throughput sequencing technologies offering the opportunity to screen first a large panel of candidate genes, then all coding region and now the whole genome for a continuously decreasing cost. This allowed improving the efficiency of the genetic testing and consequently the care management for the patient and their family members. This opportunity to always screen more genes revealed also the high prevalence of rare variants present in the genome and therefore brings us to a new challenge consisting of isolate one(s) with an impact on the cardiac phenotype.

Compliance with Ethical Standards

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This article does not contain any studies with human participants or animals performed by any of the authors.

References

Ackerman MJ. Genetic purgatory and the cardiac channelopathies: exposing the variants of uncertain/unknown significance issue. Heart Rhythm. 2015;12:2325–31.

Ackerman MJ, Priori SG, Willems S, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European heart rhythm association (EHRA). Europace. 2011;13:1077–109.

Akgün M, Bayrak AO, Ozer B, Sağıroğlu MŞ. Privacy preserving processing of genomic data: a survey. J Biomed Inform. 2015;56:103–11.

Altmann HM, Tester DJ, Will ML, et al. Homozygous/compound heterozygous triadin mutations associated with autosomal-recessive long-QT syndrome and pediatric sudden cardiac arrest: elucidation of the triadin knockout syndrome. Circulation. 2015;131:2051–60.

Andorin A, Behr ER, Denjoy I, et al. Impact of clinical and genetic findings on the management of young patients with Brugada syndrome. Heart Rhythm. 2016;13:1274–82.

Andreasen C, Nielsen JB, Refsgaard L, et al. New population-based exome data are questioning the pathogenicity of previously cardiomyopathy-associated genetic variants. Eur J Hum Genet. 2013;21:918–28.

Anselme F, Moubarak G, Savouré A, et al. Implantable cardioverter-defibrillators in Lamin a/C mutation carriers with cardiac conduction disorders. Heart Rhythm. 2013;10:1492–8.

Antzelevitch C, Pollevick GD, Cordeiro JM, et al. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation. 2007;115:442–9.

Bagnall RD, Das KJ, Duflou J, Semsarian C. Exome analysis-based molecular autopsy in cases of sudden unexplained death in the young. Heart Rhythm. 2014;11:655–62.

Barc J, Briec F, Schmitt S, et al. Screening for copy number variation in genes associated with the long QT syndrome: clinical relevance. J Am Coll Cardiol. 2011;57:40–7.

Barsheshet A, Goldenberg I, O-Uchi J, et al. Mutations in cytoplasmic loops of the KCNQ1 channel and the risk of life-threatening events: implications for mutation-specific response to β-blocker therapy in type 1 long-QT syndrome. Circulation. 2012;125:1988–96.

Baruteau A-EE, Probst V, Abriel H. Inherited progressive cardiac conduction disorders. Curr Opin Cardiol. 2015;30:33–9.

Basson CT, Cowley GS, Solomon SD, et al. The clinical and genetic spectrum of the Holt-Oram syndrome (heart-hand syndrome). N Engl J Med. 1994;330:885–91.

Bauce B, Nava A, Beffagna G, et al. Multiple mutations in desmosomal proteins encoding genes in arrhythmogenic right ventricular cardiomyopathy/dysplasia. Heart Rhythm. 2010;7:22–9.

Behr ER, Dalageorgou C, Christiansen M, et al. Sudden arrhythmic death syndrome: familial evaluation identifies inheritable heart disease in the majority of families. Eur Heart J. 2008;29:1670–80.

Bellocq C, van Ginneken AC, Bezzina CR, et al. Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. Circulation. 2004;109:2394–7.

Bezzina CR, Barc J, Mizusawa Y, et al. Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. Nat Genet. 2013;45:1044–9.

Bhonsale A, Groeneweg JA, James CA, et al. Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers. Eur Heart J. 2015;36:847–55.

Bhuiyan ZA, van den Berg MP, van Tintelen JP, et al. Expanding spectrum of human RYR2-related disease: new electrocardiographic, structural, and genetic features. Circulation. 2007;116:1569–76.

