

# European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) Expert Consensus Statement on the state of genetic testing for cardiac diseases

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## 1 | INTRODUCTION

### 1.1 | Purpose

Genetic testing has advanced significantly since the publication of the 2011 HRS/EHRA Expert Consensus Statement on the State of Genetic Testing for the Channelopathies and Cardiomyopathies.<sup>1</sup> In addition to single-gene testing, there is now the ability to perform whole-exome sequencing (WES) and whole-genome sequencing (WGS). There is growing appreciation of oligogenic disorders,<sup>2,3</sup> the role of modifier genes,<sup>2</sup> and the use of genetic testing for risk stratification, even in common cardiac diseases such as coronary artery disease or atrial fibrillation (AFib), including a proposal for a score awaiting validation.<sup>4</sup> This document reviews the state of genetic testing at the present time, and addresses the questions of what tests to perform and when to perform them. It should be noted that, as articulated in a 1999 Task Force Document by the European Society of Cardiology (ESC) on the legal value of medical guidelines,<sup>5</sup> 'The guidelines from an international organization, such as the ESC, have no specific legal territory and have no legally enforcing character. Nonetheless, in so far as they represent the state-of-the-art, they may be used as indicating deviation from evidence-based

medicine in cases of questioned liability'. In the case of potentially lethal and treatable conditions such as catecholaminergic polymorphic ventricular tachycardia (CPVT) or long QT syndrome (LQTS), it is the responsibility of the physician, preferably in conjunction with an expert genetics team, to communicate to the patient/family the critical importance of family screening, whether this be facilitated by cascade genetic testing or by broader clinical family screening.




### 1.2 | Organization of the writing committee

The writing committee included chairs and representatives nominated and approved by European Heart Rhythm Association (EHRA), Heart Rhythm Society (HRS), Asia Pacific Heart Rhythm Society (APHRS), and Latin American Heart Rhythm Society (LAHRS). Chairs and authors had no relevant relationship with industry (RWI). Details are available in Supplementary material online.

### 1.3 | Methodology and evidence review

Writing committee members were assigned topics, compiled tables of recommendations supported by appropriate text and references, and attended periodic virtual meetings. Writing committee members without relevant RWI drafted recommendations. In the arena of genetic testing, there are few if any randomized trials to provide the strongest level of scientific evidence. Recommendations were associated with a green heart symbol ('should do this') if supported by at least strong observational evidence and author consensus. A yellow heart ('may do this') was used if there was some evidence and general agreement. A red heart ('do not do this') indicated evidence or general agreement not to perform this testing (Table 1). Writing committee consensus of 80% was required. The recommendations were approved by an average of 93% of the writing committee members.

TABLE 1 Scientific rationale of consensus statements<sup>a</sup>

Definitions related to a treatment or procedure	Consensus statement instruction	Symbol
Supported by strong observational evidence and authors' consensus	'Should do this'	
Some evidence and general agreement favour the usefulness/efficacy of a test	'May do this'	
There is evidence or general agreement not to recommend a test	'Do not do this'	

<sup>a</sup>The categorization for our consensus document should not be considered directly similar to the one used for official society guideline recommendations which apply a classification (I–III) and level of evidence (A, B, and C) to recommendations.

## 1.4 | Document review and approval

After review by the writing committee, the recommendations were opened for public comment. The document was then reviewed by the scientific documents committees of EHRA, HRS, APHRS, and LAHRS. After revision, the document was sent to external reviewers nominated by the participating societies. After further revision, the document was endorsed by the collaborating societies and presented for publication.

## 1.5 | Scope of the document

This document addresses essential principles of genetic testing including modes of inheritance, different testing methodologies, and interpretation of variants. Additionally, the document presents the state of genetic testing for inherited arrhythmia syndromes, cardiomyopathies, sudden cardiac death (SCD), congenital heart disease (CHD), coronary artery disease, and heart failure. A discussion of aortopathies and hyperlipidaemia is beyond the scope of this document.

The authors discuss diagnostic, prognostic, and therapeutic implications of genetic testing in each of these syndromes, as far as these are known. The writing committee recognizes that the feasibility of genomic testing by gene panel testing or by WES or WGS depends on the availability of genomic technology and on regional reimbursement policy. Therefore, the recommendation 'should do this' can be read as 'should do this when available'.

[Table 2](#) lists previous guidelines and consensus statements that are considered pertinent for this document as they all include relevant information for the diagnosis of patients with inherited cardiovascular conditions (ICCs) and the need for genetic testing. The terms and abbreviations used in consensus statement are summarized in [Table 3](#).

## 2 | GENETIC INFLUENCES ON DISEASE AND MODES OF INHERITANCE

Research conducted, over the last three decades, has provided considerable insights into the modes of inheritance of

TABLE 2 Relevant clinical practice documents or guidelines

Title	Publication year
<b>Consensus documents/guidelines of scientific societies</b>	
APHRS/HRS expert consensus statement on the investigation of decedents with sudden unexplained death and patients with sudden cardiac arrest, and of their families <sup>6</sup>	2021
HRS/EHRA/APHRS/LAHRS Expert Consensus Statement on Catheter Ablation of Ventricular Arrhythmias <sup>7</sup>	2020
Genetic Testing for Inherited Cardiovascular Diseases: A Scientific Statement From the American Heart Association <sup>8</sup>	2020
European Recommendations Integrating Genetic Testing into Multidisciplinary Management of Sudden Cardiac Death <sup>9</sup>	2019
Pre-participation Cardiovascular Evaluation for Athletic Participants to Prevent Sudden Death: Position Paper from the EHRA and the EACPR, Branches of the ESC <sup>22</sup>	2019
HRS Expert Consensus Statement on Evaluation, Risk Stratification, and Management of Arrhythmogenic Cardiomyopathy <sup>11</sup>	2019
AHA/ACC/HRS Guideline for Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death <sup>12</sup>	2017
ESC Guidelines for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death <sup>13</sup>	2015
EHRA/HRS/APHRS Expert Consensus on Ventricular Arrhythmias <sup>14</sup>	2014
HRS/EHRA/APHRS Expert Consensus Statement on the Diagnosis and Management of Patients with Inherited Primary Arrhythmia Syndromes <sup>15</sup>	2013
HRS/EHRA Expert Consensus Statement on the State of Genetic Testing for the Channelopathies and Cardiomyopathies <sup>1</sup>	2011
Genetic counselling and testing in cardiomyopathies: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases <sup>16</sup>	2010
<b>NIH-Clinical Genome Resource Consortium (ClinGen) documents</b>	
A Multi-Centred, Evidence-Based Evaluation of Gene Validity in Sudden Arrhythmic Death Syndromes: CPVT and The Short QT Syndrome <sup>17</sup>	2022
International Evidence Based Reappraisal of Genes Associated With Arrhythmogenic Right Ventricular Cardiomyopathy Using the Clinical Genome Resource Framework <sup>18</sup>	2021
Evidence-Based Assessment of Genes in Dilated Cardiomyopathy <sup>19</sup>	2021
An International, Multicentred Evidence-Based Reappraisal of Genes Reported to Cause Congenital Long QT Syndrome <sup>20</sup>	2020
Reappraisal of Reported Genes for Sudden Arrhythmic Death: An Evidence-Based Evaluation of Gene Validity for Brugada Syndrome <sup>21</sup>	2018
Evaluating the Clinical Validity of Hypertrophic Cardiomyopathy Genes <sup>10</sup>	2017

TABLE 3 Definitions and abbreviations

Term (abbreviation)	Definition
Sudden cardiac arrest (SCA)	Sudden cessation of cardiac activity with haemodynamic collapse, typically due to sustained ventricular arrhythmia
Sudden cardiac death (SCD)	Death that occurs within 1 h of onset of symptoms in witnessed cases, and within 24 h of last being seen alive when it is unwitnessed
Sudden unexplained death (syndrome) [SUD(S)]	Unexplained sudden death occurring in an individual older than 1 year
Sudden unexplained death in infancy (SUDI) <sup>a</sup>	Unexplained sudden death occurring in an individual younger than 1 year with negative pathological and toxicological assessment
Sudden arrhythmic death (syndrome) [SAD(S)] <sup>b</sup>	Unexplained sudden death occurring in an individual older than 1 year with negative pathological and toxicological assessment
Abbreviation	
ASO	Allele-specific oligonucleotide
ACMG	American College of Medical Genetics & Genomics
aCGH	Array comparative genomic hybridization
ACM	Arrhythmogenic cardiomyopathy
ALVC	Arrhythmogenic left ventricular cardiomyopathy
ARVC	Arrhythmogenic right ventricular cardiomyopathy
AFib	Atrial fibrillation
ASD	Atrial septal defect
ASS	Atrial stand still
AD	Autosomal dominant
AR	Autosomal recessive
BrS	Brugada syndrome
CRDS	Calcium release deficiency syndrome
CCD	Cardiac conduction disease
RyR2	Cardiac ryanodine receptor
CMR	Cardiovascular magnetic resonance
CPVT	Catecholaminergic polymorphic ventricular tachycardia
CVS	Chorionic villous sample
CMA	Chromosomal microarray
CHD	Congenital heart disease
CNV	Copy number variant
DCM	Dilated cardiomyopathy
ERP	Early repolarization pattern
ECA	Extracardiac anomaly
GWAS	Genome-wide association studies
GRS	Genomic risk scores
HCM	Hypertrophic cardio-myopathy

TABLE 3 (Continued)

Term (abbreviation)	Definition
IVF	Idiopathic ventricular fibrillation
ICD	Implantable cardioverter-defibrillator
ICC	Inherited cardiovascular conditions
JLNS	Jervell and Lange-Nielsen Syndrome
LCSD	Left cardiac sympathetic denervation
LV	Left ventricular
LVH	Left ventricular hypertrophy
LVNC	Left ventricular non-compaction cardiomyopathy
LB	Likely Benign
LP/P	Likely pathogenic/pathogenic
LQTS	Long QT syndrome
MAF	Minor allele frequency
MLPA	Multiplex ligation-dependent probe amplification
NGS	Next-generation sequencing
PRS	Polygenic risk scores
PCR	Polymerase chain reaction
PNP	Polyneuropathy
PCCD	Progressive cardiac conduction disease
RCM	Restrictive cardiomyopathy
siRNA	Short interfering RNA
SQTS	Short QT syndrome
SNP	Single-nucleotide polymorphism
SNV	Single-nucleotide variant
SND	Sinus node dysfunction
SCA	Sudden cardiac arrest
SCD	Sudden cardiac death
TOF	Tetralogy of Fallot
TdP	Torsades de pointes
TKOS	Triadin knockout syndrome
UCA	Unexplained cardiac arrest
UTRs	Untranslated regions
VUS	Variants of uncertain clinical significance
VF	Ventricular fibrillation
VSD	ventricular septal defect
WES	Whole-exome sequencing
WGS	Whole-genome sequencing
WPW	Wolff-Parkinson-White syndrome
X-chr	X-chromosomal

<sup>a</sup>Synonymous with 'sudden unexplained infant death' (SUID).

<sup>b</sup>Synonymous with 'autopsy-negative sudden unexplained death'.

cardiovascular disorders and into the underlying genes and pathways. These insights were fuelled by developments in technologies for DNA sequencing and genotyping, statistical genetic



approaches, and our increased understanding of the wide spectrum of genetic variation in the general population. Two broad categories of cardiovascular disorders are recognized: Mendelian disorders that are caused by the inheritance of one or two genetic variants and that typically cluster in families, and disorders with complex inheritance, wherein multiple genetic variants contribute and for which familial clustering is less pronounced. In both categories non-genetic factors also contribute to the ultimate phenotypic expression.

Inheritance patterns for monogenic disorders include autosomal dominant (AD), autosomal recessive (AR), and sex-linked. In AD disorders, the inheritance of a single defective copy of a gene, either the maternal or the paternal copy, is sufficient to cause the disorder. In some cases, an AD condition may result from a *de novo* variant in the gene and occurs in individuals with no history of the disorder in their family. In AR disorders, both the maternal and paternal copies need to be defective to produce the disorder. X-linked disorders are caused by pathogenic variants in genes on the X chromosome. Two types of X-linked disorders are recognized, X-linked dominant and X-linked recessive. In females with an X-linked dominant condition, a pathogenic variant in one of the two copies of the gene is sufficient to cause the condition. In males, who have only one X chromosome, a pathogenic variant in the only copy of the gene causes the disorder. In X-linked recessive inheritance, in males, one defective copy of the gene is sufficient to cause the condition, whereas females are mildly affected or unaffected if only one copy of the gene is aberrant. A characteristic of both types of X-linked inheritance is that males cannot pass on the disorder to their sons. Besides Mendelian inheritance, single-gene disorders may exhibit mitochondrial inheritance. Because mitochondrial DNA is inherited from the mother, only females can pass on genetic defects residing on mitochondrial DNA. In rare cases, disease-causing variants may arise post-zygotically (during development), leading to mosaicism (the occurrence of genetically distinct cell populations). Mosaicism may be limited to somatic cells, where there would be no risk of passing the disease-variant to the offspring, or it may also affect the germ line cell population and in this way the disease variant may be passed to the offspring.

Disease-associated genetic variants likely lie on a spectrum of population frequency and phenotype effect size. Mendelian variants, when dominant, are usually characterized by an ultra-low minor allele frequency (MAF, typically <0.01%) in the population and have large effect sizes (Figure 1). Classically, genes underlying Mendelian disorders were identified by linkage studies that tracked chromosomal regions that are co-inherited with the condition in multiple affected individuals in families, followed by Sanger sequencing of the linked chromosomal interval. More recently, next-generation sequencing (NGS) and WES have been successful in identifying novel genes underlying Mendelian disorders. It is estimated that there are about 7000 single-gene inherited disorders of which causative genes have been discovered for over 4000.<sup>23</sup> Accordingly, many genes for hereditary cardiomyopathies, including dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and arrhythmogenic cardiomyopathy (ACM); hereditary arrhythmias, such as

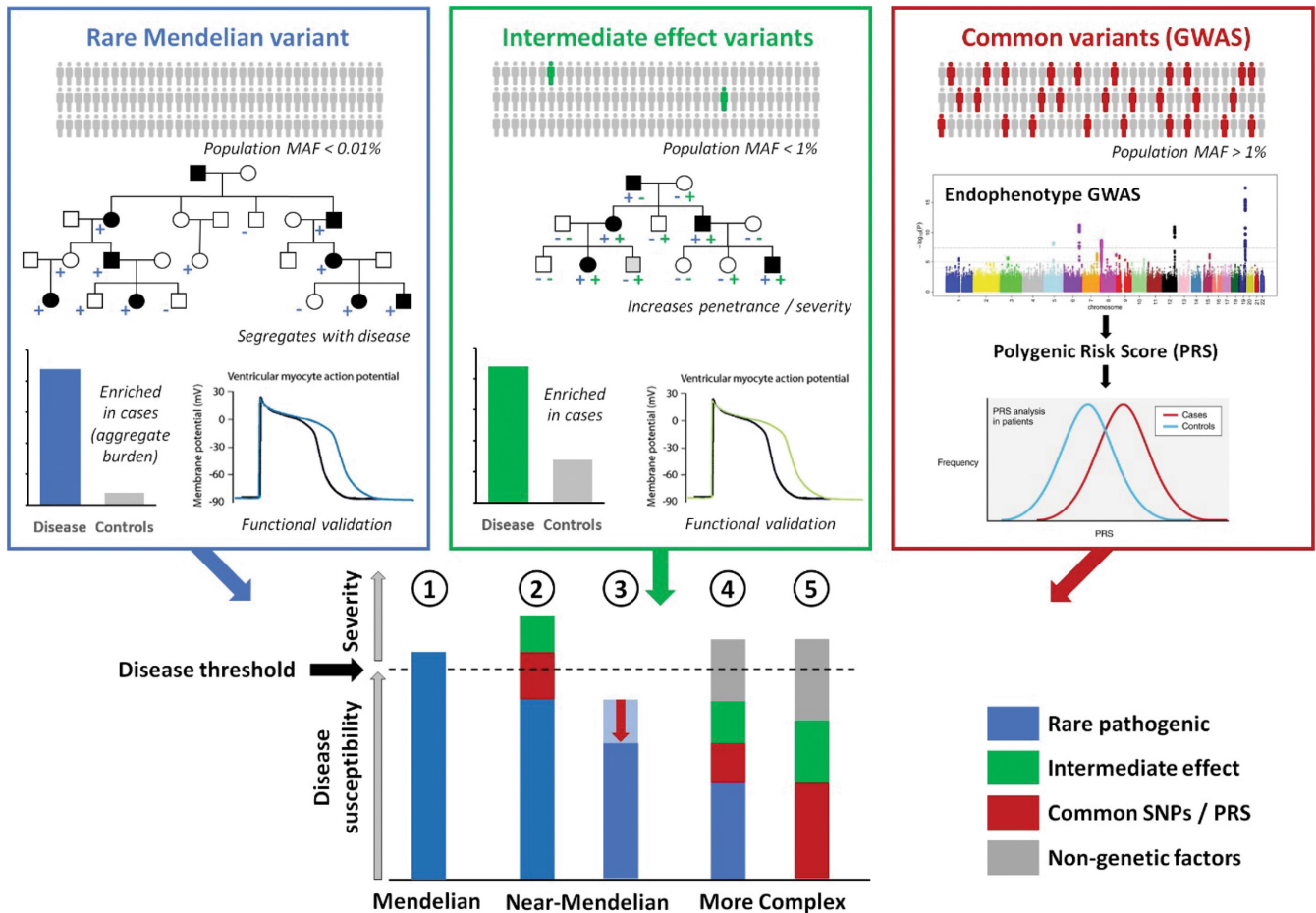
LQTSs, Brugada syndrome (BrS), short QT syndromes (SQTs), and CPVT; and cardiac conduction defects have been identified.<sup>24</sup>

In Mendelian cardiovascular disorders with potentially devastating initial manifestations, such as SCD or aortic dissection, appropriate and prompt identification of individuals at risk is imperative.<sup>25</sup> Genetic testing has been recommended for a number of inherited cardiac conditions for several years and has become a standard aspect of clinical management in affected families. The primary benefit of genetic testing is to identify at-risk carriers of the familial pathogenic variant (and non-carriers who are unlikely to develop disease) through cascade screening, assuming a genetic variant is identified that can be predicted with confidence to cause the disease. Such clinical genetic testing for these single-gene disorders has been shown to be cost-effective<sup>26</sup> and can be considered as a success story in the application of genetics into clinical practice.

Although pathogenic Mendelian genetic variants are characterized by a large effect size, they may not in isolation be sufficient to yield a disease phenotype. This is evidenced by incomplete disease penetrance where only a proportion of individuals in the same family carrying a particular genetic variant shows the disease. Another feature that characterizes Mendelian disorders is the phenomenon of variable expressivity, where different disease severity is observed among individuals carrying the same underlying genetic predisposition. What this means is that, even within pedigrees sharing the same pathogenic variant, the clinical presentation can vary from a patient having no clinical manifestation of the disease to another having severe disease. A clearly pathogenic variant can, therefore, have high diagnostic value, but low prognostic utility.<sup>27</sup> Besides non-genetic (such as environmental) factors, penetrance and expressivity of Mendelian genetic defects are influenced by the co-inheritance of other genetic factors alongside the Mendelian genetic defect, that act to exacerbate or attenuate the effect of the latter on the phenotype (often referred to as 'genetic modifiers', Figure 1).

Contrary to Mendelian disorders, where a single large-effect variant primarily determines susceptibility to the disorder, susceptibility to disorders with complex inheritance rests on the co-inheritance of multiple variants. Such variants are identified by means of genome-wide association studies (GWAS) that compare the prevalence of millions of genetic variants genome-wide between affected individuals and controls. Non-Mendelian genetic risk variants that contribute to cardiovascular disease risk and that are detectable with current approaches and study sample sizes can be broadly grouped into two categories. These comprise common variants, typically defined as having a MAF of >1–5%, which have individually small effect sizes, and intermediate effect variants (MAF <1–2%) with effect sizes and frequencies between common and Mendelian variants (Figure 1).

It is likely that a continuum of genetic complexity exists where at one end of the spectrum are Mendelian disorders determined primarily by the inheritance of an ultra-rare large-effect genetic defect, and at the other end are highly polygenic disorders determined by many genetic variants with additive effect (Figure 1). While some disorders present primarily with one form of



**FIGURE 1** The genetic aetiology of cardiovascular diseases. Mendelian disease variants (upper left panel) are ultra-rare in the population and have large effect sizes, though often not sufficient in isolation to yield a disease phenotype. Mendelian genes and variants can be identified through analysis of family pedigrees or burden analysis in case–control studies and further validated with functional assays. Common variants (upper right panel) with individually small effect sizes may collectively contribute to disease burden or modulate the effects of Mendelian variants. Intermediate effect variants (upper middle panel) are emerging variant classes that usually have population frequencies and effect sizes between rare Mendelian and common variants and may act to increase severity and penetrance. Such variants can be identified by demonstrating enrichment in case cohorts and deleterious effects in established functional assays. These different variant classes can combine to reach the threshold of disease in patients with rare cardiovascular diseases and contribute to the variable severity observed in patients. Diseases such as HCM and LQTS are often Mendelian [1] or near-Mendelian where Mendelian variants of large effect sizes can combine with other variant classes to cause disease [2] or act as protective modifiers (e.g. regulatory variants affecting the expression ratio of the mutant vs. non-mutant alleles) [3]. In contrast, diseases such as BrS and DCM may exhibit a more complex aetiology where substantial non-Mendelian genetic and non-genetic factors are required to reach disease threshold in the presence of a low penetrance rare variant [4] or in a non-Mendelian disease model [5]. blue –, individual does not harbour the familial rare pathogenic variant; blue +, individual harbours the familial rare pathogenic variant; green –, individual does not harbour that intermediate effect variant; green +, individual harbours a given intermediate effect variant; GWAS, genome-wide association study; MAF, minor allele frequency; PRS, polygenic risk score; SNP, single-nucleotide polymorphism. Adapted from Walsh *et al.*<sup>3</sup>

inheritance, different inheritance patterns may exist for the same disorder.<sup>28</sup> Emerging data suggest that common variants of small effect and intermediate effect variants may, to varying extents, influence penetrance in individuals with Mendelian genetic defects by pushing the genetic burden towards the threshold of disease, as well as influence severity of disease.<sup>29,30</sup> While their incorporation into genetic testing approaches is expected to increase the sensitivity of genetic testing, the identification of such modulatory variants is still a matter of intense research and therefore currently not clinically applicable.

### 3 | DIFFERENT METHODS OF GENETIC TESTING

#### 3.1 | Methods to interrogate genetic variation

Genomic technology has enabled efficient and comprehensive assessment of genetic variation within individuals. We each carry millions of variants in our genome, ranging in size from substitutions of a single-nucleotide (single-nucleotide variant; SNV, sometimes termed SNP) to deletions or duplications of an entire chromosome.



Smaller variants, such as SNVs, are more prevalent in our genomes. We each carry about 100 SNVs that have arisen *de novo* during our development and are private to us,<sup>31</sup> and thousands of other rare SNVs.<sup>32</sup> The largest structural variants are much less prevalent, for example aneuploidy (the presence of an abnormal number of chromosomes in a cell), affects about 1 in 300 live births.<sup>33</sup> Though *individually* smaller variants are less likely to cause disease than larger changes that are more likely to disrupt genome function, *collectively* they probably account for the majority of phenotypic variability and inherited disease.<sup>34,35</sup>

The largest genetic variants were the first to be detectable and associated with disease, with an extra copy of chromosome 21 detectable by microscopy, and recognized as causing Down's syndrome in 1959.<sup>36</sup> In 1977, *Sanger sequencing* was developed as a method for directly reading the sequence of DNA,<sup>37</sup> with the resolution to discover SNVs. It was the most widely used DNA sequencing technology for more than 30 years, underpinning the human genome project (1990–2003),<sup>38</sup> and remains an important tool today as it is fast, flexible, and remains the gold-standard for accuracy. However, it is prohibitively costly and laborious for large scale genomics, or diagnostics of ICCs at scale. The human genome, for example, is made up of ~3 billion base pairs, with about 20 000 distinct protein-coding genes. One sequencing reaction reads out up to ~1000 base pairs of sequence (equivalent to 1000 base pairs), so that typically one reaction is required per exon of a gene. Large genes require many reactions (e.g. *RYR2* has 105 exons, *TTN* has 364 exons). Furthermore, ICCs are genetically heterogeneous, so that it is often necessary to sequence many genes in an individual patient.

A 'next generation' of sequencing technologies became available in the early 2000s that used diverse strategies to make the sequencing process massively parallel, and therefore vastly more scalable.<sup>39,40</sup> Several *high-throughput sequencing* technologies are now available, each with different strengths and weaknesses (e.g. emphasizing cost, speed, accuracy or read-length), and high-throughput sequencing now is the mainstay for first-line sequencing in most diagnostic contexts.

High-throughput sequencing allows WGS, or with additional sample preparation, restriction to specific genomic regions of interest: *targeted sequencing*. The choice of target represents a trade-off of cost vs. completeness of genetic characterization. The region of interest may be restricted by gene, and/or by functional annotation (e.g. coding sequence, promotor region, cis-regulatory element, intron, etc.). Since protein-coding regions represent about 1% of the genome, but harbour ~85% of disease-causing variants,<sup>41</sup> targeted sequencing often prioritises these regions. Typical approaches are to sequence the protein-coding regions of all ~20 000 annotated genes (WES),<sup>42</sup> or a pre-specified set of genes of interest, such as genes related to a particular clinical condition (a 'gene panel'; usually exons only). Data can also be generated for a large panel of genes, or indeed all genes, but with downstream *in silico* analysis restricted to a more focused subset—sometimes described as a 'virtual panel'. In practice there is usually also a trade-off between depth and breadth of sequencing,

with broader targets (e.g. WES) leading to reduced sequencing depth and reduced sensitivity in some areas. That is for a given amount of sequencing, as the number of genes sequenced increases, the amount of data from each gene decreases. We can focus sequencing on a narrow region for maximum accuracy, or can spread across a larger region, accepting that sensitivity will decrease if sequencing is spread too thinly. Currently, more targeted sequencing often provides more complete data for the selected region. [Table 4](#) summarizes the strengths and limitations of the various genetic testing methods.

