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SPECIAL ARTICLE
Heart failure and cardiomyopathies

Integration of genetic testing into diagnostic pathways for cardiomyopathies: a clinical consensus statement by the ESC Council on Cardiovascular Genomics

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Abstract

In the modern era, cardiologists managing patients and families with cardiomyopathies need to be familiar with every stage of the diagnostic pathway from clinical phenotyping to the prescription and interpretation of genetic tests. This clinical consensus statement from the ESC Council for Cardiovascular Genomics aims to promote the integration of genetic testing into routine cardiac care of patients with cardiomyopathies, as recommended in the 2023 ESC guidelines for cardiomyopathies. The document describes the types of genetic tests currently available and provides advice on their prescription and for counselling after the return of genetic findings, including the approach in patients and families with variants of unknown significance.

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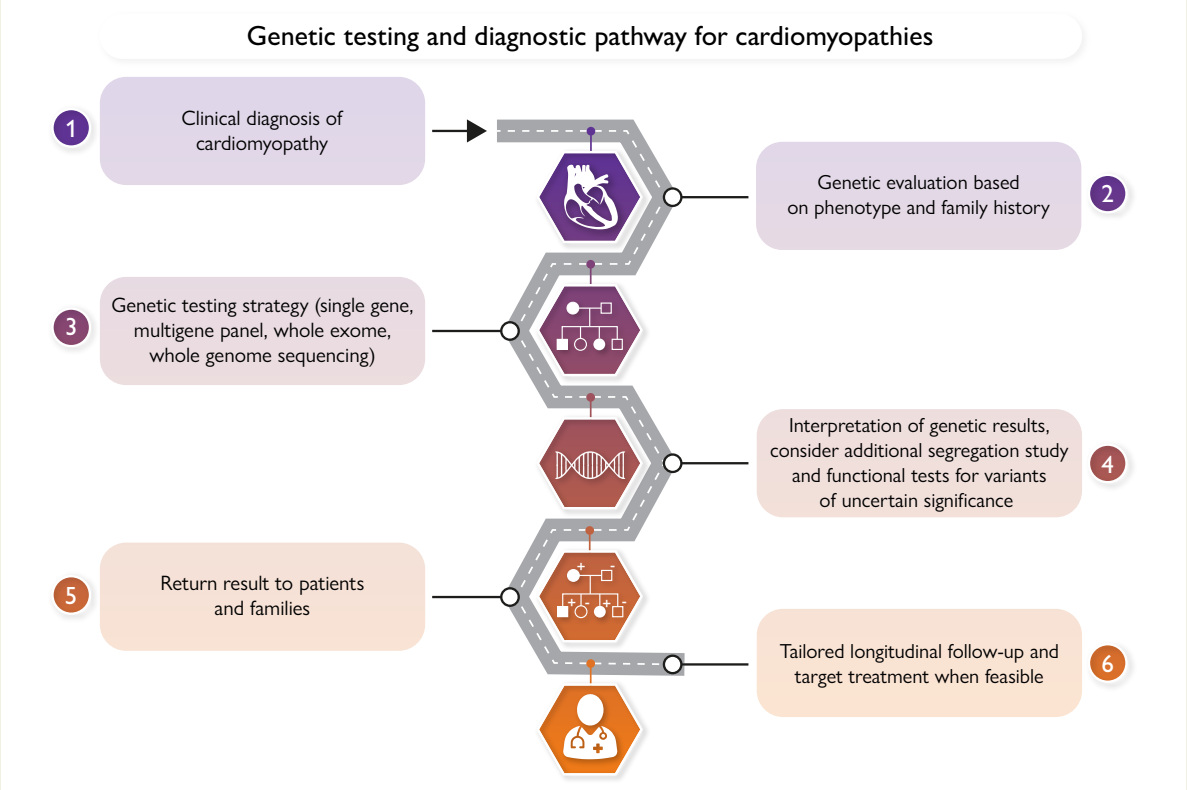
Graphical Abstract



The role of the cardiologist in genetic testing for cardiomyopathies. The figure summarizes the key roles of clinical cardiologists in the genetic work-up of cardiomyopathies.