Bjerregaard P. Proposed diagnostic criteria for short QT syndrome are badly founded. J Am Coll Cardiol. 2011;58:549–50; author reply 550–1.

Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. J Am Coll Cardiol. 1992;20:1391. Available at: PM:1309182

Brugada R, Hong K, Dumaine R, et al. Sudden death associated with short-QT syndrome linked to mutations in HERG. Circulation. 2004;109:30–5.

Campbell MJ, Czosek RJ, Hinton RB, Miller EM. Exon 3 deletion of ryanodine receptor causes left ventricular noncompaction, worsening catecholaminergic polymorphic ventricular tachycardia, and sudden cardiac arrest. Am J Med Genet A. 2015;167A:2197–200.

Chen Q, Kirsch GE, Zhang D, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature. 1998;392:293. Available at: PM:9521325

Christiaans I, van Langen IM, Birnie E, Bonsel GJ, Wilde AA, Smets EM. Quality of life and psychological distress in hypertrophic cardiomyopathy mutation carriers: a cross-sectional cohort study. Am J Med Genet A. 2009;149A:602–12.

Christiansen SL, Hertz CL, Ferrero-Miliani L, et al. Genetic investigation of 100 heart genes in sudden unexplained death victims in a forensic setting. Eur J Hum Genet. 2016;24:1797–802.

Chugh SS, Chung K, Zheng Z-JJ, John B, Titus JL. Cardiac pathologic findings reveal a high rate of sudden cardiac death of undetermined etiology in younger women. Am Heart J. 2003;146:635–9.

Corrado D, Link MS, Calkins H. Arrhythmogenic right ventricular cardiomyopathy. N Engl J Med. 2017;376:61–72.

Crotti L, Spazzolini C, Schwartz PJ, et al. The common long-QT syndrome mutation KCNQ1/A341V causes unusually severe clinical manifestations in patients with different ethnic backgrounds: toward a mutation-specific risk stratification. Circulation. 2007;116:2366–75.

Crotti L, Marcou CA, Tester DJ, et al. Spectrum and prevalence of mutations involving BrS1- through BrS12-susceptibility genes in a cohort of unrelated patients referred for Brugada syndrome genetic testing: implications for genetic testing. J Am Coll Cardiol. 2012;60:1410. Available at: PM:22840528

Crotti L, Johnson CN, Graf E, et al. Calmodulin mutations associated with recurrent cardiac arrest in infants. Circulation. 2013;127:1009–17.

Daumy X, Amarouch M-YY, Lindenbaum P, et al. Targeted resequencing identifies TRPM4 as a major gene predisposing to progressive familial heart block type I. Int J Cardiol. 2016;207:349–58.

Devalla HD, Gélinas R, Aburawi EH, et al. TECRL, a new life-threatening inherited arrhythmia gene associated with overlapping clinical features of both LQTS and CPVT. EMBO Mol Med. 2016;8:1390–408.

Donger C, Denjoy I, Berthet M, et al. KVLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome. Circulation. 1997;96:2778–81.

Eastaugh LJ, James PA, Phelan DG, Davis AM. Brugada syndrome caused by a large deletion in SCN5A only detected by multiplex ligation-dependent probe amplification. J Cardiovasc Electrophysiol. 2011;22:1073–6.

Gaita F, Giustetto C, Bianchi F, et al. Short QT syndrome: pharmacological treatment. J Am Coll Cardiol. 2004;43:1494–9.

García-Molina E, Lacunza J, Ruiz-Espejo F, et al. A study of the SCN5A gene in a cohort of 76 patients with Brugada syndrome. Clin Genet. 2013;83:530–8.

Garg V, Kathiriya IS, Barnes R, et al. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. Nature. 2003;424:443–7.

George CH, Jundi H, Thomas NL, Fry DL, Lai FA. Ryanodine receptors and ventricular arrhythmias: emerging trends in mutations, mechanisms and therapies. J Mol Cell Cardiol. 2007;42:34–50.