While exon sequencing typically also targets sufficient immediately adjacent sequence to detect non-coding variants disrupting known splice sites, it will not detect variants that create new splice sites at a distance from the usual coding sequence, and usually omits 5'- and 3'-untranslated regions and other regulatory elements which can harbour important disease-associated variants.<sup>43,44</sup>

Sequencing methods also differ in their sensitivity for different variant types. All methods are able to detect the small variants that account for the majority of the burden of ICCs (SNVs, small insertions and deletions). Larger and more complex variants, such as deletion of a whole exon, or a complex genomic rearrangement, are often harder to detect, especially if sequencing does not cover the boundary of the variant (the breakpoint). They may nonetheless be detectable in high-throughput sequence data through a change in the number of DNA reads coming from a particular region, or through a change in allele balance (loss of heterozygosity). Whole-genome sequencing offers the most comprehensive sensitivity across all variant classes, but development in computational tools continues to improve detection of structural and copy number variants (CNVs) from WES and panel sequencing.<sup>45,46</sup> However, alternative non-sequencing quantification approaches such as multiplex ligation-dependent probe amplification (MLPA) or array comparative genomic hybridization may be more sensitive as discussed below.

All sequencing approaches directly read out the DNA sequence(s) present in a sample, allowing analysis of any variation present, and can be used for both *discovery* and *detection* of variants. There are some notable additional technologies that can determine the presence or absence of a pre-specified variant, i.e. *detection only*, that have important clinical applications.

Polymerase chain reaction (PCR) methods can be used for variant detection. *Allele-specific PCR* is cheap and scalable for the detection of a specific variant and quantification of alleles in a sample, but must first be optimized for each variant to be studied. *Digital PCR* (including droplet digital PCR) allows precise quantification of the number of copies of a target DNA sequence relative to a single-copy reference locus.<sup>47</sup> It is cheap and sensitive to small differences in dose and is an important approach to confirm the presence of potential new CNVs identified by sequencing.

Other important methods are based on competitive hybridization of DNA to oligonucleotide probes with a known sequence. *DNA single-nucleotide polymorphism (SNP) arrays* can detect

TABLE 4 Different methods of genetic testing

Technology	Strengths	Limitations	Example diagnostic application
<b>Sequencing approaches</b>			
<b>Sanger sequencing</b>	<ul style="list-style-type: none"> <li>• Accuracy</li> <li>• Low cost per reaction</li> </ul>	<ul style="list-style-type: none"> <li>• Not scalable</li> <li>• Insensitive to large SVs</li> </ul>	<ul style="list-style-type: none"> <li>• Single gene test</li> <li>• Single variant testing—for a pre-specified variant during cascade family evaluation</li> </ul>
<b>Panel sequencing</b>	<ul style="list-style-type: none"> <li>• Balances reasonably comprehensive coverage (e.g. all genes associated with a particular phenotype) against cost</li> <li>• Often highly optimized for complete and uniform capture of region of interest</li> </ul>	<ul style="list-style-type: none"> <li>• Usually exonic only</li> <li>• Needs updating as knowledge changes (e.g. new gene-disease associations discovered)</li> </ul>	First line diagnostic test for proband
<b>WES</b>	<ul style="list-style-type: none"> <li>• Comprehensive coverage of all genes</li> <li>• Off-the-shelf design</li> <li>• Can run a single wet-lab workflow, and introduce specificity at analysis stage</li> <li>• Can update analysis to incorporate new knowledge without regenerating data—adaptable</li> <li>• Enables analyses for secondary findings</li> </ul>	<ul style="list-style-type: none"> <li>• Larger target requires more sequencing (c.f. panels)</li> <li>• May be less optimized than more focused panel</li> <li>• More costly and complex to store and process data (c. 10–100× more data than panel)</li> <li>• Will not detect non-coding variants</li> <li>• May not detect all variant classes</li> </ul>	<ul style="list-style-type: none"> <li>• Diagnosis in proband for very heterogeneous conditions (e.g. paediatric and syndromic cardiomyopathies)</li> <li>• Second line test if panel negative in specific circumstances, for example with informative family structure</li> </ul>
<b>WGS</b>	<ul style="list-style-type: none"> <li>• Comprehensive genetic characterization—all genes, all elements, all variant types</li> <li>• Will also detect common variants for PRS, pharmacogenetics and other applications</li> <li>• Enables analyses for secondary findings</li> </ul>	More costly and complex to store and process data (~100× more data than WES)	<ul style="list-style-type: none"> <li>• Diagnosis in proband for very heterogeneous conditions</li> <li>• Second line test if panel negative</li> <li>• Definitive and future-proof genetic characterization if funds permit—e.g. hold data in medical record for iterative targeted interpretation according to clinical needs</li> </ul>
<b>Non-sequencing approaches</b>			
<b>Allele-specific PCR</b>	Quick, cheap, accurate	Pre-specified variants only	Testing a single variant in a large family (more likely Sanger sequencing now)
<b>Array comparative genomic hybridization</b>	<ul style="list-style-type: none"> <li>• Cheap screening for SVs/CNVs</li> <li>• High-resolution (compared with cytogenetic approaches)</li> </ul>	Insensitive to other variant classes	Screening for structural variants, including aneuploidy, e.g. in structural congenital heart disease
<b>Droplet digital PCR</b>	Low cost, high-sensitivity, detection of genome dose for SV/CNV detection at a pre-specified locus	Scalability limited by multiplexing of pre-specified PCR amplicons targeting regions of interest	Confirmation of putative CNVs detected in high-throughput sequence data
<b>DNA SNP arrays</b>	<ul style="list-style-type: none"> <li>• Genome wide</li> <li>• Relatively cheap</li> </ul>	<ul style="list-style-type: none"> <li>• Pre-specified variants only</li> <li>• Accuracy poor for many rarer variants</li> </ul>	<ul style="list-style-type: none"> <li>• Recreational ancestry analysis</li> <li>• Polygenic risk</li> <li>• Pharmacogenetics</li> </ul>

CNV, copy number variant; PCR, polymerase chain reaction; PRS, polygenic risk score; SNP, single-nucleotide polymorphism; SV, structural variant; WES, whole-exome sequencing; WGS, whole-genome sequencing.

millions of variants in parallel, but each variant must be pre-specified and the hybridization optimized, and not all variants can be assayed accurately. These have minimal utility for identification of rare variants for Mendelian diagnosis but are widely used where common variants are important, for example in GWAS, calculating polygenic risk scores as detailed below, and in pharmacogenetics.<sup>48</sup> *Array comparative genomic hybridization* (aCGH) is another genome-wide hybridization-based approach used to

detect copy-number changes, of particular importance in congenital structural heart disease and individuals with syndromic ICCs. MLPA combines PCR and hybridization methods to quantify specific nucleic acid sequences quickly and efficiently, and may be used to detect many variant types, but particularly copy number changes.<sup>46</sup>

These diverse and complementary methods can then be deployed for different types of clinical genetic testing. *Confirmatory*

testing refers to genetic analysis of an individual with a diagnostic clinical phenotype to identify the underlying genetic cause. In a proband (the first presenting person in a family), there is no pre-specified variant to search for, so a direct sequencing approach is used to discover any genetic variation in the genes associated with that condition. For many ICCs, the first line test will be a high-throughput sequencing gene panel relevant to a specific disease, or a virtual panel using WES with targeted analysis. If this analysis does not identify an underlying cause, then more comprehensive genetic characterization, such as WES or WGS, may be used to interrogate additional genes, look for variant types not examined by the first line test, or assess for non-coding variants. This kind of comprehensive testing is appropriate only in experienced centres and with cautious interpretation of any variants identified. Having established the causative variant in one family member, it is appropriate to look only for this specific variant in cascade testing of subsequent family members, using Sanger sequencing or a non-sequencing approach, unless there is reason to suspect additional genetic contributors.

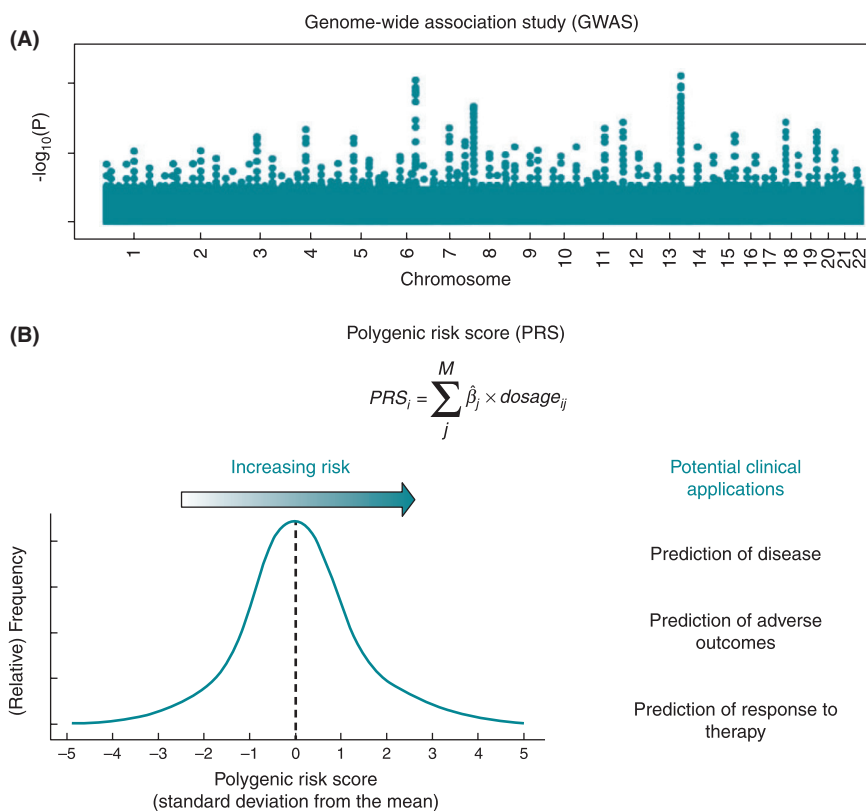
*Predictive (or cascade) testing* refers to testing of individuals with or without a phenotype, often unaffected relatives of an affected proband, with the aim of targeting clinical surveillance to individuals with the genetic predisposition. Sanger sequencing to detect the known familial variant is often used here.

WES and WGS also enable *opportunistic screening*. The American College of Medical Genetics & Genomics (ACMG) recommend that a pre-specified panel of well-characterized disease-associated

genes be interrogated whenever clinical exome or genome sequencing is undertaken, irrespective of the primary indication for genomic analysis.<sup>49</sup> This panel currently includes 73 genes ('ACMG SF v3.0'), many of which are ICC genes (Supplementary material online, Table S1).<sup>50</sup> The costs and benefits of actively seeking secondary findings remain under evaluation, and these recommendations have not been widely adopted outside the USA. Several companies also offer direct-to-consumer sequencing that includes analysis of ICC genes for individuals without symptoms or signs of disease. The costs and benefits of actively seeking secondary findings remain under evaluation, and a consensus has not been reached about these recommendations.

### 3.2 | Genome-wide association study and polygenic risk scores

Genome-wide association study is used to test associations between genetic variants and human traits or disease phenotypes (Figure 2A).<sup>51</sup> Typically, in a GWAS, each study individual is genotyped by means of a DNA SNP (SNV) array for 200 000 to 1 000 000 known SNVs, although, increasingly, whole-genome sequence data may be used. Array-based genotyping is almost invariably followed by imputation, a process of using the known linkage disequilibrium (correlation) between SNVs in order to predict (impute) unobserved genotypes that are not directly assayed on the array. This permits examination of a greater number of variants (up to 10s of millions).



**FIGURE 2** Genome-wide association studies (GWAS) test the association of common genetic variants with traits or diseases. Results are shown as a Manhattan plot (A) where the  $P$ -value (y-axis) is plotted against the genomic position (x-axis) for millions of common variants across the genome (blue markers). Polygenic risk scores (B) are generally derived from GWAS and calculated for an individual  $i$  ( $PRS_i$ ) as the sum of the products of allelic dosage ( $dosage_{ij}$ ) by the regression coefficient/weight ( $\hat{\beta}_j$ ) for all  $M$  genetic variants ( $j$ ). Created with [Biorender.com](https://biorender.com)

Each variant is then tested for association with the trait or phenotype of interest. Since the positions of the SNVs are known in the genome, the results of a GWAS in one study may be combined with others in a meta-analysis to improve statistical power. Variants with an association  $P$ -value  $< 5 \times 10^{-8}$  are generally considered statistically significant, based on multiple testing correction for the roughly 1 000 000 independent common variant tests (haplotype blocks) in the human genome.<sup>52</sup>





Similar analytic methods can be used to examine WGS and WES data. Since 2006, the GWAS approach has been successfully implemented across a broad range of phenotypes in cardiovascular genetics. It has been widely applied to identify common variants that modulate interindividual variability of quantitative cardiophysiological traits, such as electrocardiogram (ECG) parameters,<sup>53</sup> cardiovascular magnetic resonance (CMR) parameters<sup>54</sup> and blood pressure,<sup>55</sup> with the premise that the genetic variants that impinge on such traits also contribute to disease. Genome-wide association study has also been widely applied for identification of susceptibility variants for common multifactorial disorders such as coronary artery disease,<sup>56</sup> heart failure,<sup>57</sup> and AFib.<sup>58</sup> An analytic technique referred to as Mendelian randomization uses genetic information as an instrumental variable to assess for the causal relations between risk factors and diseases. For example, using this approach, GWAS studies of SCD have suggested a genetic correlation between SCD and coronary disease, traditional coronary artery disease risk factors, and electrical instability traits (QT and AFib).<sup>59</sup>







Genome-wide association studies are increasingly being used to identify common variants that contribute to susceptibility to rare/less common cardiovascular disorders such as BrS,<sup>60</sup> LQTS,<sup>28</sup> DCM,<sup>61</sup> and HCM.<sup>29,30</sup> Notably, GWAS enable the identification of many genetic variants associated with a given trait or disease, which can be used to 'score' a specific individual for their aggregate

genetic predisposition to that specific trait or disease. Such scores are referred to as *polygenic risk scores (PRS)* or genomic risk scores. Polygenic risk scores result in numeric estimates that represent the cumulative burden of genetic predisposition to a specific phenotype. The phenotype can be a disease such as DCM, or a trait such as left ventricular (LV) systolic dysfunction. The scores are typically calculated by combining the effects of many genetic variants in a mathematical framework to derive a single numeric value for an individual. The number of variants included in a PRS may range from a few to several million. The genetic variants chosen for inclusion in a PRS, and the importance or weight given to each variant, are typically derived from large-scale genetic association studies (i.e. GWAS) with the disease or trait of interest.

Since genotypes vary at each genomic position across individuals, PRS follow a distribution in the population (Figure 2B). Typically, individuals in the lower tails of a polygenic risk score have a lower risk of developing the disease or trait of interest, whereas those in the upper tails have a higher risk. Polygenic risk scores have been calculated for many conditions including cardiovascular diseases.<sup>62</sup> Both the number of conditions for which they have been calculated and the mathematical methods for selecting and weighing variants are rapidly evolving. Polygenic risk scores have been largely utilized for research purposes to date, but scores are increasingly being applied to clinical trial settings<sup>63-65</sup> indicating the potential clinical utility of using these risk markers in the management and prevention of common diseases. The potential utility of PRS in less common conditions such as inherited arrhythmias and cardiomyopathies is also being explored.<sup>28-30,66-68</sup> In the coming years, we anticipate that PRS are likely to enter the clinical practice landscape and become more widely utilized. At present, it seems too early, however. Eventually, PRS may hopefully be able to provide information not only on disease risk but also disease mechanism and therapeutic efficacy.

#### 4 | CHOICE OF GENETIC TESTS AND INTERPRETATION OF VARIANTS

Recommendation	Consensus statement instruction	Ref.
Genetic testing in patients with a potential cardiogenetic condition is performed only with appropriate genetic counselling.		Expert opinion
In patients with a clear specific phenotype, it is appropriate to perform genetic testing analysing genes with definite or strong evidence supporting disease causation.		10,17,20,21,69
In patients with a clear specific phenotype, it may be appropriate to analyse genes with moderate evidence supporting disease causation.		10,17,20,21,69
In selected cases with a definite phenotype and no genetic diagnosis after testing of the genes with definite or strong evidence supporting disease causation, broader genetic testing may be considered. Such selected cases may include familial cases, those with atypical features, such as extracardiac manifestations and those with unusual early disease onset.		17

Recommendation	Consensus statement instruction	Ref.
Variant interpretation in the clinical setting is greatly enhanced by the use of disease-specific, multi-disciplinary teams that could include clinical disease experts, clinical geneticists, or genetic counsellors and molecular geneticists.		10,70-75
Variant interpretation is best performed using standard guidelines for interpretation and can be enhanced by gene-specific rule specifications tailored for the gene and disease under consideration.		17,76,77
Reported Variants of Uncertain Clinical Significance (VUS) may be reclassified, i.e. 'upgraded' [Likely Pathogenic/Pathogenic (LP/P)] or 'downgraded' (Likely Benign/Benign), in multi-disciplinary clinics with access to molecular genetics laboratories, according to robustness of clinical phenotype and/or familial segregation evidence.		10,70-75,78
Genetic testing for genes with (i) limited, (ii) disputed, or (iii) refuted evidence should not be performed in patients with a weak (non-definite) phenotype in the clinical setting.		10,17,20,21,69
In families where a LP/P variant has been identified, detailed genetic counselling and guidance regarding inheritance patterns, variant penetrance, and risk should be offered, and cascade testing facilitated.		Expert opinion
In patients with a high probability of a specific inherited cardiac disease and a molecular screening performed in a pre-NGS era or with an incomplete NGS panel, repetition of the testing should be considered.		Expert opinion

## 4.1 | Background

A basic tenet of clinical genetic testing is that the genes evaluated should have strong scientific evidence supporting their disease association.<sup>69</sup> Given the challenge of variant interpretation,<sup>79</sup> there is risk of inaccurate information being provided to patients and families when genes with limited evidence for disease causality are tested. In the context of life-changing diagnoses which may provoke significant anxiety or aggressive treatment interventions, optimizing methods for best practice of genetic variant interpretation is essential. Recent collaborative projects involving clinical disease experts, genetic counsellors, and clinical/molecular geneticists have provided detailed evidence-based gene classifications for Mendelian arrhythmia and cardiomyopathy disorders, highlighting genes with moderate, strong or definitive evidence for disease causation, and others with limited or disputed evidence<sup>12-15,17,22</sup> (for definitions of these classifications see page 7 in: [https://clinicalgenome.org/site/assets/files/5391/gene\\_curation\\_sop\\_pdf-1.pdf](https://clinicalgenome.org/site/assets/files/5391/gene_curation_sop_pdf-1.pdf)).

In 2015, the ACMG provided a standard, criteria-based approach for the interpretation of genetic variants in clinical testing.<sup>69</sup> Criteria include the frequency of the allele in people with and without disease, the degree of familial segregation with other affected family members, topological location within relevant functional domains of the protein, and functional analysis of the variant. Importantly, no single criterion alone, including abnormal functional assay, is sufficient to conclude the pathogenicity of a genetic variant. A summation of the evidence leads to a provisional classification of the variant along a probabilistic range of categories: Pathogenic (P), Likely Pathogenic (LP), Variant of Uncertain Clinical Significance (VUS), Likely Benign (LB), Benign (B). Although challenging to quantify, according to

ACMG guidelines the terms LP and LB suggest a >90% certainty of a variant being disease-causing or benign, highlighting the significant range of probability for variants classified as VUS.

The VUS classification represents the 'Achilles Heel' of genetic variant interpretation in the clinical arena. At times, high-volume, multi-disciplinary clinics may have sufficient clinical expertise or evidence that may allow for an upgrading or downgrading of the variant to pathogenic or benign, respectively.<sup>74,77,78</sup> In contrast, the absence of segregation of a VUS interpreted variant with a robust familial phenotype may lead to re-classifying to likely benign. These examples highlight that most laboratory-based variant interpretation is done in the absence of detailed clinical phenotyping knowledge available in a multidisciplinary clinic. To minimize the burden of VUS classifications, collaborative expert teams have proposed ACMG-modified, gene-specific rules which take in to account the specific knowledge accumulated for certain genes in specific conditions.<sup>76,80</sup> Where possible, this approach may enhance variant interpretation classification.<sup>77</sup>

Genes that do not have sufficient evidence to date as single-gene causes for disease should not receive variant interpretations. Clinical testing laboratories that continue to offer these genes on their panels should clearly label their limited evidence, but may consider providing unclassified, identified variants to clinics in support of ongoing research on candidate genes.

### 4.1.1 | Use of the obtained genetic knowledge

After genetic testing, a clinically actionable result (LP/P) can provide diagnostic clarification in the proband (Table 5). It also provides

TABLE 5 Impact of genetic testing for the proband

Disease	Diagnostic	Prognostic	Therapeutic
<b>Arrhythmia syndromes</b>			
Long QT syndrome	+++	+++	+++
CPVT	+++	+	+
Brugada syndrome	+	+	+
Progressive cardiac conduction disease	+	+	+
Short QT syndrome	+	+	+
Sinus node disease	-	+	-
Atrial fibrillation	-	+	-
Early repolarization syndrome	-	-	-
<b>Cardiomyopathies</b>			
Hypertrophic cardiomyopathy	+++	++	++
Dilated cardiomyopathy	++	+++	++
Arrhythmogenic cardiomyopathy	+++	++	++
Left ventricular non-compaction	+	+	-
Restrictive cardiomyopathy	+	+	+
<b>Congenital heart disease</b>			
Syndromic CHD	+++	+	-
Non-syndromic CHD	+	-	-
Familial CHD	++	-	-

+++ : is recommended/is indicated or useful.

++ : can be recommended/can be useful.

+ : may be considered/may be useful.

- : is not recommended/is not indicated nor useful.

information relevant to prognosis and relevant to therapeutic choices in many but not all disease entities (Table 5). In addition, it offers the potential for cascade (predictive) testing of at-risk family members.<sup>81-85</sup> Cascade testing involves targeted testing of first-degree relatives for the LP/P variant found in the proband ('appropriate relatives'). When cascade testing is performed in an at-risk relative, those who are found not to carry the disease-causing gene variant can be released from further clinical surveillance in the vast majority of conditions. Some exceptions exist and are discussed at the individual disease level. In general, cascade screening is recommended when results will affect clinical management. When the results are 'only' useful for family planning, cascade screening may be considered. Recommendations for cascade screening and the age at which this should be performed are disease- and sometimes gene-specific. Those who are found to carry the disease-causing gene variant should undergo clinical screening at regular intervals. Family members of a patient where genetic testing is not done or is negative

(no likely-pathogenic or pathogenic variant is identified) also require clinical screening at regular intervals because there is considerable phenotypic heterogeneity in age of onset and disease progression within members of the same family. That being said, in some diseases, there is emerging evidence that a negative genetic test in the proband or the affected individual may indicate lower probability of monogenic disease.

In the event that a VUS is reported, a disease-specific multidisciplinary team can help to further classify the variant as LP or LB, based on the criteria outlined in detail above. A VUS that has not been upgraded to LP should not be used to facilitate cascade screening; rather, clinical screening is required. When multiple family members exhibit a characteristic phenotype, robust co-segregation of the variant with the affected family members can contribute to classification of the variant as LP or even P.

A pathogenic variant can also be identified at postmortem testing (i.e. after the usually SCD of a family member) using blood or tissue collected at autopsy. Postmortem testing is especially useful in instances where the family variant is unknown and no other affected family members are still living.<sup>86-88</sup> Access to a molecular autopsy as well as considerations related to costs and insurance coverage for this testing can vary between countries and jurisdictions. Nevertheless, identification of a LP/P variant may confirm or establish a familial diagnosis and allow cascade genetic testing of other at-risk relatives as outlined previously.

In addition, detailed genetic counselling and guidance is recommended and should start before a genetic test is performed. Families should be informed of the mode of inheritance of disease, most commonly AD inheritance whereby there is a 50% chance the variant will be passed on to offspring, regardless of sex. Families should be informed that carrying the LP/P variant does not necessarily mean development of clinical disease, reflecting variable penetrance, e.g. some gene variant carriers may never develop clinical disease (genotype positive, phenotype negative) or may only develop very mild disease and therefore be at low risk of disease complications. In all families and couples (with most conditions) where pregnancy is being planned, the above factors need to be discussed, as well as reproduction options such as prenatal genetic testing and preimplantation genetic diagnosis.






## 5 | STATE OF GENETIC TESTING FOR INHERITED ARRHYTHMIA SYNDROMES

### 5.1 | Long QT syndrome

#### 5.1.1 | Impact of genetic testing for the index case

Disease	Diagnostic	Prognostic	Therapeutic
LQTS	+++	+++	+++



Recommendations	Consensus statement instruction	Ref.
Molecular genetic testing for definitive disease associated genes (currently <i>KCNQ1</i> , <i>KCNH2</i> , <i>SCN5A</i> , <i>CALM1</i> , <i>CALM2</i> , and <i>CALM3</i> ) should be offered to all index patients with a high probability diagnosis of LQTS, based on examination of the patient's clinical history, family history, and ECG characteristics obtained at baseline, during ECG Holter recording and exercise stress test (Schwartz Score $\geq 3.5$ , Supplementary Table S2). <sup>a</sup>		20
Analysis of specific genes should be offered to patients with a specific diagnosis as follows: <i>KCNQ1</i> and <i>KCNE1</i> in patients with Jervell and Lange-Nielsen syndrome, <i>CACNA1C</i> in Timothy syndrome, <i>KCNJ2</i> in Andersen-Tawil syndrome, and <i>TRDN</i> in patients suspected to have triadin knockout syndrome.		20,89–93
An analysis of <i>CACNA1C</i> and <i>KCNE1</i> may be performed in all index patients in whom a cardiologist has established a diagnosis of LQTS with a high probability, based on examination of the patient's clinical history, family history, and ECG characteristics obtained at baseline, during ECG Holter recording and exercise stress test (Schwartz Score $\geq 3.5$ ). <sup>a</sup>		20
Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causing variant.		Expert opinion
Predictive genetic testing in related children is recommended from birth onward (any age).		Expert opinion

<sup>a</sup>The Schwartz score can be found in Supplementary material online, Table S2.