Keywords Cardiomyopathies • genetic testing

Introduction

In a recent position statement, the ESC Council on Cardiovascular Genomics outlined the critical importance of clinical and family data in the interpretation of genetic variants using cardiomyopathies as a reference.[1] In this second statement, we highlight the emerging and vital role of cardiovascular specialists in the coordination of the entire genetic testing pathway, again using cardiomyopathies as a model. Our view is that cardiologists requesting and returning genetic test results to patients and families should have a working background knowledge of the required tests, including their diagnostic yield, the limitations of analytical tools and bioinformatics pipelines, and the technical reasons for possible discrepancies between expected and observed results. These skills are vital to prevent inappropriate use of genetic testing and misinterpretation of genetic variants.

The role of the cardiologist in genetic counselling

Cardiologists involved in the care of patients and families with cardiomyopathies should, as a matter of routine, systematically collect data on family history and record detailed clinical phenotypes. This information is necessary to support pre-test hypotheses and the indications for genetic testing as well as the selection of testing strategies. The possibility of a genetic origin of cardiomyopathy and the reasons for offering a genetic test should be communicated to patients together with the implications for their relatives should the test be positive. Current best practice is that individuals should provide informed and documented consent for a genetic test. After completion of genetic testing, cardiologists working alone or in partnership with other appropriately trained care providers (e.g. geneticists, genetic counsellors) are required to return the results and to explain their clinical actionability and consequences.[1]

The role of the cardiologist in genetic testing

The preferred approach to genetic counselling and testing for cardiomyopathies is described in the 2023 ESC guidelines for the management of cardiomyopathies.[2] While many cardiomyopathies are genetic diseases, their diagnosis still rests on morphological and functional criteria that are used to define different clinical subtypes.[3,4] A diagnosis of familial or genetic disease can often be inferred by the presence of affected relatives and/or phenotypical traits consistent with a genetic origin.[5] Genetic testing follows the clinical evaluation,[1,6] and any identified genetic variants are then classified according to

internationally accepted criteria as pathogenic (P) or likely pathogenic (LP), variants of uncertain significance (VUS), and likely benign (LB) or benign (B) variants,[7] taking into account continuous gene-specific refinements in designation.[8]

Cardiovascular specialists play a role at every stage of the genetic pathway: specifically, phenotyping of patients and relatives;[5] decisions on whether genetic testing should be initiated;[6] prescription of the appropriate genetic test;[9] interpretation of genetic variants;[1] return of results to patients; and the tailoring of family screening and management (Graphical Abstract). Increasingly, at least some cardiologists will also have to participate in decisions about functional analysis of rare VUS using, for example, RNA and protein studies, particularly when there is clear evidence of familial disease. Cardiologists should also be prepared to manage secondary genetic findings, the analysis and reporting of which are recommended by the American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP).[10–13] Finally, in syndromic diseases causing cardiomyopathy, clinicians should be aware of other non-cardiac manifestations so that appropriate multidisciplinary assessment can be arranged. The complexity of this whole process makes it highly desirable that genetic evaluation is co-ordinated by specialists and teams with the necessary experience in genetic disease. The essentials of effective genetic testing are summarized in Supplementary data online, *Figure S1*.

Genetic testing: methods

A 'diagnostic' genetic test for cardiomyopathies analyses all validated disease genes and aims to detect disease-related genetic causes. A negative result occurs when there is truly no monogenetic explanation for the disease but can also be explained by limitations of the test type or design (*Tables 1* and *2*).[14–17]

Single-gene testing is performed using either conventional sequencing covering exons and exon–intron boundaries, or whole single-gene (exons and whole introns) sequencing. The latter may be appropriate when tested genes have a high prevalence of pathogenic deep intronic variants (e.g. *MYBPC3*)[18,19] in hypertrophic cardiomyopathy (HCM).

Single-gene testing is commonly used for confirmation of genetic variants detected using next-generation sequencing, but, even in the modern era, it still has some value as a fast and low-cost test in the evaluation of cardiomyopathies where the pre-test probability of a unique genetic diagnosis is strongly supported by distinct clinical, imaging, and pathology features.[20] Examples include rare syndromes such as Danon disease[21] as well as more common diseases like Transthyretin amyloidosis.[22] In other scenarios, single-gene testing may be confirmatory following the detection of biochemical or tissue disease signatures. Specific examples include Fabry disease, haemochromatosis, and familial amyloidosis.[23,24] Definite or suspected X-linked inheritance in males with dilated cardiomyopathy (DCM) phenotypes associated with evidence for skeletal muscle involvement (including isolated increases in creatine kinase) should prompt consideration of genetic testing of genes like dystrophin (*DMD*); up to 80% of patients with dystrophinopathies carry single or multi-exon deletions (70%) or duplications (10%) that are routinely detected using first-line multiplex ligation-dependent probe amplification.[25–27]