Ghouse J, Have CT, Skov MW, et al. Numerous Brugada syndrome-associated genetic variants have no effect on J-point elevation, syncope susceptibility, malignant cardiac arrhythmia, and all-cause mortality. Genet Med. 2017;19:521–8.

Giustetto C, Schimpf R, Mazzanti A, et al. Long-term follow-up of patients with short QT syndrome. J Am Coll Cardiol. 2011;58:587–95.
Goldenberg I, Moss AJ. Long QT syndrome. J Am Coll Cardiol. 2008;51:2291–300. Available at: PM:18549912
Goldenberg I, Horr S, Moss AJ, et al. Risk for life-threatening cardiac events in patients with genotype-confirmed long-QT syndrome and normal-range corrected QT intervals. J Am Coll Cardiol. 2011;57:51–9.
Gollob MH, Redpath CJ, Roberts JD. The short QT syndrome: proposed diagnostic criteria. J Am Coll Cardiol. 2011;57:802–12.
Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. 2013;15:565–74.
Gussak I, Brugada P, Brugada J, et al. Idiopathic short QT interval: a new clinical syndrome? Cardiology. 2000;94:99–102.
Hashemi SM, Hund TJ, Mohler PJ. Cardiac ankyrins in health and disease. J Mol Cell Cardiol. 2009;47:203–9.
Hayashi M, Denjoy I, Extramiana F, et al. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. Circulation. 2009;119:2426–34.
Hendriks KS, Hendriks MM, Birnie E, et al. Familial disease with a risk of sudden death: a longitudinal study of the psychological consequences of predictive testing for long QT syndrome. Heart Rhythm. 2008;5:719. Available at: PM:18452877
Hertz CL, Christiansen SL, Ferrero-Miliani L, et al. Next-generation sequencing of 34 genes in sudden unexplained death victims in forensics and in patients with channelopathic cardiac diseases. Int J Legal Med. 2015;129:793–800.
Imboden M, Swan H, Denjoy I, et al. Female predominance and transmission distortion in the long-QT syndrome. N Engl J Med. 2006;355:2744. Available at: PM:17192539
Imbrici P, Liantonio A, Camerino GM, et al. Therapeutic approaches to genetic ion channelopathies and perspectives in drug discovery. Front Pharmacol. 2016;7:121.
Jervell A, Lange-Nielsen F. Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death. Am Heart J. 1957;54:59–68.
Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2017;19(2):249–55.
Kapplinger JD, Tester DJ, Salisbury BA, et al. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. Heart Rhythm. 2009;6:1297. Available at: PM:19716085
Kapplinger JD, Tester DJ, Alders M, et al. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. Heart Rhythm. 2010;7:33–46.
Kingsmore SF, Saunders CJ. Deep sequencing of patient genomes for disease diagnosis: when will it become routine? Sci Transl Med. 2011;3:87ps23.
Kokunai Y, Nakata T, Furuta M, et al. A Kir3.4 mutation causes Andersen-Tawil syndrome by an inhibitory effect on Kir2.1. Neurology. 2014;82:1058–64.
Konigstein M, Rosso R, Topaz G, et al. Drug-induced Brugada syndrome: clinical characteristics and risk factors. Heart Rhythm. 2016;13:1083–7.
Koopmann TT, Beekman L, Alders M, et al. Exclusion of multiple candidate genes and large genomic rearrangements in SCN5A in a Dutch Brugada syndrome cohort. Heart Rhythm. 2007;4:752–5.
Larsen MK, Berge KE, Leren TP, et al. Postmortem genetic testing of the ryanodine receptor 2 (RYR2) gene in a cohort of sudden unexplained death cases. Int J Legal Med. 2013;127:139–44.
Le SS, Bhasin N, Vieyres C, et al. Dysfunction in ankyrin-B-dependent ion channel and transporter targeting causes human sinus node disease. Proc Natl Acad Sci U S A. 2008;105:15617. Available at: PM:18832177

Le Scouarnec S, Karakachoff M, J-BB G, et al. Testing the burden of rare variation in arrhythmia-susceptibility genes provides new insights into molecular diagnosis for Brugada syndrome. Hum Mol Genet. 2015;24:2757–63.