### 5.1.2 | Background

The congenital LQTS is a genetically transmitted channelopathy, characterized by prolongation of the QT interval on the baseline ECG, usually associated with T-wave abnormalities (i.e. notched T waves, biphasic T waves).<sup>15</sup> To make a diagnosis of congenital LQTS it is essential to exclude secondary causes, i.e. QT-prolonging drugs or electrolyte imbalances.<sup>94</sup> Prolongation of action potential duration favours early afterdepolarizations and torsades de pointes (TdP) is the typical arrhythmia in this disease.<sup>95–98</sup> Torsades de pointes, frequently triggered by pauses and/or adrenergic stimulation,<sup>98</sup> can cause self-terminating dizziness or syncopal events or can degenerate into ventricular fibrillation (VF) and SCD. Electrocardiogram characteristics associated with high risk of life-threatening arrhythmias, include T wave alternans and functional 2:1 atrioventricular block, which are frequently present in patients who present perinatally. To make a diagnosis of LQTS, it may be important to evaluate not only basal ECG but also the behaviour of QTc during exercise stress test and 24-h, preferably 12-lead, Holter recording.<sup>99,100</sup> Diagnostic criteria have been developed to support the diagnosis of the disease, i.e. the 'Schwartz score'.<sup>101</sup>

Long QT syndrome has a prevalence of at least 1:2500 people<sup>102</sup> and clinical manifestations tend to occur during childhood or teenage years. Among symptomatic index cases, the untreated 10-year mortality is ~50%.<sup>103,104</sup>

### 5.1.3 | Summary of the major long QT syndrome genes

Table 6 (and Supplementary material online, Table S3) summarize all genes associated with LQTS and their ClinGen classification.<sup>20</sup> Long QT syndrome genes can be divided in three main groups: those genes in which pathogenic variants reduce potassium outward

currents, those in which pathogenic variants increase sodium inward current, and those in which pathogenic variants increase calcium inward current.

**Potassium channel-related LQTS:**<sup>95,96</sup> pathogenic variants in potassium channels genes are responsible for the vast majority of LQTS cases and *KCNQ1* and *KCNH2*, encoding for the alpha subunit of potassium channels conducting the  $I_{Ks}$  and  $I_{Kr}$  currents, respectively, account for 80% of all genetically explained LQTS cases.<sup>95,96</sup> Homozygous or compound heterozygous pathogenic variants in *KCNQ1* and *KCNE1* cause the recessive Jervell and Lange-Nielsen syndrome (JLNS), in which the cardiac phenotype is combined with congenital deafness.<sup>105</sup> *KCNE1* is strongly associated with acquired-LQTS (aLQTS),<sup>94</sup> as is *KCNE2*, and it also causes an uncommon subtype usually associated with low penetrance and with a mild phenotype.<sup>106</sup> Finally, in this subgroup, the Andersen-Tawil syndrome (ATS), caused by pathogenic variants in the *KCNJ2* gene, is generally included,<sup>95,96,107</sup> although it should be questioned whether ATS is actually a subform of LQTS.<sup>74,107</sup> Patients with ATS frequently also present with extra cardiac features, including skeletal myopathy (periodic muscular weakness) and several skeletal and facial dysmorphic features.<sup>95,96</sup>

**Sodium channel-related LQTS:** pathogenic variants in *SCN5A*, causing an increase of sodium inward current, are the third most frequent cause of LQTS and have a predominant role in forms with malignant perinatal presentation.<sup>108,109</sup> Overlapping phenotypes (LQTS, BrS, and cardiac conduction defects) are described.<sup>110</sup> Other components of the Na channel complex have been proposed as candidate genes for LQTS, but there is insufficient evidence to confirm an association.<sup>20</sup>

**Calcium channel-related LQTS:** pathogenic variants causing an increase of calcium inward current are associated with rare but malignant forms of LQTS, some with associated syndromic features. Specifically, Timothy syndrome, caused by the pathogenic

TABLE 6 Genes implicated in long QT syndrome (LQTS)

Gene	Locus	Phenotype—syndrome	Protein (functional effect)	Frequency	ClinGen classification
KCNQ1	11p15.5	LQTS, JLNS	Loss-of- $I_{Ks}$ channel function	40–55%	Definitive
KCNH2	7q35-36	LQTS	Loss-of- $I_{Kr}$ channel function	30–45%	Definitive
SCN5A	3p21-p24	LQTS	Increase in $I_{Na1.5}$ channel function	5–10%	Definitive
CALM1	14q32.11	LQTS	L-type calcium channel (↑)	<1%	Definitive
CALM2	2p21	LQTS	L-type calcium channel (↑)	<1%	Definitive
CALM3	19q13.32	LQTS	L-type calcium channel (↑)	<1%	Definitive
TRDN	6q22.31	Recessive LQTS	L-type calcium channel (↑)	<1%	Strong
KCNE1	21q22.1	LQTS, JLNS, a-LQTS	Loss-of- $I_K$ channel function	<1%	Strong in aLQTS, definitive in JLNS
KCNE2	21q22.1	a-LQTS	Loss-of- $I_K$ channel function	<1%	Strong in aLQTS
KCNJ2	17q23	ATS	Loss-of- $I_{K1}$ channel function	<1%	Definitive in ATS
CACNA1C	12p13.3	TS, LQTS	L-type calcium channel (↑)	<1%	Definitive in TS, moderate in LQTS

Functional effect: (↓) loss-of-function or (↑) gain-of-function at the cellular *in vitro* level.

a-LQTS, acquired-long QT syndrome; ATS, Andersen–Tawil syndrome; JLNS, Jervell and Lange-Nielsen syndrome; RWS, Romano–Ward syndrome; TS, Timothy syndrome.

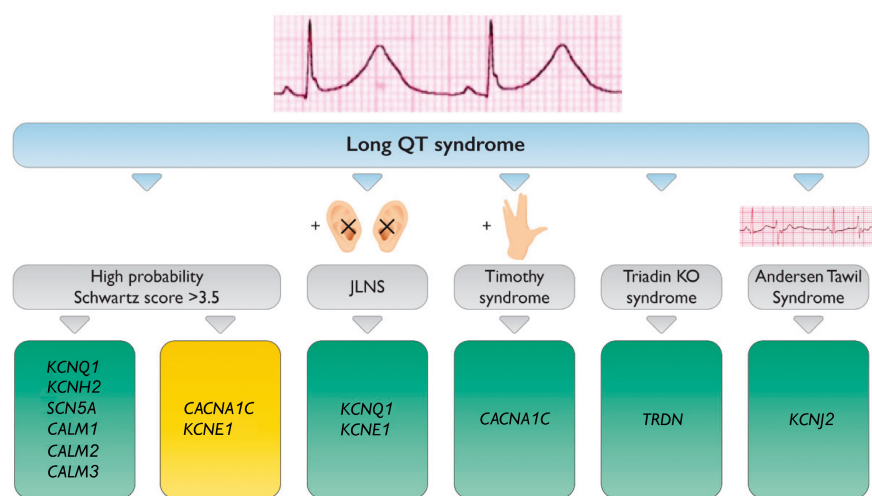


FIGURE 3 Clinical algorithm for genetic testing and family screening in long-QT syndrome

G406R variant in *CACNA1C*<sup>89</sup> is characterized by a perinatal presentation of life-threatening arrhythmias frequently associated with syndactyly, CHDs, cognitive abnormalities, and autism. Long QT syndrome caused by any of the three *CALM* genes<sup>90,111</sup> represents another malignant form of the disease and data from the International Calmodulinopathy Registry show life-threatening arrhythmias in 78% of the cases, mean QTc of almost 600 ms and a perinatal presentation in 58%.<sup>91</sup> Some of these cases show neurological features unrelated to cardiac arrest, and cardiac structural abnormalities.<sup>91</sup> The triadin knockout syndrome (TKOS)<sup>92,93</sup> is a recessive syndrome caused by pathogenic variants in *TRDN*; data from the International Registry show that cardiac arrest is the first clinical manifestation in 71% of patients, and transient QT prolongation, sometimes with T-wave inversion in V1–V3/V5 is frequently observed.<sup>93</sup> Patients in this category also frequent present with neuromuscular involvement. All these forms, which cause QT prolongation secondary to abnormal calcium handling, have in common an early malignant presentation and a poor response to conventional medical therapies.

#### 5.1.3.1 | Index cases (proband)

In LQTS patients with a high probability of LQTS, based on examination of the patient's clinical history, family history, and ECG characteristics obtained both in baseline, during ECG, Holter recording and exercise stress test (Schwartz Score  $\geq 3.5$ ), molecular testing is recommended with a different level of strength depending of the type of gene. In genes with definitive evidence, currently *KCNQ1*, *KCNH2*, *SCN5A*, *CALM1*, *CALM2*, and *CALM3*, the testing is strongly recommended in all probands<sup>20</sup> (Figure 3), including an analysis of CNV, and a disease-causing variant is identified in around 70–85% of cases.<sup>95,112</sup> A possible exception is an active athlete with a prolonged QTc. Indeed, not rarely athletes develop significant QT prolongation which is fully reversible on detraining.<sup>113</sup> In such cases the diagnosis of LQTS should not be made.<sup>113</sup> Another strong recommendation is provided in the context of specific syndromes for causative genes, i.e. *KCNE1* in patients with JLNS,<sup>105</sup> *CACNA1C* in patients with Timothy syndrome,<sup>89</sup> *KCNJ2* in patients with ATS,<sup>95</sup> and *TRDN* in patients with Triadin Knock-out syndrome<sup>92,93</sup> (Figure 3). *CACNA1C* and *KCNE1* that have a moderate evidence in the context of LQTS, the testing may be

considered in patients with a high probability of diagnosis. Only in this subgroup of patients with high probability of LQTS may a broader genetic testing be considered if no disease-causing variant is identified in established genes, and only in experienced centres and with a careful interpretation of the variant identified. However, in these cases a negative genetic test does not exclude the disease, already established clinically. In patients with an intermediate probability of LQTS (e.g. prolonged QTc with a Schwartz score 1.5–3.0), testing of genes with limited, disputed and refuted evidence should not be performed, while testing of the established genes may be considered, mostly to help rule out the diagnosis after extensive phenotypic investigation.

#### 5.1.3.2 | Family screening

Cascade screening in family members is indicated whenever a disease-causing variant is identified in the index case. Indeed, low penetrance and variable expressivity, do not allow one to exclude the diagnosis only on the basis of a normal baseline ECG.<sup>114,115</sup> Early identification of affected family members is important to establish preventive measures, as the risk of life-threatening arrhythmias is not negligible even among those with a normal baseline QTc.<sup>116</sup>



#### 5.1.4 | Prognostic and therapeutic implications of long QT syndrome genetic testing

In LQTS, the identification of a disease-causing variant contributes to risk stratification. Indeed, the identification of a pathogenic variant in *KCNQ1*, *KCNH2*, or *SCN5A* has a role together with the length of the QTc in identifying the risk of life-threatening arrhythmias in asymptomatic subjects.<sup>117</sup> Also, the location of the variant across the protein is important. In fact, location in the pore region of *KCNH2*,<sup>118</sup> the transmembrane location,<sup>118</sup> the S6 segment specifically,<sup>119</sup> and dominant-negative effect for *KCNQ1*, are independent risk factors for cardiac events.<sup>120</sup> Furthermore, some specific pathogenic variants are associated with unusually high clinical severity (high penetrance, long QTc, high incidence of SCD), such as the *KCNQ1*-A341V<sup>121</sup> or the *SCN5A*-G1631D.<sup>108</sup> Others, such as *SCN5A*-D1790G and the E1784K, that not only causes LQTS, but it is also associated with BrS and sinus node dysfunction (SND)<sup>110</sup> are relatively benign.<sup>122</sup> Thus, when managing families with the latter pathogenic

variant, the possibility of an overlap syndrome should be considered. In the recessive JLNS, it matters whether there are two pathogenic variants in *KCNE1* or in *KCNQ1*, with the former presenting with a more benign disease course in terms of risk of life-threatening arrhythmias.<sup>105</sup> Finally, there are some specific genetic subtypes that are at particular high risk of SCD in paediatric age, as patients carrying a pathogenic variant in one of the *CALM* genes<sup>90,91</sup> and despite no systematic studies, the available data suggest that whenever the variants affect the calcium current, the phenotype tends to be more complex and severe.<sup>89–93,111</sup> The role of SNVs as genetic modifier has also been documented, but its evaluation has not yet entered clinical practice in a standardized manner.<sup>2,123</sup>

The amazing progress in understanding the genotype-phenotype correlation has allowed LQTS to become the first disease for which initial steps for gene-specific management have become possible and are already usefully implemented. Patients with a pathogenic variant in *KCNQ1* are at higher risk during sympathetic activation (e.g. during exercise, swimming and emotional stress), and antiadrenergic intervention such as beta-blockers<sup>124,125</sup> and left cardiac sympathetic denervation (LCSD)<sup>126,127</sup> are particularly effective. An implantable cardioverter-defibrillator (ICD) is rarely needed and certainly not for primary prevention, in contrast to the other subtypes where the predicted risk in patients with very long QTc may lead to an earlier primary ICD implantation.<sup>116</sup> In *KCNH2*-LQTS patients, it is essential to preserve adequate potassium levels, and oral potassium may help.<sup>128</sup> Also, these patients are at higher risk when aroused from sleep or rest by a sudden noise<sup>129,130</sup> and in the post-partum phase.<sup>131</sup> Removal of telephones and alarm clocks from their bedrooms is recommended. The realization that *SCN5A* variants producing LQTS have a 'gain-of-function' support the use of late sodium current blockers, in particular mexiletine, in those patients with a QTc >500ms, if their QTc shortens by more than 40ms after oral loading test.<sup>132–134</sup> Recently, mexiletine was shown to shorten QTc also in a significant percentage of *KCNH2* patients<sup>135</sup> opening the possibility of its clinical use also in this genetic subgroup. Finally, very preliminary data, showed that a drug combining lumacaftor and ivacaftor, already in clinical use for cystic fibrosis, could have a role in patients carrying *KCNH2* variants causing a trafficking defect, but data on more patients are still needed.<sup>136,137</sup> All LQTS patients should avoid QT-prolonging drugs (see [www.crediblemeds.org](http://www.crediblemeds.org)).

#### 5.1.5 | Acquired long QT syndrome

Recommendations	Consensus statement instruction	Ref.
Molecular genetic testing for definitive disease associated genes (currently <i>KCNQ1</i> , <i>KCNH2</i> , <i>SCN5A</i> , <i>KCNE1</i> , and <i>KCNE2</i> ) should be offered to all patients with acquired LQTS who experienced drug-induced TdP, are aged below 40 years and have a QTc >440ms (males) and >450ms (females) in the absence of culprit drug		20,94
Cascade family screening for the presence of pertinent variants should be considered when QT prolonging drugs are or could be prescribed		Expert opinion

The acquired LQTS, is a clinical condition characterized by QT prolongation (usually defined as >500ms or >60–70ms drug-induced change from baseline) sometimes associated with TdP, which is induced by QT-prolonging drugs and more rarely hypokalaemia or bradycardia.<sup>94</sup> The probability of developing an acquired LQTS depends on two major factors: (i) the intrinsic risk conferred by a given drug, which is provided by CredibleMeds website (<https://crediblemeds.org>); (ii) the repolarization reserve of a subject in which genetic factors play a role.<sup>2</sup> The genetic predisposition to acquired LQTS includes both ultra-rare,<sup>138</sup> rare,<sup>139</sup> and common genetic variants.<sup>140</sup> The role of molecular testing in the isolated setting of drug-induced LQTS requires individualized consideration. In the study by Itoh *et al.*,<sup>94</sup> the probability of identifying a LP/P variant in patients with acquired LQTS was mainly dependent on three variables, i.e. age below 40years, QTc (at baseline) >440ms and presence of TdP/symptom. When all three variables were present, a LP/P variant was identified in more than 60% of the patients.<sup>94</sup> Molecular genetic screening in older individuals has a much lower yield and can therefore not be recommended on a standard basis.<sup>94</sup>

Variants which are unequivocally associated with drug-induced LQTS (e.g. D85N in *KCNE1*) should be reported as a relevant result.<sup>73</sup> Active family screening for the presence of these variants should be considered when QT prolonging drugs are or could be prescribed (expert opinion).

## 5.2 | Catecholaminergic polymorphic ventricular tachycardia

### 5.2.1 | Impact of genetic testing for the index case






Disease	Diagnostic	Prognostic	Therapeutic
CPVT	+++	+	+

### 5.2.2 | Background

Catecholaminergic polymorphic ventricular tachycardia (VT) is an uncommon inherited arrhythmia syndrome with an unknown prevalence [estimated to be in the 1:20 000 range (personal guess, AW)]. It is characterized by polymorphic (rarely documented but typically bidirectional) ventricular arrhythmias in young individuals with structurally normal hearts. Catecholaminergic polymorphic ventricular tachycardia-associated arrhythmias are mediated adrenergically (i.e. occur during exercise or emotional stress), are often asymptomatic but may also cause syncope, syncope followed by generalized seizures, sudden cardiac arrest, and SCD.<sup>17,152,153</sup> Importantly, the occurrence of exercise-induced arrhythmias may be variable, so with a strong clinical suspicion more than one exercise test is warranted. CPVT is less common than other conditions causing SCD, yet disproportionately accounts for a high percentage (10–15%) of SCD cases in the young,<sup>154–156</sup> in  $\pm 6\%$  of those labelled as idiopathic ventricular fibrillation (IVF)<sup>157</sup> and in  $\pm 1\%$  of sudden infant death syndrome,<sup>158</sup> although the latter association is hard to confirm.

### 5.2.3 | Diagnostic implications of catecholaminergic polymorphic ventricular tachycardia genetic testing

Catecholaminergic polymorphic ventricular tachycardia usually segregates as an AD trait but AR segregation is also possible (Table 7). Compared to LQTS, there is also a higher frequency of sporadic *de novo* variants, particularly with the most common CPVT-causative gene, *RYR2*.<sup>159,160</sup> This gene encodes the cardiac ryanodine receptor (RyR2), also called the calcium release channel and is responsible for release of calcium from the sarcoplasmic reticulum into the cytosol. Catecholaminergic polymorphic ventricular tachycardia 1-associated gain-of-function pathogenic variants in *RYR2* lead to a leaky RyR2

Recommendation	Consensus statement instruction	Ref.
In any patient satisfying the diagnostic criteria for CPVT (such as Class 1 clinical diagnosis <sup>a</sup> or CPVT diagnostic score >3.5 <sup>b</sup> ), molecular genetic testing is recommended for the currently established definite/strong evidence CPVT-susceptibility genes: <i>RYR2</i> , <i>CASQ2</i> , <i>CALM1-3</i> , <i>TRDN</i> , and <i>TECL</i> .		91,141–145
In phenotype-positive CPVT patients (definition: see rec. 1) who are negative for those established CPVT-susceptibility genes, genetic testing may be considered for CPVT phenocopies resulting from pathogenic variants in the <i>KCNJ2</i> , <i>SCN5A</i> , and <i>PKP2</i> genes.		17,146–148
In patients with a modest phenotype for CPVT (i.e. CPVT diagnostic score $\geq 2$ but <3.5 <sup>b</sup> ), genetic testing may be considered for the established definite/strong evidence CPVT-susceptibility genes: <i>RYR2</i> , <i>CASQ2</i> , <i>CALM1-3</i> , <i>TRDN</i> , and <i>TECL</i> .		17,91,141–145
Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causative variant.		149,150
Predictive genetic testing in related children at risk of inheriting a P/LP variant is recommended from birth onward (any age).		Expert opinion

<sup>a</sup>Adapted from HRS/EHRA/APHR Expert consensus recommendations on diagnosis of CPVT.<sup>15</sup>

<sup>b</sup>Adapted from Giudicessi *et al.*,<sup>151</sup> see Supplementary material online, Table S4.

TABLE 7 Genes implicated in catecholamine polymorphic ventricular tachycardia (CPVT)

Gene	Locus	Phenotype— syndrome	Protein (functional effect)	Frequency	ClinGen classification
RyR2	1q43	CPVT/AD	RyR2 (↑); inappropriate Ca <sup>2+</sup> release from the SR	60–70%	Definite
CASQ2	1p13.1	CPVT/AR	Inappropriate Ca <sup>2+</sup> release from the SR	±5%	Definite
CASQ2	1p13.1	CPVT/AD	Inappropriate Ca <sup>2+</sup> release from the SR	±5%	Moderate
CALM 1–3	14q32.11 2p21 19q13.32	CPVT/AD	↑ RyR2 binding affinity resulting in inappropriate Ca <sup>2+</sup> release from the SR	<1%	Strong
TECRL <sup>a</sup>	4q13.1	CPVT/AR	Altered Ca <sup>2+</sup> homeostasis, possibly linked to fatty acid/ lipid metabolism	<1%	Definite
TRDN <sup>a</sup>	6q22.31	CPVT/AR	↓ expression leading to remodelling of the cardiac dyad/ calcium release unit	<1%	Definite
KCNJ2	17q24.3	ATS/AD	Loss-of- <i>I</i> <sub>K1</sub> channel function	<1%	Definite

AD, autosomal dominant; AR, autosomal recessive.

<sup>a</sup>TECRL and TRDN may result in a CPVT-LQTS overlap phenotype consisting of modest QTc-prolongation and adrenergically triggered ventricular arrhythmia.

protein by various mechanisms. This in turn leads to increased diastolic cytosolic calcium levels with arrhythmic consequences, in particular under adrenergic circumstances. *RyR2* variants associated with a loss-of-function cellular phenotype are associated with the calcium release deficiency syndrome (CRDS), a newly described disease entity with specific electrophysiological characteristics distinguishable from CPVT.<sup>161,162</sup>

Other genes with an AD inheritance pattern are the 3 *CALM* genes, which also associate with other phenotypes, e.g. LQTS and IVF.<sup>91</sup> Those with a CPVT phenotype present at early age.<sup>91</sup> Genes with a predominant AR trait are *CASQ2*, *TRDN*, and *TECRL*.<sup>93,141–144</sup> As expected, recessive CPVT is more severe than dominant CPVT.

A phenotype closely resembling CPVT is ATS, caused by functional loss-of-function variants in the gene *KCNJ2* encoding for the Kir2.1 inwardly rectifying potassium channel (*I*<sub>K1</sub>).<sup>163</sup> Also the *SCN5A* associated phenotype Multifocal Purkinje-related Premature Contractions (MEPPC) can mimic CPVT although usually the ectopy burden is, as in ATS, also high in the resting state.<sup>146,147</sup> Finally, the *PKP2* gene, may in an earlier stage manifest as a disease without structural alterations but with adrenergically-mediated arrhythmias.<sup>148</sup> These genes might be tested in those patients with a CPVT-like phenotype, who are genotype negative for the strong CPVT genes (Supplementary material online, Table S5).

### 5.2.3.1 | Index cases

The yield of genetic testing in CPVT is highest (60%) in patients with a strong phenotype, i.e. a typical exercise test (occasionally including bi-directional VT).<sup>145,151,164</sup> In patients with a less typical clinical presentation [adrenergically induced syncope, IVF or isolated extrasystoles during the exercise test] the yield is much lower (15–20%).<sup>145,164</sup> This is not trivial because the 'background noise' in the *RYR2* gene, i.e. the presence of benign variants, is a little over 3%. This raises the likelihood of a false-positive result

in patients with a non-typical phenotype to 1 in 6 (compared to 1:20 in cases with a strong phenotype).<sup>164</sup> The latter findings have actually been used to propose a phenotype enhanced variant re-adjudication approach.<sup>151</sup> This approach significantly reduced the number of VUS by either promoting or demoting specific variants.<sup>151</sup> Specifically, akin to the 'Schwartz score' for LQTS, Wilde and Ackerman introduced the analogous CPVT diagnostic score to improve the clinical veracity of the diagnosis of CPVT.<sup>151</sup> In patients with a CPVT diagnostic score of > 3.5 (without the genetic test result), the likelihood of CPVT1 (i.e. *RYR2*-mediated CPVT) is at least 60%. Furthermore, given that genetic test companies currently designate almost every novel missense variant in *RYR2* as a VUS because of the *in silico* challenges of assessing the pathogenicity of variants in the 4967 amino acid-containing protein, incorporation of this clinical score can assist physicians with decoding the genetic test result more accurately. For example, in a patient with a robust clinical score for CPVT but a VUS test result in *RYR2*, the genetic test ordering physician (the phenotyper) can upgrade that test with result to at least a 'likely pathogenic variant' designation with 95% confidence.<sup>151</sup>

### 5.2.3.2 | Family screening

An active family screening approach is important in all CPVT families. Family-specific, cascade genetic testing for the identified CPVT-causative, pathogenic variant should be pursued regardless of symptom status and stress test expressivity. Even asymptomatic, normal stress test individuals who are genotype positive (i.e. genotype positive/phenotype negative) may require active therapy.<sup>149,150</sup>

For many cases of CPVT2 stemming from homozygous variants in *CASQ2*, consanguinity is present. An alternative explanation is compound heterozygosity which is often the case for *TRDN*-mediated CPVT. The latter is part of the phenotypic spectrum of TKOS.<sup>93</sup> Heterozygous carriers of the relevant variants in *TRDN* and *TECRL* normally have no phenotype and do not need active

treatment. This may not be true for family members heterozygous for a variant in CASQ2-encoded calsequestrin, which seems to suggest AD segregation.<sup>165</sup> In a more recent study, one-third of the heterozygous patients fulfil the diagnostic criteria for CPVT and some of them even presented with a cardiac arrest or exercise-related syncope.<sup>166</sup> These data were not considered sufficient to upscale the monoallelic gene status beyond the moderate level.<sup>17</sup> Yet, exercise-test-guided treatment is probably warranted in these patients.