Lenegre J. Etiology and pathology of bilateral bundle branch block in relation to complete heart block. Prog Cardiovasc Dis. 1964;6:409–44.

Lev M. The pathology of complete atrioventricular block. Prog Cardiovasc Dis. 1964;6:317–26.

Li Mura IE, Bauce B, Nava A, et al. Identification of a PKP2 gene deletion in a family with arrhythmogenic right ventricular cardiomyopathy. Eur J Hum Genet. 2013;21:1226–31.

Liu N, Ruan Y, Priori SG. Catecholaminergic polymorphic ventricular tachycardia. Prog Cardiovasc Dis. 2008;51:23–30.

Liu H, El Zein L, Kruse M, et al. Gain-of-function mutations in TRPM4 cause autosomal dominant isolated cardiac conduction disease. Circ Cardiovasc Genet. 2010;3:374–85.

Makita N. Phenotypic overlap of cardiac sodium channelopathies. individual-specific or mutation-specific? Circ J. 2009;73:810–7.

Makita N, Seki A, Sumitomo N, et al. A connexin40 mutation associated with a malignant variant of progressive familial heart block type I. Circ Arrhythm Electrophysiol. 2012;5:163–72.

Marcus FI, McKenna WJ, Sherrill D, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. Eur Heart J. 2010;31:806–14.

Marsman RF, Bardai A, Postma AV, et al. A complex double deletion in LMNA underlies progressive cardiac conduction disease, atrial arrhythmias, and sudden death. Circ Cardiovasc Genet. 2011;4:280–7.

Marsman RF, Barc J, Beekman L, et al. A mutation in CALM1 encoding calmodulin in familial idiopathic ventricular fibrillation in childhood and adolescence. J Am Coll Cardiol. 2014;63:259–66.

Mayosi BM, Fish M, Shaboodien G, et al. Identification of cadherin 2 (CDH2) mutations in arrhythmogenic right ventricular cardiomyopathy. Circ Cardiovasc Genet. 2017;10

Medeiros-Domingo A, Bhuiyan ZA, Tester DJ, et al. The RYR2-encoded ryanodine receptor/calcium release channel in patients diagnosed previously with either catecholaminergic polymorphic ventricular tachycardia or genotype negative, exercise-induced long QT syndrome: a comprehensive open reading frame mutational analysis. J Am Coll Cardiol. 2009;54:2065–74.

Migdalovich D, Moss AJ, Lopes CM, et al. Mutation and gender-specific risk in type 2 long QT syndrome: implications for risk stratification for life-threatening cardiac events in patients with long QT syndrome. Heart Rhythm. 2011;8:1537–43.

Mohler PJ, Splawski I, Napolitano C, et al. A cardiac arrhythmia syndrome caused by loss of ankyrin-B function. Proc Natl Acad Sci U S A. 2004;101:9137–42.

Morita H, Wu J, Zipes DP. The QT syndromes: long and short. Lancet. 2008;372:750. Available at: PM:18761222

Moss AJ, Zareba W, Kaufman ES, et al. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. Circulation. 2002;105:794–9.

Novelli V, Gambelli P, Memmi M, Napolitano C. Challenges in molecular diagnostics of channelopathies in the next-generation sequencing era: less is more? Front Cardiovasc Med. 2016;3:29.

Nyegaard M, Overgaard MT, Sondergaard MT, et al. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. Am J HumGenet. 2012;91:703. Available at: PM:23040497

Ohno S. The genetic background of arrhythmogenic right ventricular cardiomyopathy. J Arrhythm. 2016;32:398–403.