### 5.2.4 | Prognostic and therapeutic implications of catecholaminergic polymorphic ventricular tachycardia genetic testing

While there is strong and obvious impact diagnostically with respect to CPVT genetic testing, the prognostic impact is less and the therapeutic impact is negligible currently. Prognostically, there are data to suggest that specific locations within the RyR2 (i.e. the C-terminal channel forming domain) may confer increased susceptibility to CPVT-triggered arrhythmias.<sup>167</sup> More importantly, patients with CALM-mediated CPVT are at increased risk,<sup>91</sup> and AR disease presents more often at earlier age and with more malignant arrhythmias. Therapeutically, in all CPVT genotypes,  $\beta$ -adrenoceptor blockade (preferably with the non-selective beta blockers nadolol or propranolol) is the cornerstone of therapy, with upscaling therapy dependent on the (persistent) presence of symptoms and/or of ventricular arrhythmias during an exercise test, available in the form of combination drug therapy with the addition of Flecainide,<sup>168</sup> and LCSD.<sup>169</sup> Implantable cardioverter-defibrillator therapy should whenever possible be avoided in CPVT patients.<sup>170</sup> Patients satisfying a clinical diagnosis of CPVT but who are negative for both the established CPVT-causative genes and the genes underlying the CPVT

phenocopies (i.e. genotype negative/phenotype positive) should also be treated similarly.<sup>149,167</sup>

## 5.3 | Brugada syndrome






### 5.3.1 | Impact of genetic testing for the index case

Disease	Diagnostic	Prognostic	Therapeutic
Brugada syndrome	+	+	+

### 5.3.2 | Background

Brugada syndrome is an inherited arrhythmogenic disorder characterized by ST-segment elevation in the right precordial leads and malignant ventricular arrhythmias, sometimes associated with conduction disease and atrial arrhythmias. The prevalence of BrS is estimated to be 1 in 2000 worldwide, with higher prevalence in Asia.<sup>173</sup> Symptomatic patients are typically males presenting in their fourth decade of life.<sup>174,175</sup> Brugada syndrome may be involved in ~18–28% of unexplained sudden deaths/arrests.<sup>176,177</sup>

According to the 2013 HRS/EHRA/APHR expert consensus statement,<sup>15</sup> BrS is diagnosed in patients with ST-segment elevation with type I morphology  $\geq 2$  mm in  $\geq 1$  lead among the right precordial leads V1, V2 positioned in the 4th intercostal space (standard ECG) or the 2nd and 3rd intercostal spaces (high parasternal leads),<sup>178</sup> observed either spontaneously or after provocative drug testing with a class I antiarrhythmic drug. In light of data highlighting the limited specificity of provocative testing,<sup>179</sup> the Shanghai scoring system was proposed whereby the diagnosis of definite BrS in presence of type I ECG that is only manifested with provocative testing also requires supporting clinical features (Supplementary material online, Table S6).<sup>180</sup> Brugada syndrome phenocopies such as myocardial

Recommendation	Consensus statement instruction	Ref.
Genetic testing with sequencing of <i>SCN5A</i> is recommended for an index case diagnosed with BrS with a type I ECG in standard or high precordial leads occurring either (i) spontaneously, or (ii) induced by sodium-channel blockade in presence of supporting clinical features or family history.		21,171
Rare variants in genes with a disputed or refuted gene-disease clinical validity should not be reported routinely <sup>a</sup> for BrS genetic testing in a diagnostic setting.		21
Targeted sequencing of variant(s) of unknown significance in <i>SCN5A</i> with a population allele frequency $< 1 \times 10^{-5}$ identified in an index case can be considered concurrently with phenotyping for family members, following genetic counselling, to assess variant pathogenicity through co-segregation analysis.		172
Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causative variant.		Expert opinion
Predictive genetic testing (of pathogenic <i>SCN5A</i> variants) in related children is recommended from birth onward (any age).		Expert opinion

<sup>a</sup>Unless in a research setting.



ischaemia, electrolyte disturbances and drug intoxications should be excluded before a diagnosis of BrS can be made.<sup>181</sup> Drug-induced and fever-induced Brugada ECG pattern is not considered a BrS phenocopy and in both conditions genetic testing with sequencing of *SCN5A* may be considered.

Risk stratification in BrS relies primarily on symptoms and the ECG. Patients with suspected arrhythmic syncope with a spontaneous type I ECG are at high risk of malignant arrhythmic events (~2.3%/year<sup>182</sup>) and should consider ICD implantation.<sup>15</sup> Asymptomatic patients with drug-induced type I ECG are at low risk ( $\leq 0.4\%$ /year<sup>183</sup>) and should be managed conservatively. All BrS patients should be counselled to (i) avoid drugs that impair cardiac sodium channels (brugadadrugs.org<sup>184</sup>), (ii) avoid alcohol intoxication, (iii) immediately treat fever with antipyretic drugs, and (iv) seek urgent medical attention following a syncope. The role of invasive electrophysiological testing for risk stratification remains controversial.

### 5.3.3 | Diagnostic implications of Brugada syndrome genetic testing

Disease-causing rare genetic variants in *SCN5A* that result in loss of function of the cardiac sodium channel are identified in ~20% of cases (Table 8). In families with pathogenic *SCN5A* variants, penetrance is incomplete and non-carriers of the *SCN5A* variant may show a positive provocative drug challenge,<sup>185</sup> in line with the complex heritability of BrS. Case-control GWAS in

BrS identified several genetic loci harbouring common variants associated with the disease.<sup>60</sup> Polygenic scores derived from GWAS (PRS<sub>BrS</sub>) could underlie variable disease expressivity in carriers of *SCN5A* pathogenic variants.<sup>67</sup> Brugada syndrome in the absence of rare *SCN5A* variants is largely polygenic. PRS<sub>BrS</sub> are strongly associated with response to provocative drug testing.<sup>66</sup> For instance, a PRS<sub>BrS</sub> comprised of three common variants (rs11708996, rs10428132, and rs9388451) below the 10th percentile provides a sensitivity of 99% and a negative predictive value of 93% for drug-induced type I ECG, based on a population of 1368 patients that underwent ajmaline testing for suspected BrS.<sup>66</sup> Assessment of PRS<sub>BrS</sub> that include more genetic variation associated with BrS is ongoing.

Other genes have been implicated in BrS (Supplementary material online, Table S7). However, the gene-disease validity of most of those genes (other than *SCN5A*) has been *disputed* following rigorous assessment of available data using the ClinGen framework.<sup>21</sup> Although a *disputed* ClinGen status does not challenge a role of the gene product in BrS pathophysiology, it strongly argues against reporting those genes in the diagnostic setting. An algorithm for genetic testing of index cases with BrS and family members is shown in Figure 4.

#### 5.3.3.1 | Index cases

The presence of a LP/P *SCN5A* variant confirms the diagnosis of BrS in probands with a type I ECG, but the absence of such variant does not exclude the diagnosis. In drug-induced type I BrS pattern in the absence of supporting clinical context and family history, it can be

TABLE 8 Gene implicated in Brugada syndrome

Gene	Locus	Phenotype—syndrome	Protein (functional effect)	Frequency	ClinGen classification
<i>SCN5A</i>	3p22.2	BrS/AD	Loss of $I_{Na1.5}$ channel function	15–30%	Definite

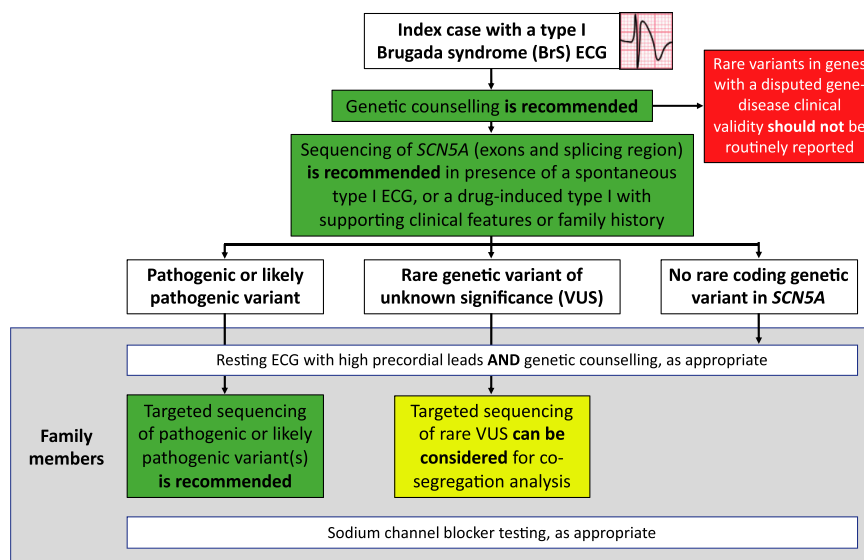


FIGURE 4 Clinical algorithm for genetic testing and family screening in Brugada syndrome

considered to perform *SCN5A* testing for the purpose of risk prediction, management and family screening. Interestingly, according to the Shanghai score, adding an *SCN5A* P/LP variant to a patient with 'isolated' drug-induced type 1 would increase his score from 2.0 to 2.5 which remains insufficient for 'probable/definite BrS'.<sup>169</sup>

### 5.3.3.2 | Family screening

Genetic testing should be offered to family members regardless of age<sup>186</sup> when a LP/P *SCN5A* variant is identified in a relative with BrS. Carriers of such variants should be instructed to take the same precautions as those with BrS (see above). Asymptomatic relatives who do not carry the *SCN5A* variant and have a completely normal resting ECG (also in the higher placed leads) can be discharged. Although phenotype positive-genotype negative family members have been described in genotype positive families,<sup>173</sup> standard provocative testing in these individuals is not supported by current data. Screening of relatives of *SCN5A* negative BrS probands should be done clinically using an ECG (also with high parasternal leads). Provocative testing can be considered based on patient's symptoms, resting ECG and personal preference, for the sake of prevention (treatment of fever, avoidance of drugs (brugadadrugs.org), and avoidance of alcohol intoxication). It should be noted and discussed with the patient prior to provocative testing that a positive provocative test in the absence of symptoms and *SCN5A* (P/LP) variant is diagnostic for BrS but is associated with a very low arrhythmic event rate, and should therefore be managed conservatively. In a large study from a single centre<sup>66</sup> which included relatives of *SCN5A* negative BrS probands,  $PRS_{BrS}$  was significantly associated with drug-induced BrS, highlighting its potential in clinical practice. Yet, further studies in other cohorts are needed before widespread use of polygenic scores in BrS.

Of note, several pathogenic *SCN5A* variants are associated with a phenotype with both right precordial ST-segment elevation as well as QTc prolongation.<sup>187</sup> Clearly, in the family screening process the QTc should also carefully be evaluated and affected individuals should also avoid drugs from the www.crediblemeds.org list.

## 5.3.4 | Prognostic and therapeutic implications of Brugada syndrome genetic testing

Brugada syndrome patients with pathogenic *SCN5A* variants exhibit more conduction abnormalities,<sup>188,189</sup> and have worse arrhythmic outcomes.<sup>171,189,190</sup> The presence of *SCN5A* pathogenic variants does not, by itself, justify prophylactic ICD implantation, but should trigger an aggressive management in presence of clinical risk markers such as (arrhythmic) syncope. Because of the risk of conduction disturbance, the presence and type of *SCN5A* pathogenic variants should also be considered when selecting an implantable device, in addition to the baseline ECG and arrhythmia documentation.





## 5.4 | (Progressive) cardiac conduction disease

### 5.4.1 | Impact of genetic testing for the index case

Disease	Diagnostic	Prognostic	Therapeutic
Cardiac conduction disease	+	+	+

### 5.4.2 | Background

Cardiac conduction disease (CCD) is a heterogeneous and often age-dependent, progressive cardiac conduction disease (PCCD) disorder characterized by a disturbed electrical impulse propagation in the atrioventricular (AV) node and His-Purkinje system. On the surface ECG, prolonged P-wave duration, AV block, and different degrees of bundle branch block (manifested as QRS fragmentation or QRS widening with normal or abnormal axis deviation) are typical features. Syncope or even cardiac arrest can occur from severe sinus

Recommendation	Consensus statement instruction	Ref.
Targeted genetic testing is recommended as part of the diagnostic evaluation for index patients with isolated cardiac conduction disease (CCD/PCCD) or with concomitant structural heart disease or extracardiac disease, when there is early age of diagnosis or a suspicion of laminopathy, especially when there is documentation of a positive family history of CCD/PCCD.		Expert opinion
Targeted genetic testing may be considered as part of the diagnostic evaluation for index patients with isolated cardiac conduction disease (CCD/PCCD) or with concomitant structural heart disease or extracardiac disease, especially in the setting of a positive family history.		Expert opinion
Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causative variant.		Expert opinion
Predictive genetic testing in related children may be considered from birth onward (any age) in specific settings.		Expert opinion

node disease (manifested with sinus bradycardia or significant sinus pauses) of from complete AV block.<sup>191,192</sup>

Fibrotic degeneration, ischaemia, infiltrative processes, valve calcifications, tumours, or thyroid dysfunction may lead to acquired dysfunction and CCD. However, in idiopathic or familial forms heritable factors significantly contribute to CCD/PCCD (Lenègre's disease). Isolated forms ('primary electrical heart diseases') can be distinguished from CCD/PCCD in the setting of cardiomyopathies (typically DCM) or of syndromic disorders, e.g. with CHD or neurological phenotypes (Table 9). Clinical disease expression may vary between pathogenic variant carriers within the family, but also between different families and often has an age-dependent course.

### 5.4.3 | Diagnostic implications of genetic testing in cardiac conduction disease/progressive cardiac conduction disease

Cardiac conduction disease/PCCD is genetically heterogeneous;<sup>193</sup> in the majority of CCD families an AD mode of inheritance is pertinent, whereas CCD/PCCD in the setting of some neuromuscular disorders is X-chromosomal linked and severely affects male patients. A *de novo* or recessive occurrence is rare.<sup>194,195</sup> Most pathogenic variants are non-synonymous or truncating pathogenic variants; so far, the frequency of small indels and CNV has not been addressed systematically.

Susceptible genes for each CCD subgroup are listed below (Table 9, Supplementary material online, Table S8). The overall and gene-specific mutation yield (sensitivity) is unknown and also for each gene; however, recent studies using targeted or WES suggested a pathogenic variant detection rate of >50% in index cases, with *SCN5A* and *LMNA* as core genes,<sup>196,197</sup> accounting for ~20% each (*TRPM4*: 5–10%). This also implies that in a measurable fraction of cases, including family clusterings of diseases, investigations of associated known heart disease genes are still insufficient to reveal the underlying substrate, suggesting that new causal genes have yet to be discovered.

#### 5.4.3.1 | Index case

Upon the ECG diagnosis of CCD/PCCD and without evidence for acquired causes, an inherited form appears likely. However, cardiac sarcoidosis is a relatively common diagnosis in isolated AV block and should be systematically excluded before a genetic diagnosis is considered in sporadic isolated AV block. Screening for cardiac sarcoidosis (using CMR or positron emission tomography-fluorodeoxyglucose) in patients younger than 60 years with unexplained second-degree (Mobitz II) or third-degree AV block can be useful.<sup>198</sup> Further routine work-up includes exercise ECG, Holter ECG and echocardiography to address presence of a cardiomyopathy or CHD. Cardiac magnetic resonance imaging (MRI) (with

gadolinium enhancement) may be considered, in particular for *LMNA* pathogenic variant carriers.<sup>193,199,200</sup> Early-onset or idiopathic forms of CCD/PCCD should prompt consideration of genetic testing, especially if the family history is indicative (CCD/PCCD, pacemaker implants, cardiomyopathy, etc.).

For the major genes associated with CCD, specialized cardiogenetic services have established targeted gene panels for CCD/PCCD testing. Four genes (*SCN5A*, *LMNA*, *GLA*, and *PRKAG2*; Table 9) are therefore recommended to be investigated.<sup>50</sup> The identification of a pathogenic variant in a disease-validated gene confirms not only the suspected diagnosis of CCD/PCCD, but also allows its classification as a genetic (and potentially heritable) disorder with or without additional clinical features.

#### 5.4.3.2 | Family investigation

A careful clinical and, if suitable [i.e. with knowledge of the pathogenic (ACMG class 4/5 i.e. LP/P) variant in a validated CCD/PCCD gene], genetic investigation is recommended and therefore indicated in family members as a part of a directed 'family cascade screening'. This includes a comprehensive assessment of the family pedigree. In relatives testing negative for this pathogenic variant, monitoring for CCD/PCCD or its development and downstream investigations in the family branch are not further needed. In contrast, pathogenic variant-positive family members should be evaluated carefully for the presence of isolated or syndromic forms of CCD/PCCD with regard to typical phenotypic features of the underlying gene (Table 9). In addition, genotype-dependant recommendations will be similar to those for the index case. Asymptomatic children in the first decade of life do not strictly need to be investigated for their genetic status, although in specific settings an earlier evaluation may be pertinent.

### 5.4.4 | Prognostic and therapeutic implications of genetic testing

Genotype may not clearly stratify risk of CCD progression, but different underlying inherited aetiologies for CCD do give prognostic information, e.g. *LMNA* for SCD risk. In addition, pathogenic variants in distinct genes (e.g. *LMNA*, *TNNI3K*) may be associated with development of heart failure, whereas other genes may exhibit extracardiac features, such as myopathy, which require additional, specialized treatment. Patients with *LMNA* pathogenic variants may develop atrial and ventricular arrhythmias as well as progressive (end-stage) heart failure and the potential need for ICD or cardiac resynchronization therapy defibrillator therapy (upon the development of phenotypic expression) or heart transplantation.<sup>200–202</sup> A risk stratification scheme has recently been proposed.<sup>200</sup> Patients with *SCN5A* pathogenic variants may also develop BrS, so avoidance of particular drugs and fever is recommended to reduce ventricular arrhythmias.

TABLE 9 Genes implicated in CCD/PCCD

Gene	Locus	Phenotype—syndrome	Protein (functional effect)	Frequency	ClinGen classification
<b>Genes for isolated SND</b>					
SCN5A	3p22.2	BrS1, SND, ASS, (LQT3) <sup>194–196,203,204</sup>	<ul style="list-style-type: none"> <li>○ Cardiac Na channel <math>\alpha</math> subunit (Nav1.5)</li> <li>○ Loss-of-function, <math>I_{Na} \downarrow</math></li> </ul>	>10%	NA/major gene; definite for LQTS, BrS1
TRPM4	19q13.33	<sup>205,206</sup>	<ul style="list-style-type: none"> <li>○ Transient receptor potential melastatin 4 channel</li> <li>○ Gain-of-function</li> </ul>	1–10%	NA/major gene
<b>Genes for syndromal disorders with CCD/PCCD</b>					
LMNA	1q22	DCM (CMD1A), AFib, SND (Emery-Dreifuss muscular dystrophy 2/3, congenital muscular dystrophy, limb-girdle myopathy, familial lipodystrophy type 2, Hutchinson-Gilford progeria, and various other disorders) <sup>197,199,200</sup>	Lamin A/C	>10%	NA/major gene; definite for DCM
DES	2q35	DCM (CMD1I), ACM, Myofibrillar myopathy (MFM1)	Desmin	()	<ul style="list-style-type: none"> <li>○ NA/rare gene;</li> <li>○ Definite for DCM, moderate for ACM</li> </ul>
DMD	Xp21.2-p21.1	DCM (CMD3B), muscular dystrophy (Becker or Duchenne type) <sup>207</sup>	Dystrophin	()	NA/rare gene
DMPK	19q13.32	DCM, myotonic dystrophy (DM1) <sup>208</sup>	Myotonic dystrophy protein kinase	()	NA/rare gene
EMD	Xq28	DCM, LVNC, SND, Emery-Dreifuss muscular dystrophy (EMD) <sup>209,210</sup>	Emerin	()	NA/rare gene
LAMP2	Xq24	HCM, DCM, LVNCDanon disease (glycogen storage disease), skeletal muscle involvement, mental retardation <sup>211</sup>	Lysosomal-associated membrane protein 2	()	<ul style="list-style-type: none"> <li>○ NA/rare gene;</li> <li>○ Definite for HCM</li> </ul>
ZNF9	3q21.3	DCM, myotonic dystrophy (DM2) <sup>212</sup>	Zink finger protein 9 (CZNP)	()	NA/rare gene
GLA	Xq22.1	Fabry disease (HCM, RCM, acral paresthesia, PNP, kidney insufficiency, angio-keratoma, anhydrosis, cornea verticillata, etc.)	Galactosidase $\alpha$	()	NA/rare gene; definite for HCM
PRKAG2	7q36.1	Cardiac preexcitation (WPW), LVH/HCM, <sup>213</sup>	AMP-activated protein kinase $\gamma$ 2-subunit	()	NA/rare gene; definite for HCM
TNNI3K	1p31.1	DCM, AFIB <sup>214</sup>	Troponin I-interacting MAP kinase	()	NA/rare gene
NKX2-5	5q35.1	ASD7, (VSD7, TOF)	Transcription factor Nkx2.5	()	NA/rare gene
GJC1	17q21.31	Bone malformations (brachyfacial pattern, finger deformity, and dental dysplasia) <sup>215</sup>	Connexin 45	()	NA/rare gene
TBX5	12q24.21	Holt-Oram syndrome (HOS) (hand-heart syndrome): ASD, hand and limb malformation (e.g., triphalangeal thumb), other CHD	Transcription factor TBX5	()	NA/rare gene
MYL4	17q21.32	AFib/conduction disease	atrial-specific myosin light chain	()	NA, rare gene
mtDNA	Mitochondrial DNA	Kearns-Sayre syndrome (KSS): Ptosis, progressive external ophthalmoplegia, ataxia, retinitis pigmentosa; Chronic progressive external ophthalmoplegia (CPEO), ptosis <sup>216</sup>	(37 mitochondrial genes)	()	NA/rare gene

Frequency: refers to mutation detection rate;<sup>25</sup> core genes: major (>10%) or minor (1–10%); rare gene (<1%); (): mutation rate unknown and/or single reports. Other phenotypes: [...], phenotype associated with gene, but unlinked with CCD/PCCD.

ClinGen: Clinical Genome Resource of NCBI; <https://clinicalgenome.org>, NA: not available = not yet curated.

ACM, arrhythmogenic cardiomyopathy; AFib, atrial fibrillation; ASD, atrial septal defect; ASS, atrial stand still; BrS, Brugada syndrome, CHD, congenital heart disease, DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQT, long-QT syndrome subtype; LVH, left ventricular hypertrophy; LVNC, left ventricular non-compaction cardiomyopathy; PNP, polyneuropathy; WPW, Wolff-Parkinson-White syndrome; RCM, restrictive cardiomyopathy; SND, sinus node dysfunction; TOF, Tetralogy of Fallot; VSD, ventricular septal defect; X-chr., X-chromosomal.

## 5.5 | Short QT syndrome

### 5.5.1 | Impact of genetic testing for the index case

Disease	Diagnostic	Prognostic	Therapeutic
SQTS	+	+	+

### 5.5.2 | Background

Short QT syndrome is a very rare channelopathy, characterized by a short QT interval on the basal ECG and by an increased risk of both atrial and ventricular arrhythmias.<sup>15,96</sup> The QT evaluation should be performed not only in basal condition but also during ECG Holter recording and exercise stress test, as typical of this disease is the reduced rate-adaptation of QT during exercise<sup>220</sup> and the evidence of a short QTc at different heart rates and not only during bradycardia.<sup>96</sup> The cut-off value of 'short' QT interval for defining SQTS remains a matter of debate as there is an overlap between healthy subjects and patients with SQTS. Short QT syndrome is usually diagnosed in the presence of a QTc consistently below 330–340ms; while between 340 and 360ms additional criteria are needed and specifically, the presence of a pathogenic variant, family history of SQTS, family history of SCD below age 40 or survival after an episode of VT/VF in the absence of heart disease.<sup>15,96</sup> No specific triggers for life-threatening arrhythmias have been recognized and age at presentation is quite variable.

### 5.5.3 | Diagnostic implications of short QT syndrome genetic testing





Short QT syndrome is a genetically heterogeneous AD disease. Four SQTS-susceptibility potassium channel genes, *KCNH2*,<sup>221</sup>

*KCNQ1*,<sup>222</sup> *KCNJ2*,<sup>217</sup> and *SLC4A3*<sup>223</sup> have been identified (Table 10). Only the first two genes have a definite or strong disease association.<sup>17</sup> Pathogenic variants in the first three genes yield a gain-of-function to their encoded potassium channel. A missense mutation in *SLC4A3*<sup>223</sup> encoding the anion exchange protein 3 (AE3) has been identified in two large families with SQTS by WES. Although the functional change of the mutation supports a contribution to the accelerated repolarization, further study will be necessary. Loss of function type mutations in L-type calcium channel related genes, *CACNA1C*, *CACNA2b*,<sup>224</sup> and *CACNA2D1*<sup>218</sup> have been linked to SQTS<sup>17</sup> (Supplementary material online, Table S10), frequently showing overlapping BrS features.<sup>224</sup> However, the evidence of these genes is limited at most.

#### 5.5.3.1 | Index cases

Short QT syndrome is diagnosed clinically in index patients<sup>13,15,219</sup> and the presence of a disease-causing variant is a key finding to support the diagnosis above all in cases in which the QTc is short, but not below 330–340ms.<sup>13,15,219</sup> Genetic screening for two potassium channel genes (*KCNQ1* and *KCNH2*) is recommended and for two other genes (*KCNJ2* and *SLC4A3*) may be considered for index cases<sup>17</sup> (Figure 5). Compared to loss-of function mutations identified in LQTS, the reported number of mutations in SQTS is very small.<sup>225</sup> All other genes should be screened in patients with a high probability of the disease and only in experienced centres as variant interpretation may be critical. If a SQTS patient shows an overlapping phenotype with BrS, mutations in L-type calcium channel related genes may be involved.