Orgeron GM, Calkins H. Advances in the diagnosis and management of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Curr Cardiol Rep. 2016;18:53.

Pagon RA, Adam MP, Ardinger HH, et al. GeneReviews(®). Seattle: University of Washington; 1993.

Paludan-Müller C, Ahlberg G, Ghouse J, et al. Integration of 60,000 exomes and ACMG guidelines question the role of Catecholaminergic polymorphic ventricular tachycardia-associated variants. Clin Genet. 2017;91:63–72.

Patel C, Yan G-XX, Antzelevitch C. Short QT syndrome: from bench to bedside. Circ Arrhythm Electrophysiol. 2010;3:401–8.

Pipilas DC, Johnson CN, Webster G, et al. Novel calmodulin mutations associated with congenital long QT syndrome affect calcium current in human cardiomyocytes. Heart Rhythm. 2016;13:2012–9.

Postema PG, Wolpert C, Amin AS, et al. Drugs and Brugada syndrome patients: review of the literature, recommendations, and an up-to-date website (www.brugadadrugs.org). Heart Rhythm. 2009;6:1335–41.

Priori SG, Napolitano C. Cardiac and skeletal muscle disorders caused by mutations in the intracellular Ca2+ release channels. J Clin Invest. 2005;115(8):2033.

Priori SG, Schwartz PJ, Napolitano C, et al. Risk stratification in the long-QT syndrome. N Engl J Med. 2003;348:1866–74.

Priori SG, Napolitano C, Schwartz PJ, et al. Association of long QT syndrome loci and cardiac events among patients treated with beta-blockers. JAMA. 2004;292:1341–4.

Priori SG, Pandit SV, Rivolta I, et al. A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. Circ Res. 2005;96(7):800.

Priori SG, Wilde AA, Horie M, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and Management of Patients with inherited primary arrhythmia syndromes expert consensus statement on inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in may 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. Heart Rhythm. 2013;19:e75. Available at: PM:24011539

Priori SG, Blomström-Lundqvist C, Mazzanti A, et al. 2015 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC). Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). Eur Heart J. 2015;36:2793–867.

Probst V, Kyndt F, Potet F, et al. Haploinsufficiency in combination with aging causes SCN5A-linked hereditary Lenègre disease. J Am Coll Cardiol. 2003;41:643–52.

Probst V, Wilde AA, Barc J, et al. SCN5A mutations and the role of genetic background in the pathophysiology of Brugada syndrome. Circ Cardiovasc Genet. 2009;2:552. Available at: PM:20031634

Probst V, Veltmann C, Eckardt L, et al. Long-term prognosis of patients diagnosed with Brugada syndrome: results from the FINGER Brugada syndrome registry. Circulation. 2010;121:635. Available at: PM:20100972

Pua CJ, Bhalshankar J, Miao K, et al. Development of a comprehensive sequencing assay for inherited cardiac condition genes. J Cardiovasc Transl Res. 2016;9:3–11.

Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.

Rigato I, Bauce B, Rampazzo A, et al. Compound and digenic heterozygosity predicts lifetime arrhythmic outcome and sudden cardiac death in desmosomal gene-related arrhythmogenic right ventricular cardiomyopathy. Circ Cardiovasc Genet. 2013;6:533–42.

Risgaard B, Jabbari R, Refsgaard L, et al. High prevalence of genetic variants previously associated with Brugada syndrome in new exome data. Clin Genet. 2013;84:489–95.

Roberts JD, Herkert JC, Rutberg J, et al. Detection of genomic deletions of PKP2 in arrhythmogenic right ventricular cardiomyopathy. Clin Genet. 2013;83:452–6.

Roden DM. Clinical practice. Long-QT syndrome. N Engl J Med. 2008;358:169–76.

Romano C, Gemme G, Pongiglione R. Rare cardiac arrythmias of the pediatric age. II. Syncopal attacks due to paroxysmal ventricular fibrillation (presentation of 1st case in Italian pediatric literature). Clin Pediatr (Bologna). 1963;45:656–83.