A short QTc is also found in patients with the AR primary systemic carnitine deficiency syndrome, which is characterized by hypoketotic hypoglycaemia, hyperammonaemia, liver dysfunction, hypotonia, and cardiomyopathy and caused by variants in *SLC22A5*.<sup>226</sup> Indeed, homozygote or compound heterozygote variants have been identified in unexplained SCD or resuscitated cardiac arrest cases without overt extra-cardiac manifestations.<sup>227,228</sup> The

Recommendation	Consensus statement instruction	Ref.
In any patient satisfying the diagnostic criteria for SQTS (such as Class 1 clinical diagnosis <sup>a</sup> or SQTS diagnostic score >4 <sup>b</sup> ), molecular genetic testing is recommended for the definitive disease associated genes (currently <i>KCNH2</i> , <i>KCNQ1</i> ).		17
Testing of <i>KCNJ2</i> and <i>SLC4A3</i> may be performed in all index patients in whom a cardiologist has established with a high probability a diagnosis of SQTS, based on examination of the patient's clinical history, family history, and ECG characteristics obtained at baseline or during ECG Holter recording and exercise stress test (SQTS diagnostic score ≥4).		17,217,218
Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causative variant.		Expert opinion
Predictive genetic testing in related children may be considered in specific settings.		Expert opinion

<sup>a</sup>Adapted from HRS/EHRA/APHR Expert consensus recommendations on diagnosis of SQTS.<sup>15</sup>

<sup>b</sup>Adapted from Gollob *et al.*,<sup>219</sup> see Supplementary material online, Table S9.

TABLE 10 Genes implicated in short QT syndrome (SQTS)

Gene	Locus	Phenotype— syndrome	Protein (functional effect)	Frequency	ClinGen classification
KCNH2	7q35-36	SQTS/AD	Increase in $I_{K_r}$ channel function	<10%	Definite
KCNQ1	11p15.5	SQTS/AD	Increase in $I_{K_s}$ channel function	5%	Strong
KCNJ2	17q23	SQTS/AD	Increase in $I_{K1}$ channel function	$\pm$ 1%	Moderate
SLC4A3	2q35	SQTS/AD	pH ( $\uparrow$ ) and Cl <sup>-</sup> ( $\downarrow$ )	<1% <sup>a</sup>	Strong-moderate <sup>b</sup>

Functional effect: ( $\downarrow$ ) loss-of-function or ( $\uparrow$ ) gain-of-function at the cellular *in vitro* level.

<sup>a</sup>Might be significantly higher (personal communication AAMW and MG).

<sup>b</sup>Classification discussed between members of the Clin Gen curation panel. Maybe become strong based on new data (personal communication AAMW and MG).

BrS, Brugada syndrome; SQTS, short QT syndrome.

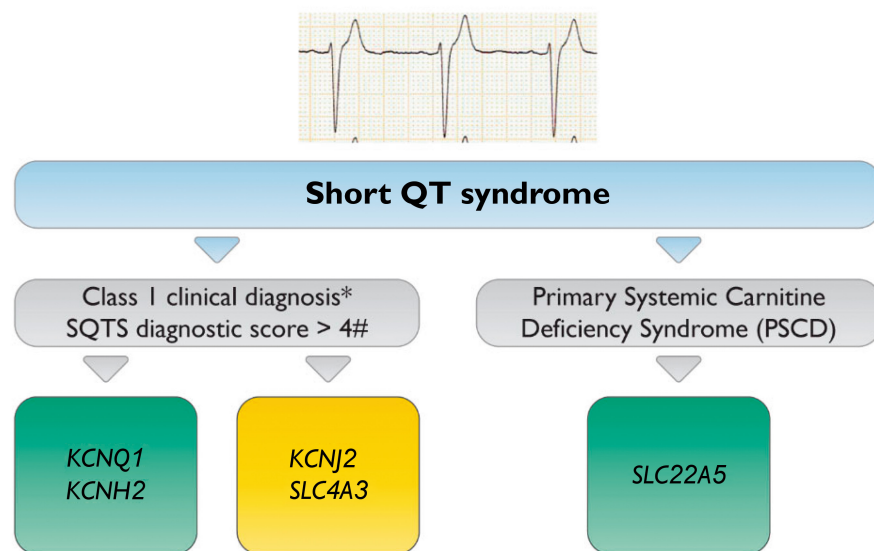


FIGURE 5 Clinical algorithm for genetic testing and family screening in short-QT syndrome. <sup>a</sup>Adapted from HRS/EHRA/APHRS Expert consensus recommendations on diagnosis of SQTS.<sup>15</sup> <sup>b</sup>Adapted from Gollob *et al.*,<sup>219</sup> see Supplementary material online, Table S9

QT interval in these patients is responsive to carnitine supplementation treatment.<sup>227,228</sup>

#### 5.5.3.2 | Family screening

Cascade screening in family members is indicated whenever a definite disease-causing variant is identified in the index case. However, results should be managed carefully.

#### 5.5.4 | Prognostic and therapeutic implications of short QT syndrome genetic testing

Implantation of an ICD with/without hydroquinidine is recommended for high-risk patients independent of genetic status. In the long-term follow-up of SQTS patients, hydroquinidine prevented events, and the QT prolongation effect was more relevant in KCNH2-based patients.<sup>229</sup> In asymptomatic patients and family members with pathogenic variants, hydroquinidine prolonged QT intervals, though its efficacy for preventing life-threatening arrhythmias still needs to be proved.<sup>230,231</sup> There are some phenotypic differences among different genotypes. The onset of arrhythmias in KCNH2-based patients seems to occur later in life than in other

subtypes.<sup>232</sup> while the occurrence of AFib is more frequent in this subtype.<sup>233</sup> However, life-threatening arrhythmias are equally frequent among different genotypes.<sup>232</sup>

## 5.6 | Atrial fibrillation




### 5.6.1 | Impact of genetic testing for the index case

Disease	Diagnostic	Prognostic	Therapeutic
Atrial fibrillation	-	+	-

### 5.6.2 | Background

Atrial fibrillation is the most common cardiac arrhythmia worldwide, and it may be associated with an unfavourable prognosis, depending on the clinical profile and access to treatment. Atrial fibrillation is characterized by uncoordinated electrical activity in the atria. This causes a rapid and irregular heartbeat and increases the risk of stroke and sudden death. Its prevalence is around 0.4% in the general population and increases to approximately 6% in



Recommendation	Consensus statement instruction	Ref.
An analysis of <i>SCN5A</i> , <i>KCNQ1</i> , <i>MYL4</i> and truncating <i>TTN</i> variants may be performed in all index patients in whom the diagnosis of familial (young = age < 60) AF, is established, based on examination of the patient's clinical history, family history, and ECG characteristics.		234–243 Expert opinion
Variant-specific genetic testing may be recommended for family members and appropriate relatives following the identification of the disease-causative variant.		Expert opinion
Predictive genetic testing in related children may be considered in specific settings.		Expert opinion

those over 65 years of age. The incidence of the familial form of AFib is unknown. The incidence of AFib increases together with the numbers of affected individuals with early onset AFib in the family.<sup>244</sup> Today, familial AFib is more commonly diagnosed. In a cohort study of 914 patients with AF, 36% had lone AFib. A positive family history for AFib was present in 15% of those lone AFib patients (5% of all AFib patients).<sup>245</sup> Atrial fibrillation is also commonly related to dilated or hypertrophic cardiomyopathies,<sup>246</sup> LQTS,<sup>247</sup> or SQTS,<sup>230,248</sup> BrS,<sup>234</sup> CPVT,<sup>249,250</sup> familial amyloidosis,<sup>251</sup> congenital cardiac abnormalities,<sup>252</sup> and pre-excitation syndromes.<sup>213,253</sup>

The prognosis for AFib patients is determined by assessing associated cardiovascular disease and identifying patients with genetic predisposition to AFib may have important clinical implications. Furthermore, testing to identify genes that play a role in the initiation of AFib may provide new understanding and new therapeutic

options. Also, early recognition of AFib patients at risk may reduce morbidity and mortality.<sup>254</sup>

### 5.6.3 | Genetic forms of atrial fibrillation

There has not yet been a consensus curation for isolated familial AFib (despite the fact that AFib is a well-established feature of many inherited cardiac syndromes, and the existence of some monogenic forms of isolated AFib). *Table 11* summarizes the existing evidence for genes implicated in AF. Evidence supporting AFib as a single-gene disease has emerged over the last decade. Genetic forms of AFib may be observed in association with other phenotypes (Brugada, conduction disease, cardiomyopathy), or may be isolated, probably particularly in young individuals.<sup>242,243</sup> Genes involved include those encoding both ion channels and sarcomere-related proteins.

TABLE 11 Genes implicated in atrial fibrillation

Gene	Locus	Phenotype—syndrome	Protein (functional effect)	Frequency	ClinGen classification
<i>SCN5A</i>	3p22.2	AFib/conduct.	Decrease in $I_{Na1.5}$ channel function	()	NA, major gene
<i>KCNQ1</i>	11p15.5	AFib/SQTS	Increase in $I_{Ks}$ channel function	()	NA, rare gene
<i>KCNH2</i>	7q35-36	AFib/SQTS	Increase in $I_{Kr}$ channel function	()	NA, rare gene
<i>TBX5</i>	12q24.21	AFib/Holt Oram syndr.	T-Box transcription factor 5	()	NA, rare gene
<i>GJA5</i>	1q21.1	AFib/atrial standstill	Decrease in Connexin 40 function	()	NA, rare gene
<i>MYL4</i>	17q21.32	AFib/conduction disease	atrial-specific myosin light chain	()	NA, rare gene
<i>TTN</i>	2q31.2	AFib/DCM	Titin	()	NA, rare gene
<i>KCN5A</i>	12p13.32	AFib	Decrease in Ultrarapid component of the atrial-specific delayed rectifier potassium current ( $I_{kur}$ )	()	NA, rare gene
<i>GJC1</i>	17q21.31	AFib	decrease in Connexin 45 function	()	NA, rare gene
<i>NPPA</i>	1p36.22	AFib	Atrial natriuretic protein (ANP), loss of interaction with the ANP receptor	()	NA, rare gene
<i>LMNA</i>	1q22	AFib/conduction disease DCM (CMD1A), (Emery-Dreifuss muscular dystrophy 2/3, congenital muscular dystrophy, limb-girdle myopathy, familial lipodystrophy type 2, Hutchinson-Gilford progeria, and various other disorders) <sup>197,199,200</sup>	Lamin A/C	()	NA/rare gene 'Definitive' for DCM

() : mutation rate unknown and/or single reports.

From a purely electrical or ion channel perspective, loss-of-function genetic variants in the *SCN5A* gene may provoke an AFib phenotype, commonly in patients who also manifest BrS and/or conduction system disease.<sup>234–236</sup> Additionally, gain-of-function mutations in *SCN5A* may cause AFib in isolation.<sup>237</sup> In a large Chinese family with AFib segregating as an AD trait, a gain-of-function variant in *KCNQ1* (S140G) was identified.<sup>238</sup> Similarly, a loss-of-function variant in the *KCN5A* gene encoding the ultrarapid component of the atrial-specific delayed rectifier potassium current (Ikur) has been described in a large pedigree with familial AFib.<sup>255</sup> Two additional potassium channels, *KCNJ2* and *KCNH2*, have been reported to cause AFib in patients with associated SQTS.<sup>256,257</sup> Lastly, genetic defects effecting gap junction function (*GJA5*, *GJC1*) may also provoke AFib.<sup>258</sup> The association of AFib with variants in other genes like *KCNE2*, *RYR2*, and *SCN1B* are not yet strong enough to warrant routine genetic screening outside a research setting.

Genes encoding sarcomeric proteins may also provoke AFib in the absence of ventricular involvement. The *MYL4* gene, encoding the atrial-specific myosin light chain, has been described as a cause of early-onset AFib and conduction system disease.<sup>239</sup> Similarly, mutations in *LMNA* and *TTN* (in particular A-band localizing variants) commonly provoke atrial arrhythmias.<sup>240,241,243</sup>

Finally, a more rare and unique form of familial AFib has been reported secondary to a genetic defect in the *NPPA* gene, which encodes the atrial natriuretic peptide, implicating neurohormonal dysregulation in provoking AFib.<sup>259</sup>

## 5.7 | Sinus node disease

### 5.7.1 | Impact of genetic testing for the index case

Disease	Diagnostic	Prognostic	Therapeutic
Sinus node disease	-	+	-

### 5.7.2 | Background

Sinus node dysfunction (for diagnostic criteria, see ref.<sup>260</sup>) is an aetiologically and thereby clinically heterogeneous, often age-dependent disorder. Sinus node dysfunction is commonly acquired;

inherited ('idiopathic' or familial) forms are less common, in particular in elder patients where ischaemia or age-related degeneration of the sinoatrial (SA) node occur. Infiltrative disorders (e.g. sarcoidosis, amyloidosis, hemochromatosis, collagen vascular disease or metastatic cancer), cardiac procedures, infections (e.g. bacterial endocarditis and Chagas disease), and obstructive sleep apnoea commonly result in SND. External causes are abnormally increased vagal tone, autonomic dysfunction, hypothyroidism, hyperkalaemia, hypokalaemia, hypocalcaemia, hypoxia and hypothermia, cardiac surgery, as well as increased intracranial pressure or medications.



Isolated (i.e. otherwise unexplained) or familial forms ('primary electrical heart diseases') can be distinguished from syndromal forms (heritability of SND and heart rate is meanwhile noted from several large studies).<sup>261–264</sup> In the surface ECG, sinus bradycardia (<50 b.p.m.) is a typical feature; significant bradycardia or pauses may result in dizziness, syncope or rarely cardiac arrest.<sup>192,265</sup> Other ECG signs are chronotropic incompetence, sinus pause (>3 s) or sinus arrest, various degrees of SA exit block, atrial fibrillation, and AV node blockade.

### 5.7.3 | Diagnostic implications of genetic testing in sinus node dysfunction

Sinus node dysfunction is genetically heterogeneous. There has not yet been a consensus curation for sinus node disease. The overall variant detection rate (sensitivity) for 'idiopathic' or familial forms is unknown, but currently estimated <25%. The majority of SND patients have an AD mode of inheritance; *de novo* occurrence and other modes (X-chromosomal, recessive occurrence, digenic traits, or CNVs) are rare. Susceptible genes for each SND subgroup are listed below (Table 12 and Supplementary material online, Table S11). Core genes for SND include *SCN5A*, *HCN4*, and *LMNA*.

#### 5.7.2.1 | Index case with sinus node dysfunction

Upon the ECG diagnosis of SND and without evidence for acquired causes, an inherited form appears likely, particularly when it is found in younger individuals (<age 60). Routine work-up includes exercise ECG, Holter ECG, and echocardiography to address presence of a cardiomyopathy or CHD. Cardiac MRI (with gadolinium application) may be considered, in particular for *LMNA* pathogenic variant carriers.<sup>193,199,200</sup>

Recommendations	Consensus statement instruction	Ref.
(Targeted) Genetic testing may be considered as part of the diagnostic evaluation for index patients with familial or isolated, but otherwise unexplained sinus node dysfunction (SND) or with SND and concomitant atrial fibrillation, cardiac conduction disease (CCD), structural heart disease or with SND and extracardiac disease (syndromal forms), especially in the setting of a positive family history.		Expert opinion
Interrogation for a putative family history and family cascade screening including clinical screening and variant-specific genetic testing, are recommended for appropriate relatives.		Expert opinion

For the major genes associated with SND (Table 12), specialized cardiogenetic services have established targeted gene panels for SND and/or CCD/PCCD testing. Two genes (*SCN5A* and *LMNA*) are part of the medically actionable gene list (currently 73 genes) of the ACMG and are therefore recommended to be investigated.<sup>49</sup> The identification of a pathogenic variant in a disease-validated gene confirms not only the (suspected) diagnosis of SND, but also allows its classification as a genetic (and potentially heritable) disorder with or without additional clinical features.

#### 5.7.2.2 | Family investigation

A careful clinical and, if suitable [i.e. with knowledge of the pathogenic (ACMG class 4/5) variant in a validated SND], genetic investigation (testing for the relevant variant) is recommended and therefore indicated in family members as a part of a directed 'family cascade screening'. This includes a comprehensive assessment of the family pedigree.

In relatives without this pathogenic variant, monitoring for SND or its development and downstream investigations in the family branch

TABLE 12 Genes implicated in sinus node disease (SND)

Gene	Locus	Phenotype/syndrome	Protein (functional effect)	Frequency	ClinGen classification
<b>Genes for isolated SND</b>					
<i>SCN5A</i>	3p22.2	BrS1, SND, ASS, LQT3 <sup>195,266,267</sup>	Cardiac Na <sup>+</sup> channel $\alpha$ subunit (Nav1.5) (loss-of-function, $I_{NaI}$ )	1–10%	NA/major gene 'Definitive' for LQTS, BrS
<i>HCN4</i>	15q24.1	Familial SND, ST, left ventricular non-compaction. <sup>268,269</sup>	Hyperpolarization-activated cyclic nucleotide-gated K <sup>+</sup> channel 4 (loss-of-function, $I_f \downarrow$ )	1–10%	NA/major gene
<i>GNB2</i>	7q22.1	Familial SND <sup>270</sup>	G-protein $\beta$ subunit 2 (gain-of-function, $I_{K^+ ACh} \uparrow$ )	<1%	NA/rare gene
<i>KCNQ1</i>	11p15.4	SQTS, [LQT1], AFib, SND <sup>271,272</sup>	K <sup>+</sup> voltage-gated channel (subfamily Q, 1) (Kv7.1) (Gain-of-function, $I_{Ks} \uparrow$ )	<1%	NA/rare gene. 'Definitive' for LQTS
<i>KCNJ5</i>	11q24.3	Familial SND <sup>273,274</sup>	G-protein gated inwardly rectifying K <sup>+</sup> (GIRK) channel 5 (Kv3.4) (Gain-of-function, $I_{K, ACh} \uparrow$ )	<1%	NA/rare gene
<i>RYR2</i>	1q43	CPVT, SND <sup>249,275</sup>	Ryanodine receptor 2 (gain-of-function)	<1%	NA/rare gene 'Definitive' for CPVT
<b>Genes for syndromal disorders with SND</b>					
<i>LMNA</i>	1q22	DCM (CMD1A), Afib (Emery-Dreifuss muscular dystrophy 2/3, congenital muscular dystrophy, limb-girdle myopathy, familial lipodystrophy type 2, Hutchinson-Gilford progeria, and various other disorders) <sup>197,199,200</sup>	Lamin A/C	1–10%	NA/rare gene 'Definitive' for DCM
<i>CACNA1D</i>	3p21.1	+ Inner ear deafness <sup>276,277</sup> (neurodevelopmental disorders, autism spectrum disorder with epilepsy; primary aldosteronism)	L-type calcium voltage-gated channel subunit alpha 1-D (Cav1.3)	()	NA/rare gene
<i>GNB5</i>	15q21.2	+ Developmental delay, speech defects, severe hypotonia, pathological gastro-oesophageal reflux, retinal disease <sup>278</sup>	G-protein $\beta$ subunit 5, (inhibitory G-protein signaling)	()	NA/rare gene
<i>SGOL1</i>	3p24.3	CAID syndrome; cohesinopathy with chronic atrial and intestinal dysrhythmia <sup>279</sup>	Nuclear protein for chromosome segregation	()	NA/rare gene
<i>EMD</i>	Xq28	DCM, LVNC, AFib, Emery-Dreifuss muscular dystrophy (EMD) <sup>209,280</sup>	Emerin	()	NA/rare gene

Frequency: refers to mutation detection rate<sup>29</sup>; core genes: major (>10%) or minor (1–10%); rare gene (<1%); (): mutation rate unknown and/or single reports. Other Phenotypes: [...], phenotype associated with gene, but unlinked with SND.

ClinGen: Clinical Genome Resource of NCBI; <https://clinicalgenome.org>.

ASS, atrial stand still; AFib, atrial fibrillation; ASD, atrial septal defect; BrS, Brugada syndrome, CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; LQT, long-QT syndrome type; LVNC, left ventricular non-compaction cardiomyopathy; SND, sinus node dysfunction; ST, sinus tachycardia; X-chr., X-chromosomal.

are not further needed. In contrast, pathogenic variant-positive family members shall be carefully evaluated for presence of isolated or syndromal forms of SND with regard to typical phenotypic features of the underlying gene (Table 12). In addition, genotype-depending recommendations will be similar as for the index case. Asymptomatic children in the first decade of life are not strictly needed to be investigated for their genetic status (in the presence of normal findings during routine cardiological investigation).

### 5.7.4 | Prognostic and therapeutic implications of genetic testing

There is no genotype-based risk stratification for patients with SND. However, mutations in distinct genes (e.g. *LMNA*, *SCN5A*, *KCNQ1*) may be associated with other overlapping phenotypes (e.g. BrS, SQTS) or with the development of heart failure and arrhythmias (i.e. *LMNA*), whereas other genes may exhibit particular extracardiac features. This has impact for the mode of monitoring during follow-up (which should include regular imaging studies in families with f.e. *LMNA* and *SCN5A* variants).

Patients with *LMNA* variants may develop atrial and ventricular arrhythmias as well as progressive (end-stage) heart failure and the potential need for ICD therapy or heart transplantation. A risk stratification scheme has recently been proposed.<sup>200</sup> Patients with *SCN5A* pathogenic variants may also develop BrS; avoidance of particular drugs and fever are recommended to reduce ventricular arrhythmias.

## 5.8 | Early repolarization syndrome

### 5.8.1 | Impact of genetic testing for the index case

Disease	Diagnostic	Prognostic	Therapeutic
Early repolarization syndrome	-	-	-

### 5.8.2 | Background



The presence of a J wave, a positive deflection immediately following the QRS complex, in f.e. the inferolateral ECG leads is known as

early repolarization pattern (ERP). Early repolarization pattern is a common ECG finding (estimated incidence 1–13%), usually considered innocent amongst healthy asymptomatic young individuals and athletes.<sup>281</sup> Case-control and epidemiological studies have, however, described an association between J waves and unexplained cardiac arrest (UCA).<sup>282–284</sup>

Haïssaguerre *et al.* found that ERP was present in 31% of 206 case subjects with IVF cases and 5% of 412 matched subjects without heart disease.<sup>284</sup> The link between ERP and malignant arrhythmias is also supported by the accentuation of the J wave before the onset of VF, an association with VF storms and the observation of triggering PVCs coincident with the J wave.<sup>284–286</sup> The term early repolarization syndrome (ERS) has since been used to identify UCA survivors with an ECG with a suggestive/suspicious ERP.<sup>15</sup>

According to animal models and an early ECG imaging study, an imbalance in myocyte currents in favour of enhanced outward currents ( $I_{to}$  and  $I_{KATP}$ ) during phase 2 of the action potential causes premature myocardial repolarization and variable loss of the action potential dome, which is most marked in the epicardial myocardium. In turn, epicardial heterogeneity in repolarization duration and transmural heterogeneity is most marked in the inferior LV wall resulting in localized steep gradients of repolarization and inferior J point elevation.<sup>287,288</sup> Increasing evidence supports an alternative hypothesis, according to which the J point elevation typical of ERP could be an expression of delayed depolarization.<sup>289–291</sup>

Early repolarization pattern shows at least moderate heritability in nuclear families<sup>292</sup> and across general population studies.<sup>293</sup> It is over-represented in families of UCA survivors<sup>294</sup> and autopsy negative SCD families.<sup>295,296</sup> There has not yet been a consensus curation for ERS. *SCN5A* variants with loss-of-function (determined by patch clamping expression studies) have been identified in 2–10% of patients with ERS, the patients showed signs of conduction slowing, supporting a depolarization phenotype.<sup>297–299</sup> Two paediatric ERS cases have been identified with a duplication and a *de novo* missense variant in *KCND3* responsible for  $I_{To}$ .<sup>300,301</sup> Furthermore, a recent general population GWAS has associated ERP with a genome-wide significant SNP tagging the *KCND3* locus (encoding the  $I_{To}$  current alpha subunit), suggesting the possibility of polygenic heritability.<sup>302</sup> There is, however, absence of other highly penetrant, reproducible and truly rare single gene causes of ERS. For example, the p.S422L

Recommendation	Consensus statement instruction	Ref.
In unexplained cardiac arrest survivors diagnosed clinically with ERS, molecular genetic testing may be appropriate.		Expert opinion
In asymptomatic individuals with only an ECG-based early repolarization pattern, genetic testing should not be performed.		Expert opinion

variant in *KCNJ8* responsible for  $I_{KATP}$ , has been implicated frequently in ERS but has too high a population frequency to cause a rare monogenic disorder.<sup>303,304</sup> Supplementary material online, Table S12 summarizes all genes which have been associated with ERS.

## 5.9 | Wolff–Parkinson–White syndrome

### 5.9.1 | Background

WPW is a condition where an extraconnection in the heart, called an accessory pathway (AP), is present, resulting in a pattern of pre-excitation during sinus rhythm. The most common arrhythmia associated with WPW is a paroxysmal supraventricular tachycardia, where the impulse uses the AP either from atrium to ventricle (antidromic circus movement tachycardia) or, more common, vice versa (orthodromic circus movement tachycardia). Resulting symptoms include dizziness, a sensation of fluttering or pounding in the chest (palpitations), shortness of breath, pre-syncope and syncope. In rare cases, arrhythmias associated with WPW can lead to cardiac arrest and sudden death.

Wolff–Parkinson–White affects 1 to 3 in 1000 people worldwide and is the second most common cause of paroxysmal supraventricular tachycardia in most parts of the world. Complications of WPW can occur at any age, although many individuals born with an AP in the heart never experience any health problems associated with the condition.

### 5.9.2 | Genetics of Wolff–Parkinson–White

#### 5.9.2.1 | Non-syndromic cases

Most cases of WPW occur in people with no apparent family history of the condition. These cases are described as sporadic and are usually not inherited. Familial WPW accounts for only a small percentage of all cases of this condition.<sup>305</sup> The familial form of the disorder typically has an AD pattern of inheritance. No specific genes have been identified for non-syndromic pre-excitation to date.

#### 5.9.2.2 | Syndromic cases

Wolff–Parkinson–White often occurs with other structural abnormalities of the heart or underlying heart disease. The most common heart defect associated with the condition is Ebstein's anomaly, which affects the tricuspid valve and right ventricle. In at least 10% of patients with Ebstein's anomaly, one or more APs are present.<sup>306</sup> Other genetic syndromes associated with APs include hypokalaemic periodic paralysis (a condition that causes episodes of extreme muscle weakness), Pompe disease (a disorder characterized by the storage of excess glycogen), Danon disease (a condition that weakens the heart and skeletal muscles and causes intellectual disability), and tuberous sclerosis complex (a

condition that results in the growth of non-cancerous tumours in many parts of the body).

An important subset of syndromic WPW associates with HCM. The locus for this (combined) condition, consisting of pre-excitation, HCM and (progressive) conduction abnormalities, was first identified in 1995<sup>307</sup> and the gene, *PRKAG2*, encoding for the enzyme AMP-activated protein kinase (AMPK), was identified in 2001, resulting in glycogen storage abnormalities in the heart.<sup>308</sup> In a recent relatively large series one-third of individuals carrying a pathogenic *PRKAG2* variant had evidence of pre-excitation and approximately two-thirds had an increased wall thickness.<sup>309</sup>

In conclusion, only in the presence of the combination of pre-excitation and HCM and/or progressive CCD is genetic testing pertinent (see above and State of genetic testing for cardiomyopathies section). The vast majority of WPW cases, however, will be isolated and not based on a genetic cause.

## 6 | STATE OF GENETIC TESTING FOR CARDIOMYOPATHIES











### 6.1 | Hypertrophic cardiomyopathy

#### 6.1.1 | Impact of genetic testing for the index case

Disease	Diagnostic	Prognostic	Therapeutic
HCM	+++	++	++

#### 6.1.2 | Background

Hypertrophic cardiomyopathy is a relative common inherited cardiac condition characterized by hypertrophy of the LV wall, not explained by other conditions (i.e. hypertension or valvular heart disease). Typically, the hypertrophy is asymmetric and confined to the intraventricular septum. Clinical sequelae of HCM include diastolic dysfunction, heart failure, atrial arrhythmias (with associated thrombotic events), and malignant ventricular arrhythmias. Genetic testing provides an opportunity to improve care of patients with HCM and their family members. Offspring of carriers have a 50% chance of inheriting the same disease-causing genetic variant.<sup>16,81,326</sup> It is essential to take a multigenerational family history of HCM including those suspected of dying suddenly. Engaging patients and family members means discussing the role of genetic testing including appropriate pre- and post-test genetic counselling, and its impact on psychological, social, legal, ethical, and professional implications of a positive test. Genetic assessment should ideally be performed in a specialized multidisciplinary HCM centre.<sup>16,326</sup> Next-generation sequencing led to an expansion in the number of genes included in diagnostic gene panel. However, inclusion of genes with limited gene-disease association, diminish the efficacy of genetic counselling by adding uncertainty and misinterpretation, among others leading to false positive res

Recommendation	Consensus statement instruction	Ref.
For genetic testing in a proband with HCM (including those cases diagnosed post-mortem), the initial tier of genes tested should include genes with definitive or strong evidence of pathogenicity (currently <i>MYH7</i> , <i>MYBPC3</i> , <i>TNNI3</i> , <i>TPM1</i> , <i>MYL2</i> , <i>MYL3</i> , <i>ACTC1</i> , and <i>TNNT2</i> ).		10
For genetic testing in a proband with HCM, the initial tier of genes tested may include genes with moderate evidence of pathogenicity ( <i>CSRP3</i> , <i>TNNC1</i> , <i>JPH2</i> ).		10,310–314
In patients with HCM, genetic testing is recommended for identification of family members at risk of developing HCM.		315–318
In patients with atypical clinical presentation of HCM, or when another genetic condition associated with unexplained hypertrophy is suspected (e.g. HCM phenocopy) genetic testing is recommended.		10,253,308,319–324
Predictive genetic testing in related children is recommended in those aged >10–12 years.		82,85,318
In patients with HCM who harbour a variant of uncertain significance, the usefulness of genetic testing of phenotype-negative relatives for the purpose of variant reclassification is uncertain.		10,315,325
Predictive genetic testing in related children aged below 10–12 years may be considered, especially where there is a family history of early-onset disease.		82 85
In patients with HCM who harbour a variant of uncertain significance, testing of affected family members for the purpose of variant classification may be considered.		Expert opinion
For patients with HCM in whom genetic testing found no LP/P variants, cascade genetic testing of family relatives is not recommended.		10,315–317,325
Ongoing clinical screening is not recommended in genotype-negative relatives in most families with genotype-positive HCM		10,315,316,325

ults.<sup>10,82,315–317,327–329</sup> Recommendation for genes with a definite, strong or moderate evidence of pathogenicity of HCM and phenocopies are depicted in [Table 13](#).

### 6.1.3 | Diagnostic implications of genetic testing

#### 6.1.3.1 | Index case

Hypertrophic cardiomyopathy is predominantly a disease of the sarcomere. First-line genetic testing primarily includes panel testing for genes with strong evidence for being disease-causing in HCM.<sup>10</sup> Gene panels generally (and are recommended to) include 8 sarcomere genes, including *MYH7*, *MYBPC3*, *TNNI3*, *TNNT2*, *TPM1*, *MYL2*, *MYL3*, and *ACTC1*, and typically identify a disease-causing variant in approximately 30% of sporadic and 60% of familial cases.<sup>10,315,316,328–330</sup> Variants in *TNNC1* (troponin C1) have moderate evidence of pathogenicity<sup>310,331</sup> ([Table 13](#)). A number of non-sarcomeric pathogenic variants with moderate to strong evidence of pathogenicity may be included in the initial tier of genes tested, including *CSRP3*, *JPH2*, *ALPK3*, and *FHOD3*.<sup>311–314</sup>

Expanding to larger panels, including the genes summarized in Supplementary material online, Table S13, usually does not add diagnostic value.<sup>69,315</sup> Initial genetic testing is usually performed in the index case (proband).<sup>315</sup> In up to 40% of patients with HCM, no sarcomere variant is identified, and there is no family history of disease.<sup>332</sup>

Genes associated with HCM phenocopies may be included in first-tier genetic testing if there is clinical suspicion based on phenotype evaluation of a syndromic disorder, including *PRKAG2* (glycogen storage disease),<sup>253,308,319</sup> *LAMP2* (Danon disease),<sup>320</sup> *GLA* (Fabry disease),<sup>321</sup> and relevant genes for transthyretin amyloid cardiomyopathy,<sup>322</sup> and Pompe disease.<sup>333–335</sup> In some circumstances, the genetic test result may alter the management of the index case, such as enzyme replacement therapy in patients with Fabry disease or more aggressive clinical management of patients with Danon disease, or increased awareness for sinus bradycardia and AV block in *PRKAG2*.<sup>323,324</sup>

Postmortem testing for HCM-associated variants using blood or tissue collected at autopsy has been reported, particularly in instances where the family variant is unknown and no other affected



TABLE 13 Genes implicated in hypertrophic cardiomyopathy

Gene	Locus	Syndrome	Protein (functional effect)	Frequency	ClinGen classification
MYBPC3	11p11.2	Familial HCM	↓contractility due to ↓Ca <sup>2+</sup> sensitivity	40–45%	Definite
MYH7	14q11.2-q12	Familial HCM	↓contractility due to ↓Ca <sup>2+</sup> sensitivity	15–25%	Definite
TNNI3	19q13.4	Familial HCM	Loss of function (inhibitory)	1–7%	Definite
TNNT2	1q32.1	Familial HCM	Increase oxygen consumption	1–7%	Definite
TPM1	15q22.2	Familial HCM	Loss-of-function of the thin filament	1–2%	Definite
ACTC1	15q.14	Familial HCM	Gain-of-function causing high contractile phenotype	1–2%	Definite
MYL2	12q24.11	Familial HCM	Loss-of-function	1–2%	Definite
MYL3	3p21.31	Familial HCM	Loss-of-function	1–2%	Definite
<b>Intrinsic cardiomyopathy genes</b>					
ACTN2	1q43	LVH, LVNC, DCM, and idiopathic VF	Loss-of-function	<1%	Moderate
PLN	6q22.31	HCM, DCM, and ARVC	Loss-of-function of SERCA (Ca <sup>2+</sup> overload) mitochondrial disease	<1%	Definite
JPH2	20q13.12	Familial HCM/DCM	Unknown	<1%	Moderate
FHOD3	18q12.2	Familial HCM/DCM	Actin filament polymerization disruption	0.5–2%	Not curated by ClinGen
CSRP3	11p15.1	Late onset familial HCM, DCM	Unknown (non-sarcomeric gene)	<1%	Moderate
TNNC1	3p21.1	Familial HCM	Disruption of Ca <sup>2+</sup> handling	<1%	Moderate
<b>Syndromic genes, where isolated LVH may be seen</b>					
CACNA1C	12p13.33	Timothy syndrome, BrS, LQTS	Intracellular Ca (2+) overload	<1%	Definite
DES	2q35	Desminopathy (DCM), myofibrillar myopathy	Dysfunction through Z-disk and myofibril disintegration, followed by abnormal accumulation of intracellular proteins	<1%	Definite
FHL1	Xq26.3	Emery-Dreifuss MD, cardiac conduction abnormalities, arrhythmias, HCM	Dysfunction through Z-disk and myofibril disintegration, followed by abnormal accumulation of intracellular proteins	<1%	Definite
FLNC	7q32.1	Myofibrillar myopathy, HCM, RCM, distal myopathy	Dysfunction through Z-disk and myofibril disintegration, followed by abnormal accumulation of intracellular proteins	<1%	Not curated by ClinGen
GLA	Xq22.1	Fabry disease	Loss-of-function	<1%	Definite
LAMP2	Xq24	Danon disease	Loss-of-function	<1%	Definite
PRKAG2	7q36.1	PRKAG2 cardiomyopathy	Dysfunction of AMPK	1–2%	Definite
PTPN11	12q24.13	Noonan syndrome	RASopathy	<1%	Definite
RAF1	3p25.2	Noonan syndrome	RASopathy	<1%	Definite
RIT1	1q22	Noonan syndrome	RASopathy	<1%	Definite
TTR	18q12.1	Transthyretin amyloidosis	Loss-of-function causing amyloid deposition in peripheral nerves and heart	1–2%	Definite
ALPK3	15q25.3	Infant-onset HCM/DCM	Biallelic loss-of-function	<1%	Strong
<b>Syndromic genes, where LVH is occurs together with other syndromic features</b>					
ABCC9	12p12.1	Cantu syndrome	Reduce ATP-mediated potassium channel inhibition (gain-of-function)	<1%	Definite
BAG3	10q26.11	Myofibrillar myopathy	Dysfunction through Z-disk and myofibril disintegration, followed by abnormal accumulation of intracellular proteins	<1%	Definite
CAV3	3p25.3	Caveolinopathy	Disruption of caveolae formation	<1%	Definite
COX15	10q24.2	Leigh syndrome	Loss-of-function of SERCA (Ca <sup>2+</sup> overload) mitochondrial disease	<1%	Strong
CRYAB	15	Alpha-B crystallinopathy	Dysfunction through Z-disk and myofibril disintegration, followed by abnormal accumulation of intracellular proteins	<1%	Definite

(Continues)

TABLE 13 (Continued)

Gene	Locus	Syndrome	Protein (functional effect)	Frequency	ClinGen classification
FXN	9q21.11	Friedreich ataxia	Loss-of-function of mitochondrial protein	<1%	Definite
GAA	17q25.3	Pompe disease	Loss-of-function	<1%	Definite
LDB3/ZASP	10q23.2	Myofibrillar myopathy	Dysfunction through Z-disk and myofibril disintegration, followed by abnormal accumulation of intracellular proteins	<1%	Moderate
MYO6	6q14.1	Bilateral hearing loss	Disruption of the structural integrity of inner ear hair cells	<1%	Definite
SLC25A4	4q35.1	Mitochondrial disease	RASopathy	<1%	Definite

ACM, arrhythmogenic cardiomyopathy; BrS, Brugada syndrome; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LQTS, long QT syndrome.

family members are still living.<sup>86–88</sup> Access to a molecular autopsy as well as considerations related to costs and insurance coverage for this testing can vary between jurisdictions. Nevertheless, identification of a LP/P variant not only confirms the diagnosis of HCM but allows cascade genetic testing of other at-risk relatives as outlined previously.

#### 6.1.3.2 | Family screening

After genetic testing, a clinically actionable result (likely-pathogenic or pathogenic) can provide diagnostic clarification in the proband and offers the potential for cascade (predictive) testing of at-risk family members.<sup>81–85</sup> Cascade testing involves targeted testing of first-degree relatives for the LP/P variant found in the proband. When cascade testing is performed in an at-risk relative, those who are found not to carry the disease-causing gene variant can be released from further clinical surveillance. Those who are found to carry the disease-causing gene variant should undergo clinical screening at regular intervals. Family members of a patient where genetic testing is not done or is negative (no likely-pathogenic or pathogenic variant is identified) also require clinical screening at regular intervals because there is considerable phenotypic heterogeneity in age of onset and disease progression within members of the same family.

#### 6.1.4 | Prognostic and therapeutic implications of genetic testing

Although there is some evidence that individuals who carry >1 LP/P variant may have more severe disease, including SCD, the role of the genetic test result in the determination of risk in SCD remains uncertain and is therefore not clinically useful. Similarly, a genetic result *per se* does not influence decisions related to implanting an ICD in patients with HCM. Several studies have reported that patients with HCM who carry LP/P sarcomere variants have a worse prognosis compared to sarcomere variant negative patients. This includes earlier onset of disease, higher incidence of SCD, higher incidence of AFib and ventricular arrhythmias, HF, and overall mortality.<sup>83,329,336–338</sup> However, there remains considerable intra- and inter-familial

heterogeneity with variants in the same gene that currently limits the application of genetic information for clinical decision-making, including risk stratification for SCD in the proband.<sup>318,339</sup> Early data on polygenic risk scores suggests they may correlate with disease severity.<sup>29,30</sup> Discovery of an HCM phenocopy may modify therapeutic options, such as enzyme replacement therapy in Fabry patients.










## 6.2 | Dilated cardiomyopathy

### 6.2.1 | Impact of genetic testing for the index case

Disease	Diagnostic	Prognostic	Therapeutic
DCM	++	+++	++

### 6.2.2 | Background

Dilated cardiomyopathy is defined by the presence of LV or biventricular dilatation and systolic dysfunction in the absence of abnormal loading conditions (hypertension, valve disease) or coronary artery disease sufficient to cause global systolic impairment.<sup>351</sup> A new category of *hypokinetic non-dilated cardiomyopathy* was also proposed<sup>352</sup> to characterize patients with systolic dysfunction but without LV dilatation. Dilated cardiomyopathy encompasses a broad range of genetic or acquired disorders and careful diagnostic work-up should be performed to identify the underlying cause and then consider an aetiology-oriented approach to therapy.<sup>352</sup> In the pre-molecular era, systematic cardiac screening of the relatives of patients with DCM identified probable familial disease in about 20–35% of cases.<sup>353–355</sup> Subsequently, identification of DCM-related genes and development of high-throughput sequencing technologies led to the identification of pathogenic variants in up to 50% of DCM patients<sup>340,355</sup> including a non-marginal yield in sporadic DCM.<sup>356</sup> Moreover, there are more and more situations in which genetic predisposition interacts with extrinsic or environmental factors resulting in mixed genetic/environmental causes, such as myocarditis, as well as peripartum, alcoholic, or chemotherapy-related cardiomyopathies.<sup>341,342,357,358</sup>

Recommendation	Consensus statement instruction	Ref.
Genetic testing is recommended for probands with DCM and family history of DCM, and the initial tier of genes tested should include genes with definitive or strong evidence of pathogenicity (currently <i>BAG3</i> , <i>DES</i> , <i>FLNC</i> , <i>LMNA</i> , <i>MYH7</i> , <i>PLN</i> , <i>RBM20</i> , <i>SCN5A</i> , <i>TNNC1</i> , <i>TNNT2</i> , <i>TTN</i> , <i>DSP</i> ).		19,340
For genetic testing in a proband with DCM, the initial tier of genes tested may include genes with moderate evidence of pathogenicity ( <i>ACTC1</i> , <i>ACTN2</i> , <i>JPH2</i> , <i>NEXN</i> , <i>TNNI3</i> , <i>TPM1</i> , <i>VCL</i> ).		19
Genetic testing is recommended for patients with DCM and family history of premature unexpected sudden death or in a DCM patient with clinical features suggestive of a particular/rare genetic disease (such as atrioventricular block or sinus dysfunction or creatine phosphokinase elevation).		340
Genetic testing can be useful for patients with apparently sporadic DCM, particularly in the presence of either severe systolic dysfunction (left ventricular ejection fraction <35%), or a malignant arrhythmia phenotype (e.g. sustained ventricular tachycardia/fibrillation), or particularly at a younger age.		340
Genetic testing may be considered for patients with DCM related to an acquired or environmental cause that may overlap with a genetic cause (such as peripartum or alcoholic cardiomyopathy).		341,342
Genetic testing is useful for patients with DCM to improve risk stratification and guide therapy.		201,343–348
Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causative variant.		16,340,349
Predictive genetic testing in related children is recommended in those aged >10–12 years.		16,350
Predictive genetic testing in related children aged below 10–12 years may be considered, especially where there is a family history of early-onset disease.		16,350

### 6.2.1.1 | Summary of the common dilated cardiomyopathy genes

About 100 genes have been reported to be possibly related to DCM (main genes in [Table 14](#)). The disease-specific metrics designed by the Clinical Genome Resource (ClinGen), reclassified many of these genes to limited or disputed evidence<sup>19</sup> (Supplementary material online, Table S14). Truncating variants in titin gene (*TTN*) are the most frequent in DCM, accounting for up to 20% of cases.<sup>24</sup> A case-control study demonstrated that variants in *TTN*, *DSP*, *MYH7*, *LMNA*, *BAG3*, *TNNT2*, *TNNC1*, *PLN*, *ACTC1*, *NEXN*, *TPM1*, and *VCL* are significantly enriched in DCM cases.<sup>359</sup> Mutated genes are most often related to sarcomeric genes, z-disc/cytoskeleton, intercalated disc, and ion flux in large series with large panels indicating partial overlap with other cardiomyopathy subtypes [such as ACM (arrhythmogenic right ventricular cardiomyopathy, ARVC)] as well as with channelopathies.<sup>340</sup>

Series also suggest that the single-variant Mendelian disease model is insufficient to explain some DCM cases, since multiple variants (mainly compound heterozygous may be observed in up to 38% in DCM patients).<sup>340</sup> Preliminary data suggest a complex polygenic architecture for some DCM patients with a combination of rare and frequent variants and interactions with environmental factors.<sup>29,30,360,361</sup>

### 6.2.3 | Diagnostic implications of dilated cardiomyopathy genetic testing

#### 6.2.3.1 | Index cases

The yield of genetic study in DCM is variable and depends on familial context (familial vs. sporadic DCM, history of SCD), presence of particular associated cardiac or extra-cardiac signs, type of genetic testing selection, and stringency of variant interpretation. It can be grossly estimated to be 20–50% and is the highest in DCM with familial forms or with particular associated cardiac or extra-cardiac signs.<sup>340,349,356</sup> As in other conditions genetic testing in an index patient, and identification of a pathogenic variant, may have several impacts since the information is able to confirm the genetic origin and mode of inheritance, can distinguish DCM from other cardiomyopathies such as ACM and is useful for appropriate aetiology-management of patients. Genetic testing is therefore useful in all DCM patients, is recommended in DCM patients with the highest yield of pathogenic variant screening and should be considered even in the absence of familial context or associated clinical features (<60 years of age). High-throughput sequencing with targeted sequencing panels of genes is the most cost-effective approach and recommended technique.<sup>362</sup> Panels

TABLE 14 Genes implicated in dilated cardiomyopathy

Gene	Locus	Phenotype-syndrome	Protein (functional effect)	Frequency	ClinGen classification
TTN	2q31.2	DCM	Titin	~15-25%	Definitive
LMNA	1q22	DCM, ACM	Lamin A/C	~4-7%	Definitive
MYH7	14q11.2	HCM	Bêta Myosin heavy chain	~3-5%	Definitive
TNNT2	1q32.1	HCM, DCM	Troponin T	~2%	Definitive
RBM20	10q25.2	DCM	RNA-binding motif protein 20	~2%	Definitive
PLN	6q22.31	DCM, ACM	Phospholamban	~1% (more in Netherlands)	Definitive
FLNC	7q32.1	DCM»BiVACM	Filamin-C	~3%	Definitive
BAG3	10q26.11	DCM, myopathy	BAG family molecular chaperone regulator 3	~2%	Definitive
DSP	6p24.3	ARVC, DCM	Desmoplakin	1-3%	Strong
TPM1	15q22.1	HCM, DCM	alpha-tropomyosin	~1-2%	Moderate
ACTC1	15q11q14	HCM, DCM	Cardiac alpha-actin	<1%	Moderate
ACTN2	1q43	HCM, DCM, LVNC	Alpha-actinin-2	<1%	Moderate
DES	2q35	DCM, Myopathy, ACM	Desmin	<1%	Definitive
JPH2	20q13.12	DCM, HCM	Junctophilin 2	<1%	Moderate
NEXN	1p31.1	DCM, HCM	Nexilin	<1%	Moderate
SCN5A	3p22.2	LQTS, Brugada, DCM, ACM	Sodium channel protein type 5 subunit alpha	<1%	Definitive
TNNC1	3p21.1	DCM, HCM	Cardiac Troponin C	<1%	Definitive
TNNI3	19q13.4	HCM, DCM	Cardiac troponin I	<1%	Moderate
VCL	10q22.2	DCM	Metavinculin	<1%	Moderate

ACM, arrhythmogenic cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy.

should include validated genes in DCM (see Table 14), with most prevalent genes such as TTN as well as genes with prognostic or therapeutic implications, such as LMNA or FLNC. Genetic testing/panel can be oriented by the presence of a particular extra-cardiac phenotype such as neuromuscular diseases, mitochondrial diseases, congenital syndromes.<sup>363</sup>








#### 6.2.3.2 | Family screening

Most genetic DCM inheritance follows an AD pattern, although X-linked, recessive, and mitochondrial patterns of inheritance occur (see genetic influences on disease and modes of inheritance section).<sup>360,364</sup> Penetrance in AD DCM is age-dependent. Therefore, an individual who carries a disease-causing variant is more likely to show a disease phenotype with increasing age, and a normal phenotypic assessment by echocardiogram and ECG does not exclude the possibility of later onset disease. The identification of a LP/P in the index case allows specific cascade genetic screening to identify gene carriers among relatives.<sup>16,350,365</sup> Relatives who do not carry the pathogenic variant are reassured and cardiac follow-up is no longer required. Relatives who carry the pathogenic variant must be periodically investigated for early detection of the phenotype, to allow optimal management and prevention of the complications. A genetic diagnosis can be useful for reproductive counselling and planning, including options for prenatal or pre-implantation genetic testing to prevent the transmission of DCM.<sup>366</sup>

#### 6.2.4 | Prognostic and therapeutic implications of dilated cardiomyopathy genetic testing

The identification of a specific genetic substrate can help to manage the patients and guide clinical decisions. Patients with pathogenic LMNA variants have consistently been associated with a poor prognosis, especially with a high risk of SCD related either to conduction defect or ventricular arrhythmia.<sup>201,343,344</sup> There are, however, exceptions for particular founder pathogenic variants.<sup>367</sup> Preventive pacemaker (PM) or ICD therapy should be considered early in LMNA carriers, and algorithms for ICD implantation include the pathogenic variant mechanism (truncating vs. missense variant) as associated with higher SCD risk.<sup>201,343,344</sup> Higher risk of SCD is also associated with pathogenic variants, especially truncated variants, in *FLNC*, *DES*, *RBM20*, and *PLN* genes,<sup>345-348,368</sup> so that preventive ICD implantation may also be considered in these patients. Desmosomal pathogenic variants in patients with DCM or biventricular cardiomyopathy are also associated with a greater risk of life-threatening ventricular arrhythmias/SCD.<sup>369</sup> Patients with DCM are also at greater risk for heart failure and heart transplantation when they are carriers of pathogenic variants in *LMNA*, *RBM20*, and *DSP* genes.<sup>345,369</sup> Preventive PM implantation related to conduction defect should also be considered in patients with DCM and muscular dystrophy related to dystrophin, *DES* and *EMD* genes.<sup>345,348</sup>

### 6.3 | Arrhythmogenic cardiomyopathy

Disease	Diagnostic	Prognostic	Therapeutic
ACM	+++	++	++
Recommendations	Consensus statement instruction		Ref.
Comprehensive genetic testing is recommended for all patients with consistent phenotypic features of ACM, including those cases diagnosed post-mortem, whatever familial context.			<a href="#">370</a>
Genetic testing of first tier definitive disease-associated genes (currently <i>PKP2</i> , <i>DSP</i> , <i>DSG2</i> , <i>DSC2</i> , <i>JUP</i> , <i>TMEM43</i> , <i>PLN</i> , <i>FLNC</i> , <i>DES</i> , <i>LMNA</i> ) is recommended.			<a href="#">370,371</a>
Owing to the possibility of complex genotypes, in families with multiple affected members, the case with the more severe and/or earlier phenotype may be considered the 'genetic proband' and be tested first.			<a href="#">362</a>
In patients with a borderline ACM phenotype, comprehensive genetic testing may be considered. The identification of a LP/P genetic variant would be useful to confirm the diagnosis.			<a href="#">372</a>
Variant-specific genetic testing is recommended for family members and appropriate relatives following the identification of the disease-causative variant.			<a href="#">370,373</a>
Predictive genetic testing in related children is recommended in those aged >10–12 years.			<a href="#">370,374</a>
Predictive genetic testing in related children aged below 10–12 years may be considered, especially where there is a family history of early-onset disease.			Expert opinion

#### 6.3.1 | Background

Arrhythmogenic cardiomyopathy is mainly characterized by fibro or fibrofatty myocardial replacement which can cause progressive global/regional ventricular dysfunction, and high burden of ventricular arrhythmias.<sup>375</sup> Structural alterations can affect left, right, or both ventricles which lead to three recognized phenotypic variants: the dominant-right ('the classic arrhythmogenic right ventricular cardiomyopathy'—ARVC) variant, the biventricular variant (Biv ACM), and the dominant-left variant (also known as 'arrhythmogenic left ventricular cardiomyopathy'—ALVC). The identification of a LP/P genetic variant is a major diagnostic criterion in all types and can be a necessary requirement for the ALVC variant.<sup>372</sup> The most common pattern of inheritance in monogenic ACM is AD. However, Naxos disease and Carvajal syndrome, which lead to the identification of the desmosomal cause of the disease are both recessive conditions.<sup>373</sup>

#### 6.3.2 | Diagnostic implications of arrhythmogenic cardiomyopathy genetic testing

Arrhythmogenic right ventricular cardiomyopathy is predominantly associated with variants in desmosomal genes. Haploinsufficiency

is a well-recognized molecular mechanism in these genes, and loss-of-function variants (nonsense, frameshift and splicing site) have the strongest evidence for pathogenicity.<sup>374</sup> The interpretation of missense or in-frame insertion/deletion variants is generally challenging and segregation with the phenotype in the families is usually mandatory for establishing their causality. Nearly 50% of patients with ARVC have one or more desmosomal pathogenic variants, with *PKP2* the most common mutated gene.<sup>371,376</sup> The number of variants that could be considered pathogenic in *JUP* is anecdotally besides Naxos disease. Non-desmosomal gene variants represent a minority of ARVC causes, and have been reported in a limited number of cases. Familial segregation studies are limited in some of the new proposed genes and the evidence supporting their causality is limited.