Rooryck C, Kyndt F, Bozon D, et al. New family with catecholaminergic polymorphic ventricular tachycardia linked to the triadin gene. J Cardiovasc Electrophysiol. 2015;26:1146–50.
Roux-Buisson N, Cacheux M, Fourest-Lieuvin A, et al. Absence of triadin, a protein of the calcium release complex, is responsible for cardiac arrhythmia with sudden death in human. Hum Mol Genet. 2012;21:2759–67.
Schott JJ, Charpentier F, Peltier S, et al. Mapping of a gene for long QT syndrome to chromosome 4q25-27. Am J Hum Genet. 1995;57:1114–22.
Schott JJ, Benson DW, Basson CT, et al. Congenital heart disease caused by mutations in the transcription factor NKX2-5. Science. 1998;281:108–11.
Schott JJ, Alshinawi C, Kyndt F, et al. Cardiac conduction defects associate with mutations in SCN5A. Nat Genet. 1999;23:20–1.
Schwartz PJ, Moss AJ, Vincent GM, Crampton RS. Diagnostic criteria for the long QT syndrome. An update. Circulation. 1993;88:782–4.
Schwartz PJ, Priori SG, Spazzolini C, et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. Circulation. 2001;103:89–95.
Schwartz PJ, Stramba-Badiale M, Crotti L, et al. Prevalence of the congenital long-QT syndrome. Circulation. 2009;120:1761–7.
Schwartz PJ, Crotti L, Insolia R. Long-QT syndrome: from genetics to management. Circ Arrhythm Electrophysiol. 2012;5:868–77.
Selga E, Campuzano O, Pinsach-Abuin ML, et al. Comprehensive genetic characterization of a Spanish Brugada syndrome cohort. PLoS One. 2015;10:e0132888.
Sen-Chowdhry S, Syrris P, Ward D, Asimaki A, Sevdalis E, McKenna WJ. Clinical and genetic characterization of families with arrhythmogenic right ventricular dysplasia/cardiomyopathy provides novel insights into patterns of disease expression. Circulation. 2007;115:1710–20.
Shimizu W, Moss AJ, Wilde AA, et al. Genotype-phenotype aspects of type 2 long QT syndrome. J Am Coll Cardiol. 2009;54:2052–62.
Sisakian H. Cardiomyopathies: evolution of pathogenesis concepts and potential for new therapies. World J Cardiol. 2014;6:478–94.
Skinner JR, Crawford J, Smith W, et al. Prospective, population-based long QT molecular autopsy study of postmortem negative sudden death in 1 to 40 year olds. Heart Rhythm. 2011;8:412–9.
Spazzolini C, Mullally J, Moss AJ, et al. Clinical implications for patients with long QT syndrome who experience a cardiac event during infancy. J Am Coll Cardiol. 2009;54:832–7.
Spjuth O, Bongcam-Rudloff E, Dahlberg J, et al. Recommendations on e-infrastructures for next-generation sequencing. Gigascience. 2016;5:26.
Spoonamore KG, Ware SM. Genetic testing and genetic counseling in patients with sudden death risk due to heritable arrhythmias. Heart Rhythm. 2016;13:789–97.
Stallmeyer B, Koopmann M, Schulze-Bahr E. Identification of novel mutations in LMNA associated with familial forms of dilated cardiomyopathy. Genet Test Mol Biomarkers. 2012;16:543–9.
Steffensen AB, Refaat MM, J-PP D, et al. High incidence of functional ion-channel abnormalities in a consecutive long QT cohort with novel missense genetic variants of unknown significance. Sci Rep. 2015;5:10009.
Sudmant PH, Rausch T, Gardner EJ, et al. An integrated map of structural variation in 2,504 human genomes. Nature. 2015;526:75–81.
Tan HL, Bezzina CR, Smits JP, Verkerk AO, Wilde AA. Genetic control of sodium channel function. Cardiovasc Res. 2003;57:961. Available at: PM:12650874
Tan HL, Hofman N, van Langen IM, van der Wal AC, Wilde AA. Sudden unexplained death: heritability and diagnostic yield of cardiological and genetic examination in surviving relatives. Circulation. 2005;112:207–13.
Te Riele AS, Agullo-Pascual E, James CA, et al. Multilevel analyses of SCN5A mutations in arrhythmogenic right ventricular dysplasia/cardiomyopathy suggest non-canonical mechanisms for disease pathogenesis. Cardiovasc Res. 2017;113:102–11.