Biventricular ACM is also frequently associated with desmosomal genetic variants. Specific variants in *PLN* (p.Arg14del) and *TMEM43* (p.Ser358Leu) are highly relevant in some countries where a founder effect has been demonstrated.<sup>377,378</sup> The identification of other pathogenic variants in these two genes associated with ACM is quite rare. Initial investigations postulated *RYR2* gene as part of the genetic substrate of ARVC.<sup>379</sup> However, after decades of their initial descriptions, and after investigation of thousands of patients, evidence no longer supports these associations.

Desmoplakin (*DSP*) is by far the most commonly mutated desmosomal gene in patients with ALVC. *DSG2* and *DSC2* genes

variants have also been described in ALVC patients but represent a significantly lower number of cases.<sup>369</sup> Non-desmosomal genes can be more relevant in the left-dominant variant of the disease. Truncations in *FLNC*, *RBM20*, and some *DES* variants were consistently associated with this phenotype often without overt skeletal myopathy, which is traditionally related to these genes.<sup>346,380,381</sup> The yield of genetic study in ACM is highly variable and depends on several factors (type of ventricle affected, familial clustering, ethnicity of the cohort and selection criteria, type of genetic testing selection, and the stringency of variant interpretation) but can be grossly estimated in the 50–60% range.

### 6.3.2.1 | Index cases

Genetic testing is indicated in a proband with consistent phenotypic features of ACM, including those cases diagnosed post-mortem.<sup>370,375,382</sup> The identification of a LP/P genetic variant would also be useful to confirm the diagnosis in patients with a borderline phenotype. In those cases with isolated LV compromise, the demonstration of a pathogenic genetic variant could be necessary to link the electrical and/or structural manifestations with the diagnosis of ACM.<sup>372</sup> In families with multiple affected members, the case with the more severe and/or earlier phenotype must be considered the 'genetic proband' and be tested first to enhance the detection of complex genotypes causing the disease (homozygous or compound/double heterozygous situations).<sup>372</sup> Nowadays, the recommended genetic test for ACM must include a minimal number of genes that

have clinically demonstrated their association with the disease (see [Table 15](#)). Genes with limited or disputed evidence are summarized in Supplementary material online, Table S15. High-throughput sequencing has demonstrated a high level of accuracy and is the recommended technique. Targeted sequencing panels of genes is the most cost-effective approach.<sup>362</sup> Copy number variation's analysis should be included, since this type of variant can be found in 1–4% of negative studies.<sup>371</sup> Whole-exome/genome sequencing must assure adequate coverage in causative genes, and its application without filtering against genes of interest should be considered only in research contexts. Owing to the limited yield of genetic testing in ACM, a negative result does not rule out the diagnosis. The high genetic noise based on the prevalence of rare variants in ACM genes (especially missense changes in desmosomal genes) in the general population strengthens the importance of interpretation of the results by experts in cardiovascular molecular genetics.<sup>374</sup>

### 6.3.2.2 | Family screening

The identification of a LP/P variant in the index case allows specific cascade genetic screening to identify gene carriers among relatives.<sup>370,373</sup> Incomplete penetrance and highly variable clinical expression associated with most ACM-related genes must be considered in the interpretation of the results, genetic counselling and clinical management.<sup>383,384</sup> Clinical and genetic evaluations of older generations in the family is also recommended and could be valuable for phenotype delineation associated with a particular genotype.

TABLE 15 Genes implicated in arrhythmogenic cardiomyopathy

Gene	Locus	Phenotype/syndrome	Protein (Cellular complex)	Frequency	ClinGen classification
<i>PKP2</i>	12p11.21	Classic ARVC. BiVACM and ALVC in a minority of cases.	Plakophilin 2 (desmosome)	20–45%	Definite
<i>DSP</i>	6p24.3	Frequent BiVACM and ALVC. Occasional hair and skin features. Rare homozygous variants—Carvajal Syndrome.	Desmoplakin (desmosome)	2–15%	Definite
<i>DSG2</i>	18q12.1	Frequent BiVACM and ALVC.	Desmoglein 2 (desmosome)	4–15%	Definite
<i>DSC2</i>	18q12.1	ARVC. Less frequent BiVACM and ALVC.	Desmocollin 2 (desmosome)	2–7%	Definite
<i>FLNC</i>	7q32.1	ALVC. Right ventricular involvement is rare	Filamin-C (cytoskeleton)	3%	Definite <sup>a</sup>
<i>JUP</i>	17q21.2	Naxos disease (cardioectodermal)	Plakoglobin (desmosome)	<1% (higher in Naxos, Greece)	Definite
<i>TMEM43</i>	3p25.1	ARVC and BiVACM	Transmembrane protein 43 (nuclear envelope)	<1% (higher in Newfoundland)	Definite
<i>PLN</i>	6q22.31	Frequent ALVC/DCM	Phospholamban (sarcoplasmic reticulum; calcium handling)	1% (10–15% in Netherlands)	Definite <sup>a</sup>
<i>DES</i>	2q35	<ul style="list-style-type: none"> <li>○ Frequent ALVC. Right ventricular involvement is also possible.</li> <li>○ Conduction system abnormalities common. Skeletal myopathy possible.</li> </ul>	Desmin (cytoskeleton)	1–2%	Moderate

ALVC, arrhythmogenic left ventricular cardiomyopathy; ARVC, arrhythmogenic right ventricular cardiomyopathy; BiVACM, bi-ventricular arrhythmogenic cardiomyopathy; DCM, dilated cardiomyopathy.

<sup>a</sup>Genes with a clear association with ALVC and included also in the ClinGen classification for DCM.



The identification of relatives without the family pathogenic variant allows psychological relief and optimizes the clinical resources. On the other hand, variant-carrier relatives must be investigated periodically should be advised of the benefit of life-style modifications.





### 6.3.3 | Prognostic and therapeutic implications of arrhythmogenic cardiomyopathy genetic testing

Arrhythmogenic cardiomyopathy is characterized by highly variable intra/interfamilial phenotype severity and the influence of environmental factors is probably more determinant than in other cardiomyopathies.<sup>385</sup> Some investigations have suggested that ACM patients with an identifiable causative genetic variant do not have significant differences in disease course and prognosis from gene elusive patients.<sup>383</sup> Nevertheless, identification of the specific genetic substrate can guide the clinical decisions in some scenarios. Preventive (early) ICD implantation may be considered in ACM patients with truncations in *FLNC*, *DSP*, *LMNA*, *DES* and *PLN* pathogenic variants, who present with reduced LV systolic function.<sup>370,380,381</sup> Arrhythmogenic cardiomyopathy patients with cadherin-2 (*CDH2*) pathogenic variants have a higher incidence of ventricular

arrhythmias, while development of heart failure is rare.<sup>386</sup> Since the ClinGen curation of genes for ACM, new evidence supports *CDH2* as a disease gene in a small subset of ACM patients.<sup>387</sup> Indeed, in ACM severe ventricular arrhythmias may present before ventricular dysfunction or structural manifestations are evident, that is why the detection of P/LP variant in an index case will allow through familial cascade screening early detection and prompt stratification of arrhythmic risk of those mutation carriers.

For *LMNA*, *PLN* and ACM caused by desmosome gene variants (mainly *PKP2*) specific calculators have been developed.<sup>344,388,389</sup> Those patients initially diagnosed with DCM where a pathogenic desmosomal variant is identified could have a greater risk of life-threatening ventricular arrhythmias and sudden death, regardless of the LV ejection fraction.<sup>369</sup> Patients with complex genotypes (homozygous and compound/double heterozygous) carrying clearly disease-causing variants, have a worse prognosis (considering ventricular arrhythmias and ventricular dysfunction) compared with single pathogenic variant carriers.<sup>376,390,391</sup> Competitive or high-level leisure sport has been demonstrated to increase penetrance, incidence of ventricular arrhythmias and progression to ventricular dysfunction in carriers of pathogenic desmosomal variants.<sup>392,393</sup>

## 6.4 | Left ventricular non-compaction cardiomyopathy

Disease	Diagnostic	Prognostic	Therapeutic
LVNC	+	+	-
Recommendation	Consensus statement instruction		Ref.
LVNC cardiomyopathy genetic testing may be useful for patients in whom a cardiologist has established a clinical diagnosis of LVNC based on examination of the patient's clinical history, family history, and electrocardiographic/echocardiographic/MRI phenotype.			387,394-396
Genetic testing may be useful for patients with a clinical diagnosis of LVNC cardiomyopathy associated with other cardiac or non-cardiac syndromic features.			397-399
Genetic testing should not be performed in isolated (incidental) LVNC with normal LV function, no associated syndromic features and no family history.			387,394-400
Variant specific genetic testing may be considered for family members and appropriate relatives following the identification of the disease-causative variant.			Expert opinion

### 6.4.1 | Background

Left ventricular non-compaction (LVNC) is a phenotype that can present as a cardiomyopathy characterized by prominent LV trabeculations with deep intertrabecular recesses and thinning of the

compact epicardium.<sup>394</sup> In children, LVNC can present with severe heart failure and life threatening arrhythmias. In adults, the clinical presentation and significance is less clear, particularly when the diagnosis is made outside the context of an affected family. Patients with LVNC can present with isolated LV trabeculations with no LV

TABLE 16 Genes implicated in left ventricular noncompaction in adults

Gene	Locus	Syndrome	Protein (functional effect)	Frequency	ClinGen classification
<i>MHY7</i>	14q11.2	LVNC, DCM or HCM	Beta myosin heavy chain	10–15%	NA/major gene
<i>MYBPC3</i>	11p11.2	LVNC, DCM or HCM	Myosin binding protein C	5–15%	NA/major gene
<i>TTN</i>	2q31.2	LVNC, DCM	Titin	5–10%	NA/major gene
<i>ACTC1</i>	15q11.14	LVNC, DCM or HCM	Cardiac alpha-actin	1–5%	NA/rare gene
<i>RYR2</i>	1q43	LVNC, DCM	Ryanodine receptor type 2	1–2%	NA/rare gene
<i>PRDM16</i>	1p36	LVNC	PR domain zinc finger protein 16	1–2%	NA/rare gene
<i>LBD3</i>	11p15.1	LVNC, DCM	LIM domain binding 3	1–2%	NA/rare gene
<i>TBX5</i>	12q24.1	LVNC, Holt-Oram syndrome	T-box transcription factor 5	1–2%	NA/rare gene
<i>NKX2-5</i>	5q35.1	LVNC, DCM, conduction disease	Homeobox protein Nkx2-5	1–2%	NA/rare gene
<i>HCN4</i>	15q24.1	LVNC, conduction disease	Hyperpolarization-activated cyclic nucleotide-gated K <sup>+</sup> channel 4	1–2%	NA/rare gene
<i>TAZ</i>	Xp28	LVNC, Barth syndrome	Tafazzin	1–2%	NA/rare gene

DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular noncompaction.

dysfunction, LVNC associated with other cardiomyopathies such as HCM or DCM, or can present with LVNC associated with other cardiac (e.g. conduction disease) or non-cardiac systemic features (e.g. skeletal abnormalities in Holt-Oram syndrome).<sup>387,394–396,400,401</sup>

Major adverse events in adults include life-threatening arrhythmias, thromboembolism, and heart failure. Genetic testing in LVNC, therefore, is strongly guided by a comprehensive clinical evaluation of the patient and their family. Isolated LVNC, with no LV dysfunction and detected incidentally on MRI will have a very low genetic testing yield compared to LVNC associated with other cardiomyopathies and LV dysfunction, syndromic features, and/or a strong family history where the genetic testing yield will be significantly higher.<sup>394,396–399,401,402</sup>

Left ventricular non-compaction is most commonly inherited as an AD trait in families, although AR, X-linked, and mitochondrial inheritance is also seen, often in children. Studies of the genetic causes of LVNC have primarily identified variants in cardiomyopathy genes and specifically sarcomere genes, including *MYH7*, *MYBPC3*, and *TTN* with reported genetic testing yields between 17% and 41% (Table 16).<sup>394</sup> Other genetic diseases where LVNC is part of a clinical syndrome are also important to consider, such as *LDB3* (LIM-domain binding protein 3) with DCM and myopathy, *TBX5* in Holt-Oram syndrome, *NKX2-5* with conduction disease, and *TAZ* (tafazzin) associated with Barth syndrome in males<sup>387,394–399</sup> (Table 16). The choice of which genes to test in LVNC is strongly guided by the clinical phenotype, including presentation (symptomatic vs. incidental finding on cardiac MRI), association with other cardiomyopathies, other systemic cardiac or non-cardiac features, and presence of a family history of LVNC or other inherited cardiomyopathies.<sup>402</sup> There are not many known 'LVNC only' genes, so genetic testing is guided by the other cardiomyopathies such as HCM or DCM (see Table 16). Most

commonly a broad cardiomyopathy panel will represent the first step of genetic testing, with additional selection of genes guided by the phenotype. Left ventricular non-compaction in the setting of physiological changes such as during pregnancy or in athletes, as well as LVNC diagnosed incidentally on imaging studies, has a high prevalence in normal adult populations leading to overdiagnosis of LVNC as a pathogenic entity.<sup>403</sup> Therefore, genetic testing should rarely be considered in these settings and may lead to more harm than benefit related to uncertain genetic findings including variants of uncertain significance.




#### 6.4.2 | Diagnostic implications of left ventricular non-compaction genetic testing

The main benefit of genetic testing in LVNC is for diagnosis in the index cases and to then use this genetic diagnosis for cascade testing in family members.<sup>394</sup> The identification of a genetic cause may also be useful in guiding reproductive decisions such as pre-implantation genetic diagnosis.

#### 6.4.3 | Prognostic and therapeutic implications

Currently, no significant genotype-phenotype correlations have been associated with LVNC alone and therefore, little prognostic information is available based on the genetic findings. There are some emerging data suggesting that specific genotypes such as *MYH7* pathogenic variants or multiple pathogenic variants in patients with LVNC and LV dysfunction may be associated with worse clinical outcomes compared to sporadic cases.<sup>394</sup>

## 6.5 | Restrictive cardiomyopathy

Disease	Diagnostic	Prognostic	Therapeutic
RCM	+	+	+
Recommendation	Consensus statement instruction		Ref
RCM genetic testing may be considered for patients in whom a cardiologist has established a clinical diagnosis of RCM based on examination of the patient's clinical history, family history, and electrocardiographic/echocardiographic phenotype.			402,404–406
Genetic testing specifically for <i>TTR</i> pathogenic variants is recommended for patients with RCM and a clinical diagnosis of cardiac <i>TTR</i> amyloidosis.			407,408
Variant-specific genetic testing may be considered for family members and appropriate relatives following the identification of the disease-causative variant.			Expert opinion

### 6.5.1 | Background

Restrictive cardiomyopathy (RCM), defined by the presence of impaired LV filling and diminished diastolic volume with normal or near-normal LV wall thickness and ejection fraction, is a relatively rare cardiomyopathy which can have both genetic and non-genetic causes. These causes generally relate to infiltrative (e.g. amyloidosis), non-infiltrative (e.g. myofibrillar myopathies), storage diseases (e.g. Fabry disease), and endomyocardial aetiologies such as carcinoid heart disease.<sup>404</sup> In children, RCM often presents with severe heart failure, and carries a poor prognosis with heart transplant being the only viable long-term treatment option. In adults, there is significant overlap with HCM and DCM, and patients often present with heart failure and life-threatening arrhythmias. While the genetic basis of RCM is still emerging, there are significant commonalities with the genetic causes of HCM and DCM mainly relating to sarcomere and cytoskeletal disease genes.<sup>402,405,406</sup>

The inheritance pattern of RCM spans AD, AR, X-linked, and mitochondrial forms of transmission. Detailed family history and comprehensive clinical evaluation are essential to establish both cardiac features, as well as potential syndromic manifestations seen in RCM

such as skeletal myopathies. Our knowledge of the specific genetic causes of RCM is rapidly growing. Currently, sarcomere and cytoskeletal disease genes include *MYH7*, *TNNI3*, *TNNT2*, *ACTC1*, *FLNC*, and *TTN*, reflecting the common genetic aetiologies of HCM and DCM<sup>402,405,406</sup> (Table 17). In practical terms, genetic testing for RCM incorporates gene panels used for HCM and DCM, and relevant phenocopies such as *GLA* gene in suspected Fabry disease (Table 17). The yield of genetic testing in familial RCM is difficult to estimate due to the range of aetiologies and the rare prevalence of disease, but may be up to 60%.<sup>402,405</sup> Inherited infiltrative diseases can lead to RCM, with amyloidosis being the most common, caused by pathogenic variants in the *TTR* gene which encodes transthyretin.<sup>407,408</sup> Pathological deposition of mis-folded amyloid can occur in many organs such as the liver, kidney, eyes, as well as the heart, so-called cardiac amyloidosis.<sup>407</sup>

### 6.5.2 | Diagnostic implications of restrictive cardiomyopathy genetic testing

The main benefit of genetic testing in familial RCM is for diagnosis in the index cases and to then use this genetic diagnosis for cascade testing in at-risk family members. The identification of a genetic

TABLE 17 Genes implicated in restrictive cardiomyopathy

Gene	Locus	Syndrome	Protein (functional effect)	Frequency	ClinGen classification
<i>MHY7</i>	14q11.2	RCM	Beta myosin heavy chain	10–15%	NA/major gene
<i>TTN</i>	2q31.2	RCM	Titin	5–10%	NA/major gene
<i>ACTC1</i>	15q11.14	RCM	Cardiac alpha-actin	5–10%	NA/major gene
<i>TNNI3</i>	19q13.4	RCM	Cardiac troponin I	5–10%	NA/major gene
<i>TTR</i>	18q12.1	RCM, amyloidosis	Transthyretin	1–5%	NA/major gene
<i>FLNC</i>	7Q32.1	RCM	Filamin-C	1–5%	NA/major gene
<i>TNNT2</i>	1q32.1	RCM	Cardiac troponin T	1–2%	NA/rare gene

RCM, restrictive cardiomyopathy.







cause may also be useful in guiding reproductive decisions such as pre-implantation genetic diagnosis.

### 6.5.3 | Prognostic and therapeutic implications

The genetic diagnosis may guide clinical management strategies. For example, a genetic diagnosis of Fabry disease may lead to the

introduction of enzyme replacement therapy for the deficiency in alpha-galactosidase enzyme.<sup>409</sup> Similarly, a genetic diagnosis of *TTR* cardiac amyloidosis may be amenable to newer targeted treatments that inhibit hepatic synthesis of the *TTR* protein, stabilize the tetramer, or disrupt fibrils, such as tafamidis.<sup>407,410,411</sup> As the genetic architecture of RCM is further elucidated and underlying disease mechanisms identified, the impact of the genetic diagnosis in terms of guiding clinical management and informing prognosis will become more prominent.

## 7 | STATE OF GENETIC TESTING FOR SUDDEN CARDIAC DEATH OR SURVIVORS OF UNEXPLAINED CARDIAC ARREST

Recommendations	Consensus statement instruction	Ref.
Unexpected sudden deaths should be investigated with a general autopsy, toxicology, and cardiac pathology (where possible).		6,412
If a sudden death is likely to be due to a cardiac genetic cause, or remains unexplained after pathological evaluation, EDTA blood, and/or fresh tissue (e.g. liver or spleen) should be retained for potential genetic analysis. Other sources of DNA such as blood spots and tissue stored in suitable media at room temperature may suffice.		15,413–416
When a SCD could be attributable to a likely genetic cause, post-mortem genetic testing in the deceased individual targeted to the likely cause should be performed.		414, expert opinion
When a SCD remains unexplained despite an autopsy and toxicology, post-mortem genetic testing in the deceased individual targeted to channelopathy genes should be performed when the circumstances and/or family history support a primary electrical disease.		15,415,416
When a SCD <50 years old remains unexplained despite an autopsy, toxicology and channelopathy gene panel testing, post-mortem genetic testing in the deceased individual may be extended to a wider panel including cardiomyopathy genes.		15,415,416
In a decedent with unexplained SCD or an UCA survivor, hypothesis-free (post-mortem) genetic testing using exome or genome sequencing should not be performed.		Expert opinion
In selected UCA survivors with idiopathic VF, genetic testing for founder variants, <sup>a</sup> where relevant, should be considered.		417
In UCA survivors, genetic testing of channelopathy and cardiomyopathy genes may be considered.		418–421
In relatives of UCA survivors or SCD decedents in whom a pathogenic variant has been identified, predictive genetic testing should be performed.		413,422
In relatives of UCA survivors or SCD decedents, clinical evaluation of 1st degree family members should be performed, and targeted to the index case's phenotype if present.		422–427
In decedents with SCD or survivors with cardiac arrest in whom a non-genetic cause has been identified, genetic testing of the index case and clinical evaluation of relatives should not be performed.		Expert opinion

<sup>a</sup>In this setting, a founder variant is a pathogenic variant that has a relatively high prevalence in the population in a particular geographic region due to the presence of the variant in a single ancestor or small number of ancestors.

### 7.1 | Background

Sudden cardiac death is the most common mode of death due to cardiac disease. Approximately 1–3 per 100 000 individuals under 35 years old die suddenly and unexpectedly every year within this age group.<sup>6,412</sup> A significant minority of decedents will have signs of cardiomyopathy on autopsy that may then receive a molecular diagnosis after post-mortem genetic testing.<sup>413</sup> However, 30–40% of cases of SCD in the young remain unexplained despite toxicological assessment and evaluation by an expert cardiac pathologist.<sup>15,414,415</sup> Many have had an underlying heritable cardiac channelopathy such as CPVT, LQTS, or BrS.<sup>6,15</sup> Early studies of diagnostic utility of post-mortem genetic testing, the ‘molecular autopsy’, in series of sudden arrhythmic death syndrome/sudden unexplained death decedents provided a pathogenic variant yield of 24% in the major channelopathy genes [CPVT1 (RyR2); LQT1-3 (KCNQ1, KCNH2, SCN5A) and BrS1 (SCN5A)].<sup>415</sup> A population-based NGS study then proposed a 27% burden of ‘clinically relevant’ pathogenic variants by including cardiomyopathy genes and rare subsequently disputed channelopathy genes in the panel.<sup>416</sup> Most recently, a large molecular autopsy series in an extended panel

of 77 cardiac genes detected a lower yield (13%) of LP/P variants according to the more stringent ACMG criteria.<sup>417</sup> These variants were immediately useful in guiding family evaluation and they increased the diagnostic yield by 50% when undertaken in families who were also undergoing clinical testing. Furthermore, a proportion of these variants were present in cardiomyopathy genes, indicating a concealed structural cause of SCD.<sup>417,418</sup> If focus is placed on younger cases, exertional circumstances of death and the use of exome sequencing in parent and child trios, then yields can increase substantially.<sup>419,420</sup>

When individuals survive a cardiac arrest (i.e. non-fatal cardiac arrest), they may present with a range of aetiologies including genetic disorders for which genetic testing is already described in this document. Detailed clinical screening is warranted with emphasis of finding evidence for these aetiologies.<sup>5</sup> If no cause is detectable, the subject is described as UCA, or IVF. Idiopathic ventricular fibrillation is defined as a resuscitated cardiac arrest victim with a normal ECG, preferably with documentation of VF, in whom known cardiac, respiratory, metabolic, and toxicological causes have been excluded through clinical evaluation.<sup>6,15</sup> It is estimated to account for ~5–7% of all out-of-hospital cardiac arrests.<sup>422,423</sup>

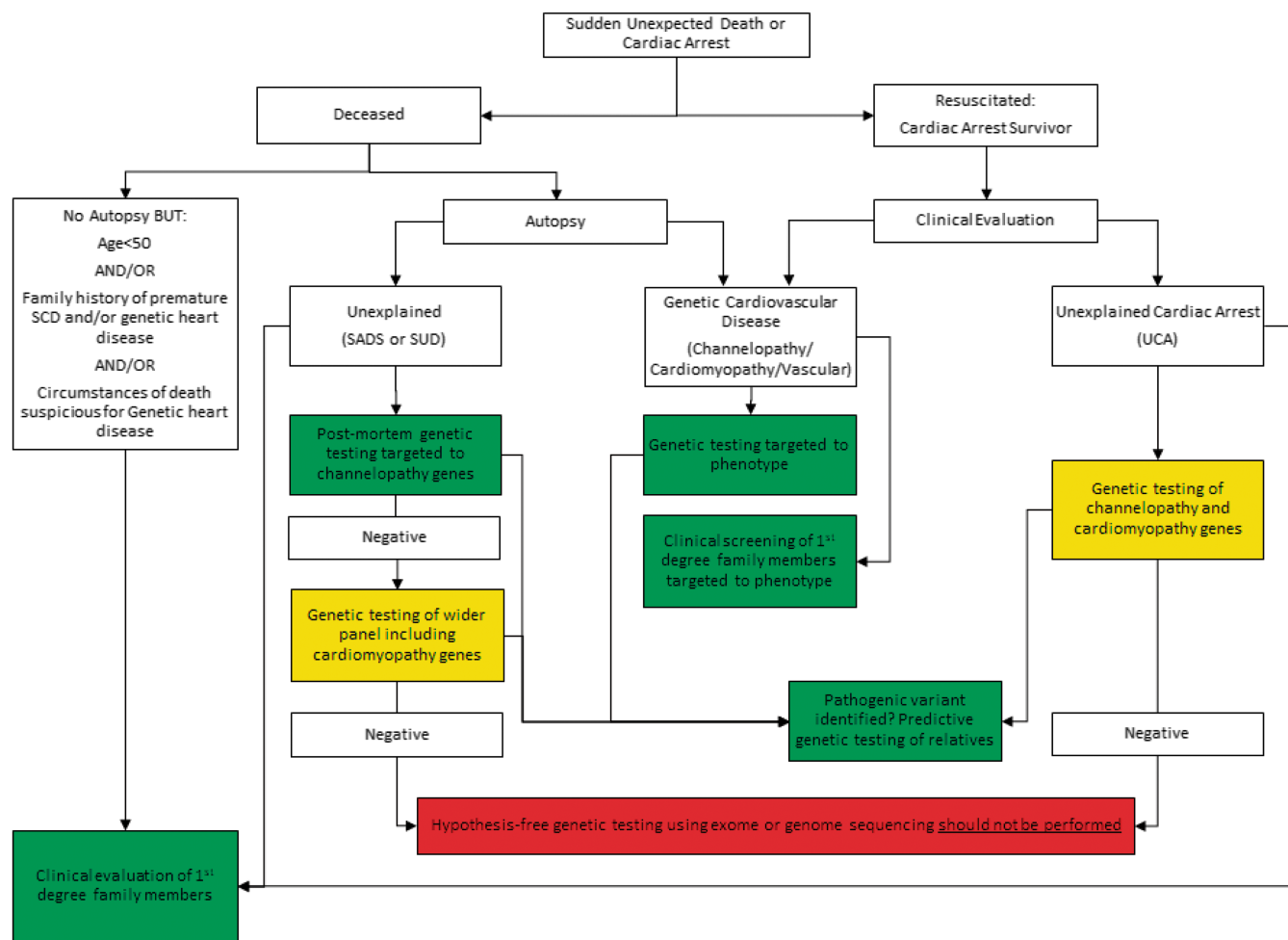


FIGURE 6 Flowchart of the work up of a sudden cardiac death or non-fatal cardiac arrest

Genetic investigation of case series of UCA survivors have employed a mixture of cardiac panels and exome sequencing, identifying a yield of channelopathy- and cardiomyopathy-associated putative pathogenic variants ranging from 3% to 27%.<sup>424–426</sup> This heterogeneity likely reflects differences in genes studied, adjudication of variant pathogenicity, patient sub-phenotypes and variability in diagnostic conclusions. Importantly, pathogenic variants in cardiomyopathy genes, especially ACM, in UCA survivors without a cardiomyopathic phenotype suggest an underlying concealed structural substrate. Phenotypes may, however, evolve over time in some cases.<sup>427</sup> The most robust genetic finding has been a Dutch founder haplotype at the *DPP6* gene associated with short-coupled-VF. No other genetic defects in or around the *DPP6* gene have been reported in other UCA populations.<sup>428</sup> In other patients with short-coupled-VF *RyR2* variants have been identified and it appears that these variants are characterized by a loss of function phenotype.<sup>161,162,429,430</sup> A term CRDS has been coined for this condition.<sup>161</sup> The role of genetic testing after a sudden unexpected death or cardiac arrest is visualized in [Figure 6](#). Recently, WES with virtual panel analysis performed systematically in 228 survivors of cardiac arrest of uncertain aetiology was shown to identify a pathogenic variant in 10% of cases.<sup>421</sup>

## 8 | STATE OF GENETIC TESTING FOR CONGENITAL HEART DISEASE

### 8.1 | Background

Genetic testing in patients with CHD ([Table 18](#)) is moving rapidly, with recent definition of patient subgroups most likely to achieve a genetic diagnosis, beyond well-known causes such as Down syndrome and velocardiofacial syndrome ([Figure 7](#)).

A genetic diagnosis usually has little impact on treatment of the CHD itself but may assist in risk stratification<sup>449</sup> and influence priorities during follow-up, such as surveillance for AV block in patients with pathogenic variants in *NKX2.5*<sup>450</sup> or *TBX5*, or screening for extracardiac features, such as immune dysfunction in 22q11 deletion syndrome or platelet dysfunction in Noonan syndrome.<sup>451</sup>

Some forms of inherited cardiovascular disease, such as the arrhythmia syndromes, involve a relatively small number of genes, with tight genotype-phenotype relationships, supportive functional data and well-established prognostic implications. In contrast, CHD has a large number of genes that are implicated in the development of CHD, with at least 130 genes identified as having a role in causation of human CHD, presenting either in isolation or in association with extra-cardiac features (see <http://chdgene.victorchang.edu.au/>). This includes genes associated with heterotaxy syndromes, which are sometimes present in patients with single ventricles and other rare CHD subtypes. Of note, no ClinGen curation yet exists for CHD genes, and for some putative CHD genes, further genomic and functional studies are required to

confirm their role in CHD. Even for established CHD genes, variant interpretation is frequently complicated by the fact that CHD often affects singletons, precluding segregation analysis, and when it aggregates in families, is associated with reduced penetrance and variable expressivity. Nevertheless, a molecular diagnosis may be relevant in pre-conception counselling and carries numerous psychological benefits as well.<sup>452</sup>

Indications for genetic testing vary according to age and mode of presentation, such as the severity of a CHD, the type of CHD, the presence of extracardiac features and the presence of non-genetic factors predisposing to CHD. The diagnosis of a monogenic cause of CHD is less likely when environmental factors occur, such as twin-to-twin transfusion, prematurity-associated patent ductus arteriosus, or maternal risk factors.<sup>453</sup> Extracardiac features, such as developmental delay, growth delay or facial dysmorphic features, are not apparent in foetuses or infants. Early genetic diagnosis can help to differentiate between syndromic and non-syndromic CHD, contributing to prognostication for cardiac and extracardiac outcome in these patients.

#### 8.1.1 | Antenatal testing

When fetal cardiac anomalies are identified on ultrasound assessments and fetal aneuploidies are excluded, chromosomal microarray (MCA) or copy number sequencing (CNV seq) on DNA derived from amniocentesis specimens or chorionic villous samples (CVS) detect pathogenic chromosomal abnormalities in about 10–15% of fetuses with CHD.<sup>465,466</sup> In those with normal CMA or CNVseq, a genetic diagnosis is made by subsequent prenatal trio whole exome sequencing (WES) in 5–12%.<sup>441</sup> The yield of prenatal CMA or WES varies according to presence of extracardiac anomalies and type of CHD.<sup>465,466</sup> Some CHD types have a low positive predictive value for being associated with chromosomal anomalies, while other CHD types have a higher likelihood of being caused by a pathogenic variant in a specific gene for syndromic or isolated CHD.<sup>465</sup> When offering prenatal genetic testing for CHD, expert advice should be sought to counsel on expected yield and on potential risks of amniocentesis or CVS, and personal goals and preferences of the parents should be prioritized.<sup>467</sup> When these conditions are met, prenatal CMA or CNVseq can be offered for fetal CHD.<sup>439–441</sup> Trio whole exome sequencing (WES) on amniocentesis or CVS can be considered in prenatal cases with syndromic and/or complex CHD where the anticipated post-natal course carries a high risk of morbidity or mortality.<sup>439–441</sup>

#### 8.1.2 | Antenatal screening

Routine antenatal testing on amniocentesis or CVS is focused on the identification of major chromosomal abnormalities including



















Recommendations	Consensus statement instruction	Ref
Genetic testing for patients with congenital heart disease (CHD) should prioritize the personal goals and preferences of the patient, parents or guardian, require pre-test genetic counseling and should be coordinated by multidisciplinary teams with expertise in genetics of CHD.		
<b>Antenatal testing</b>		
When foetal congenital heart disease (CHD) is identified on antenatal ultrasound examinations, a chromosomal microarray (CMA) or CNV sequencing (CNV seq) of foetal tissue [amniocentesis or chorionic villous sample (CVS)] should be offered.		431-433
Trio WES on amniocentesis or CVS samples may be performed in prenatal cases with syndromic and/or complex CHD.		431-433
<b>Neonates and infants requiring investigation or procedures for complex CHD</b>		
CMA or CNV seq is indicated in infants with CHD to identify pathogenic CNVs.		434, Expert opinion
Standard karyotype analysis may be performed in infants with CHD (or in parents of children with pathogenic CNVs) to identify balanced translocations.		Expert opinion
Trio WES or WGS may be performed in infants with complex CHD to identify pathogenic variants/indels, contributing to prognostication for extracardiac outcome and for cardiac outcome.		435, expert opinion
Targeted CHD gene panels to identify variants in a set of CHD-related genes may be performed in infants with complex CHD.		436, Expert opinion
<b>Patients with CHD and extracardiac anomalies</b>		
CMA or CNV seq is indicated in patients with CHD and extracardiac anomalies to identify pathogenic CNVs.		437
Trio testing for <i>de novo</i> or inherited (autosomal or X-linked recessive) pathogenic variants with either WES or WGS should be performed in patients with CHD and extracardiac anomalies.		438-440
<b>Familial forms of CHD</b>		
WES/WGS of the affected family members should be performed in families with at least two first degree relatives with heterotaxy or CHD.		441-444
Targeted analysis of a specific gene may be performed if the CHD type is highly suggestive of a specific gene.		442, Expert opinion
<b>Sporadic non-syndromic CHD (excl. neonates or infants)</b>		
CMA or CNV seq for pathogenic CNVs may be performed in older individuals with sporadic non-syndromic CHD.		434,445
Trio testing for <i>de novo</i> or inherited (autosomal or X-linked recessive) pathogenic variants with either WES or WGS in older patients with sporadic non-syndromic CHD has a low diagnostic yield and limited utility, and should not be performed routinely.		440, Expert opinion
<b>Heterotaxy</b>		
WES/WGS of the affected family members should be performed in families with at least two first degree relatives with CHD.		446, 447
Trio testing for <i>de novo</i> or inherited (autosomal or X-linked recessive) pathogenic variants with either WES or WGS should be performed in patients with syndromic heterotaxy (e.g. primary ciliary dyskinesia).		446,448
Routine trio testing for <i>de novo</i> or inherited (autosomal or X-linked recessive) pathogenic variants with either WES or WGS should not be performed in patients with sporadic non-syndromic heterotaxy.		446; expert opinion

TABLE 18 Categories of CHD

Category	Definition	Primary type/s of causative genetic variants	Diagnostic yield <sup>a</sup>		
			CMA	WES	WGS <sup>b</sup>
Syndromic (CHD+ECA)	CHD seen in conjunction with extracardiac anomalies including (but not limited to) neurological, cranio-facial, limb, growth, skeletal, and genitourinary differences	<i>de novo</i> <sup>c</sup> or inherited CNVs and SNVs	~3–25%	~25%	~41%
Non-syndromic, inherited	CHD seen without features suggestive of a genetic syndrome, often affecting multiple family members	Inherited SNVs	Unknown	~31–46%	~36%
Sporadic	CHD without a suspected hereditary component and without being associated with a known syndrome	Multiple variants contributing synergistically	~3–10%	~2–10% <sup>d</sup>	~10%

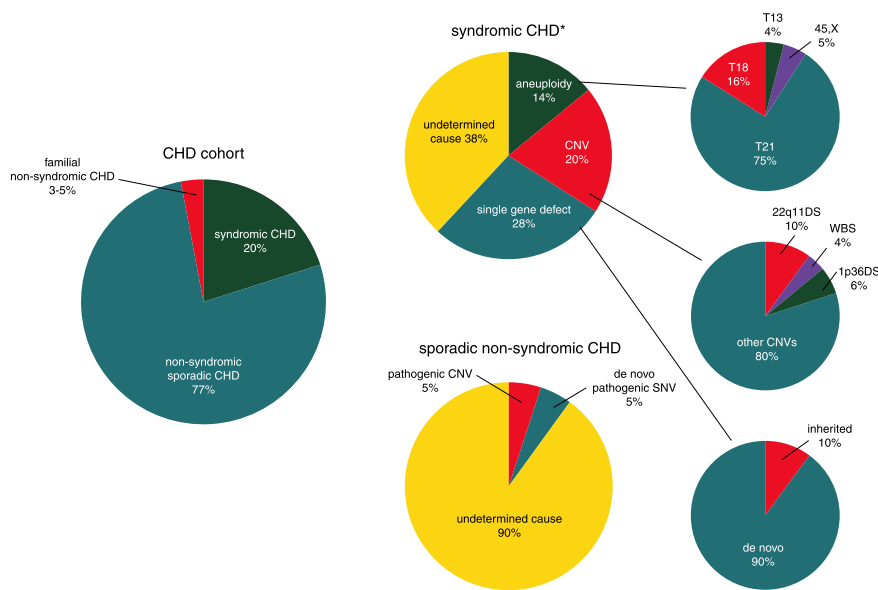
<sup>a</sup>Based on literature with clinically applicable results, i.e. studies conducting clinical evaluations of variants according to ACMG guidelines.<sup>69</sup>

<sup>b</sup>Based on our clinical experience in conjunction with Alankarage *et al.*, 2019.<sup>441</sup>

<sup>c</sup>*De novo*, not inherited from either parent.

<sup>d</sup>Based on large cohort-based studies without clinical evaluation of variants. Information presented is collated from research reported in refs<sup>439–442,444,454–457</sup> and modified from ref.<sup>458</sup>

CHD, congenital heart disease; CMA, chromosome microarray; CNV, copy number variant; ECA, extracardiac anomaly; SNV, single-nucleotide variant; WES, whole-exome sequencing; WGS, whole-genome sequencing.



**FIGURE 7** Genetic causes of congenital heart defects. Non-syndromic (lower panel) and syndromic (upper panel) cohorts. The diagram in the left panel displays the relative prevalence of the three broad CHD subgroups, namely syndromic CHD, sporadic non-syndromic CHD, and familial non-syndromic CHD. The diagrams in the central panel display the current yield of standard karyotyping, CMA and WES/WGS in the non-syndromic (lower panel) and syndromic (upper panel) cohorts, respectively, illustrating the low diagnostic yield in sporadic non-syndromic CHD, compared to the syndromic cohort. The pie diagrams at the right display the most common causes of aneuploidies and of CNVs, and the inheritance pattern of single gene defects. The percentages displayed in the diagrams are based on.<sup>440,441,445,463,464</sup> CHD, congenital heart defect; CNV, copy number variant; T13, trisomy 13; T18, Trisomy 18; T21, trisomy 21; WBS, Williams–Beuren syndrome

Trisomy 13, 18, 21 and 22q11 deletion, responsible for velocardiofacial syndrome, and should be offered to any patient pursuing invasive prenatal diagnosis without prior knowledge of cardiac or other malformations.<sup>459</sup> Cell-free DNA testing on a maternal blood

sample is emerging as a non-invasive means of aneuploidy screening for foetuses with no apparent structural abnormalities although this approach currently lacks resolution in definition of sub-microscopic chromosomal anomalies.<sup>460–462</sup>

### 8.1.3 | Neonates and infants requiring investigation or procedures for congenital heart disease

Testing for pathogenic chromosomal CNVs by CMA or CNV seq should be performed.<sup>434</sup> These techniques have essentially replaced standard karyotype analysis as first line testing, although conventional karyotyping may be performed particularly in assessment of balanced translocations. Testing for SNVs or small insertion/deletions can be considered, although yield in sporadic cases is low.<sup>435</sup> For these variants, WES or WGS are replacing 'CHD' 'panels' (usually comprising 10–40 genes<sup>436</sup>) considering the low diagnostic yield of CHD gene panels and the ability to re-interrogate WES/WGS results taking into consideration future findings.

### 8.1.4 | Patients with congenital heart disease and extracardiac anomalies

Patients with CHD and extracardiac anomalies, including additional major congenital anomalies (with functional consequences and/or requiring treatment), dysmorphism (association of at least three dysmorphic features), abnormal growth, and neurodevelopmental abnormalities, are regarded as syndromal forms of CHD, and collectively account for around 20% of the total CHD cohort. Patients with syndromic CHD should undergo CMA or CNV seq,<sup>437</sup> followed by trio testing for *de novo* or inherited (primarily autosomal or X-linked recessive) pathogenic SNVs with either WES or WGS if CMA is not diagnostic, because of the substantial rate of achieving a genetic diagnosis in ~25–40%.<sup>438</sup> Given its potential to detect both CNVs and SNVs, WGS has shown promise in becoming a first tier analysis in syndromic CHD. *De novo* variants account for ~90% of these genetic causes.<sup>439,440</sup>

### 8.1.5 | Familial forms of congenital heart disease

In patients with *familial forms* of CHD (one or more affected first degree relative), inherited single-gene defects may be identified by WES. The diagnostic yield with two affected family members is conventionally thought to be around 10% with a substantially higher yield when three or more are affected.<sup>441</sup> Families with at least two first degree relatives with CHD may benefit from WES/WGS of the affected family members.<sup>441–444</sup> In some families, the CHD type is highly suggestive of a specific gene (e.g. *ELN* pathogenic variants in supravalvar aortic stenosis without Williams–Beuren syndrome) (see [Table 19](#)). In such families targeted analysis of this specific gene can be considered, followed by WES if this initial investigation is negative.<sup>442</sup>

### 8.1.6 | Sporadic non-syndromic congenital heart disease

Congenital heart disease with no syndromal or familial pattern should be considered as being of 'undetermined cause' because only

**TABLE 19** Non-exhaustive list of high confident genes for non-syndromic human CHD

Familial non-syndromic CHD	Gene	Inheritance
Atrial septal defect	<i>GATA4</i>	AD
Atrial septal defect (with or without atrioventricular conduction block)	<i>NKX2.5</i>	AD
	<i>TBX5</i>	AD*
Atrioventricular septal defect	<i>CRELD1</i>	AD
	<i>NR2F2</i>	AD
Supravalvar aortic stenosis	<i>ELN</i>	AD
Aortic valve stenosis	<i>NOTCH1</i>	AD
	<i>TAB2</i>	AD**
Tetralogy of Fallot	<i>NOTCH1</i>	AD
	<i>FLT4</i>	AD
Patent ductus arteriosus	<i>TFAP2B</i>	AD***
Heterotaxy	<i>ACVR2B</i>	AD
	<i>CFC1</i>	AD
	<i>NODAL</i>	AD
	<i>CCDC11</i>	AR
	<i>CFAP53</i>	AR
	<i>PKD1L1</i>	AR
	<i>ZIC3</i>	XL

Pathogenic variants in *TBX5*, *TAB2*, and *TFAP2B* can cause non-syndromic CHD, or may be associated with \*hand anomalies, \*\*connective tissue disorder, or \*\*\*facial dysmorphism.

a small proportion of these patients will have single-gene variants that may be identified with WGS or WES (<5%).<sup>434,440,445</sup> Routine testing of such patients remains in the realm of research and is not currently justified in clinical practice. Some apparently non-syndromic infants will later present with syndromic associations including developmental delay, and would be considered for genetic testing, highlighting the difficulty in defining access to testing on the basis of categorization early in life.

### 8.1.7 | Heterotaxy

Diagnostic genetic testing strategy for patients with heterotaxy, defined as left-right patterning anomalies of the thoracic and/or abdominal organs, is in line with that proposed for CHD: WES or WGS should be offered to familial heterotaxy and to syndromic patients (e.g. primary ciliary dyskinesia), but is a lower priority for heterotaxy patients with no syndromic appearance or familial occurrence.<sup>446–448</sup>

We summarize recommendations for genetic testing in the different categories in the table of recommendations. These recommendations should be applied (1) in consideration of technology availability, access and health insurance issues and sociocultural differences, (2) in the light of shared decision making between a trained healthcare professional and the patient, parents or guardian, and (3) only if adequate pre- and post-test counseling can be guaranteed.

Genetic testing in the pediatric domain should be coordinated between cardiology and clinical genetics specialists, with support by genetic counselors and ideally a multidisciplinary clinic for return of results and liaison with genetic pathologists and developmental biologists. Thus, identification of congenital heart disease should prompt a referral to a center specializing in pediatric cardiovascular genetics.

## 9 | STATE OF GENETIC TESTING FOR CORONARY ARTERY DISEASE AND HEART FAILURE

Some inherited conditions may lead to coronary artery disease. For example, monogenic predisposition to familial hypercholesterolaemia is a powerful predictor of premature coronary artery disease.<sup>468</sup> The major genes are *APOB*, *LDLR*, *PCSK9*. Over the past two decades, a widespread contribution of polygenic risk to coronary artery disease susceptibility has been demonstrated.<sup>469,470</sup> Novel genetic susceptibility mechanisms including clonal haematopoiesis of indeterminate potential, a somatic rather than germline genetic process, have also been shown to play a role in coronary artery disease susceptibility recently.<sup>471</sup> Genetic evaluation in clinical practice is currently directed at identifying individuals with an inherited predisposition to coronary artery disease that may enable a mechanistic understanding of the disease, and inform carrier testing. Although research indicates that genetic predisposition may be useful for risk prediction both in primary and secondary prevention settings,<sup>4,63,64,472</sup> the predictive utility of polygenic risk scores for coronary artery disease are debated<sup>473,474</sup> and such scores are not routinely used in clinical practice. Data have also emerged to indicate that risk reduction after treatment with statins<sup>475</sup> or proprotein convertase subtilisin/kexin type 9 (*PCSK9*) inhibitors<sup>63,64</sup> may be greatest for individuals with the highest inherited burden of polygenic predisposition to coronary artery disease. Despite rapid innovations in the understanding of both inherited and somatic genetic variation that may underlie coronary artery disease, and despite increasing development of comprehensive polygenic risk assays for coronary artery disease and component clinical risk factors, clinical genetic testing is largely focused on addressing low-density lipoprotein, an underlying treatable clinical risk factor for coronary artery disease.

Genetic testing for heart failure is in some sense a superset of the earlier sections on genetic testing for cardiomyopathy. In patients with ischaemic cardiomyopathy, testing should be considered according to the recommendations in the paragraph above for coronary artery disease; there is currently no further indication for testing with respect to the presentation of heart failure as a result of coronary artery disease. In cases where patients present with heart failure with preserved or reduced ejection fraction with an apparent explanatory cause such as uncontrolled hypertension or valve disease there is also currently no indication for genetic testing. Heart failure that is unexplained should always lead to a detailed

family history and if a Mendelian pattern of inheritance is suggested, then panel testing for cardiomyopathy should proceed as described earlier in this document. In (young) cases of heart failure with no apparent cause and no family history, or in cases where alcohol or pregnancy appear to be co-factors, many would consider Mendelian panel testing, particularly because of the demonstrated contribution from modifying effects of titin loss-of-function variants. While genome wide association studies for heart failure and for LV remodelling are now published, and while this polygenic tail would be expected to modify Mendelian causes of heart failure, such tools have yet to be translated into predictive scores that would provide utility in a clinical setting.

## 10 | CONCLUSION AND FUTURE DIRECTIONS

In the past decade, we have seen significant progress in genetic testing of the inherited cardiovascular diseases. Understanding of the genetic basis of disease has improved both in terms of new disease genes, as well as new genetic mechanisms such as oligogenic disease and the emergence of polygenic risk scores. At the present time, cardiovascular genetic testing already offers numerous benefits in terms of more diagnostic precision, influencing therapeutic options, and informing prognosis. Indeed 'genetic cardiology' is recognized as a new field, with such sub-specialty experts needed to facilitate the translation of genetic findings into improved clinical care. While great progress has been made, new challenges and gaps in our knowledge remain, including the accurate classification and interpretation of variants, robust curation of potentially new disease genes, and understanding variable phenotype penetrance both within and between families. Furthermore, understanding the genetic landscape of cardiovascular diseases in other ethnic populations with different genetic backgrounds will be important to ensure the benefits of genetic testing are realized on a truly global scale.

Looking to the future, with the advances being made in the field of gene therapy, the identification of the patient's fundamental disease-causative substrate may enable not only genotype-guided therapies but also gene-specific, even pathogenic variant-specific therapies.<sup>23,476</sup> For AR disorders like TKOS, a molecular diagnosis could permit 'gene replacement' therapies. However, most genetic heart conditions are AD conditions resulting in either haploinsufficiency or a dominant negative state. For some, allele-specific oligonucleotide/short interfering RNA (siRNA) therapies to knock down the mutant allele may be sufficient.<sup>477,478</sup> This gene therapy strategy requires a novel therapeutic for each pathogenic variant however. Similarly, gene-editing with CRISPR/Cas9-based strategies requires a unique effort for each pathogenic variant.<sup>479</sup> For those genetic heart diseases with hundreds of unique disease-causative variants within each disease-susceptibility gene, a gene-editing

solution may not be feasible. Most recently, proof-of-principle for a gene-specific gene therapy solution has been provided. This therapy, called Suppression-Replacement (SupRep) gene therapy envisions the AAV9 delivery (or some future iteration) of the therapeutic cargo containing a single, gene-specific siRNA to knockdown both the mutant allele and the wild type allele, followed by a bio-engineered complementary DNA (cDNA) of the gene of interest that is immune to siRNA-mediated knockdown.<sup>480</sup> Regardless of the underlying gene therapy strategy being explored, numerous obstacles will need to be overcome before these promising *in vitro* data will be translated into available therapies in humans.

## CONFLICT OF INTEREST

None declared.

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