Tennessen JA, Bigham AW, O'Connor TD, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science. 2012;337:64–9.

Tester DJ, Ackerman MJ. Novel gene and mutation discovery in congenital long QT syndrome: let's keep looking where the street lamp standeth. Heart Rhythm. 2008;5:1282–4.

Tester DJ, Ackerman MJ. Genetics of long QT syndrome. Methodist Debakey Cardiovasc J. 2014;10:29–33.

Tester DJ, Medeiros-Domingo A, Will ML, Haglund CM, Ackerman MJ. Cardiac channel molecular autopsy: insights from 173 consecutive cases of autopsy-negative sudden unexplained death referred for postmortem genetic testing. Mayo Clin Proc. 2012;87:524–39.

Tomaselli GF, Barth AS. Ion channel diseases: an update for 2016. Curr Treat Options Cardiovasc Med. 2016;18:21.

Tully I, Atherton J, Hunt L, Ingles J, Semsarian C, McGaughran J. Rarity and phenotypic heterogeneity provide challenges in the diagnosis of Andersen-Tawil syndrome: two cases presenting with ECGs mimicking catecholaminergic polymorphic ventricular tachycardia (CPVT). Int J Cardiol. 2015;201:473–5.

Van der Werf C, Wilde AA. Catecholaminergic polymorphic ventricular tachycardia: from bench to bedside. Heart. 2013;99:497–504.

Veltmann C, Borggrefe M. Arrhythmias: a "Schwartz score" for short QT syndrome. Nat Rev Cardiol. 2011;8:251–2.

Ward OC. A new familial cardiac syndrome in children. J Irish Med Assoc. 1964;54:103–6.

Watanabe H, Koopmann TT, Le Scouarnec S, et al. Sodium channel β1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. J Clin Invest. 2008;118:2260–8.

Wijeyeratne YD, Behr ER. Sudden death and cardiac arrest without phenotype: the utility of genetic testing. Trends Cardiovasc Med. 2017;27(3):207–13.

Wilde AA, Behr ER. Genetic testing for inherited cardiac disease. Nat Rev Cardiol. 2013;10:571–83.

Wilde AA, Bhuiyan ZA, Crotti L, et al. Left cardiac sympathetic denervation for catecholaminergic polymorphic ventricular tachycardia. N Engl J Med. 2008;358:2024–9.

Winkel BG, Holst AG, Theilade J, et al. Nationwide study of sudden cardiac death in persons aged 1-35 years. Eur Heart J. 2011;32:983–90.

Winkel BG, Larsen MK, Berge KE, et al. The prevalence of mutations in KCNQ1, KCNH2, and SCN5A in an unselected national cohort of young sudden unexplained death cases. J Cardiovasc Electrophysiol. 2012;23:1092–8.

Wolf CM, Berul CI. Inherited conduction system abnormalities--one group of diseases, many genes. J Cardiovasc Electrophysiol. 2006;17:446–55.

Wolf CM, Wang L, Alcalai R, et al. Lamin a/C haploinsufficiency causes dilated cardiomyopathy and apoptosis-triggered cardiac conduction system disease. J Mol Cell Cardiol. 2008;44:293–303.

Zawistowski M, Reppell M, Wegmann D, et al. Analysis of rare variant population structure in Europeans explains differential stratification of gene-based tests. Eur J Hum Genet. 2014;22:1137–44.