Clinically similar but genetically heterogeneous cardiomyopathies are easily tested with phenotype-directed *multigene panels* and are the current first-line genetic testing for cardiomyopathies. Although the design of multigene panels may vary from a few tens to hundreds of genes,[28] it is important to highlight that increasing the number of genes tested in a panel has only a small effect on the yield of testing[29] at the expense of generating a greater number of VUS.[30,31,32] Commercial or customized clinical gene panels should always include validated genes associated with cardiomyopathies and should be expanded when new disease genes are discovered and confirmed.[33] Although evidence of familial disease is not an absolute criterion for genetic testing, the diagnostic yield is higher in familial disease than in singleton cases. Different scores have been proposed to identify patients with higher probabilities of a positive genetic testing result.[34–37] However, as yet, these do not substantially influence the decision to perform a genetic test in most settings.

Whatever the design of a diagnostic multigene panel for cardiomyopathies, it must include HCM, DCM, and arrhythmogenic right ventricular cardiomyopathy genes classified as definitive, strong, and moderate by ClinGen (the NIH-funded resource dedicated to building a central resource that defines the clinical relevance of genes and variants for use in precision medicine and research, https://clinicalgenome.org/).[28] This minimum criterion assures diagnostic utility and enables uniform implementation of multicentre registries, surveys, and gene-based clinical trials. Restrictive cardiomyopathy (RCM) remains problematic as RCM genes are not yet curated in ClinGen and because so-called restrictive ventricular physiology—the key diagnostic marker—is common in different morphological subtypes.

Whole-exome sequencing (WES) analyses the coding and adjacent intronic regions across the genome (1%–2% of the genome) that contain most defects associated with Mendelian diseases (>85%). The attraction of this approach is that all genes known to be disease-related can be tested. It also provides potentially useful information on novel candidate genes and thus represents an investment for future automated re-analysis that takes into account new information on VUS and genes of uncertain significance.[38] Nevertheless, WES is not the current first-line test for cardiomyopathies (*Table 1*) as most data suggest that the diagnostic yield of WES in adult patients with cardiomyopathy is similar to multigene panels.[39] This situation may be different in paediatric patients, where WES appears to have a higher diagnostic yield compared with multigene panels.[40] Other limitations of whole-exome testing include the need to manage secondary findings,[10,11] the identification of many more VUS of dubious clinical relevance,[12] and the lack of detection of copy number variations (CNV) in routine interpretation.

Whole-genome sequencing (WGS) provides the sequence of all coding, non-coding, and intergenic regions of the genome,[41] offering coverage uniformity and the possibility of detecting deep intronic variants and CNV or other structural variants. As with WES, WGS enables future re-analysis to incorporate advances in knowledge.[42] In a recent WGS study including 209 children with cardiomyopathy, 39% of cases harboured pathogenic coding variants in known cardiomyopathy genes, and 5% had high-risk loss-of-function variants in additional candidate cardiomyopathy genes.[43] In familial DCM, a first-line WGS test has demonstrated high variant detection, accuracy, and capacity to identify structural variants, but the incremental yield of clinically actionable variants was limited by the paucity of functional evidence for DCM association.[44] As with WES, the drawbacks of WGS include secondary findings as well as VUS with a low probability of clinical actionability and the need for functional tests proving the effects of variants on the mutated protein.

Genotype–phenotype discordance

An increasingly common concern for the clinician is genotype–phenotype discordance[45] (i.e. an identified genetic variant is associated with a

Table 1 **Main advantages and limitations of different strategies for DNA sequencing**

	Single gene/few genes	Multi gene panels	Whole (clinical) exome	Whole genome
When and Who	• Cardiomyopathies with minimal locus heterogeneity and high pre-test probability. • Most common: single-gene coding exons sequencing (e.g. *TTR*) or copy number variations test (e.g. *DMD*) • Whole-gene sequencing (exons and introns) of genes with known pathogenic intronic variants (e.g. *MYBPC3*)	• First-line screening • Phenotypically similar Cardiomyopathies with high locus heterogeneity • Cardiomyopathies with no gene-specific pre-test hypothesis • Genocopies: possible but not otherwise tested	• Depending on prescriptors and labs: first-line screening in children • Expanding previous negative genetic test • Trios (proband and biological parents) testing. • Both diagnostics (clinical exomes) and research.	• Further expansion of previously negative tests. • (Research) epidemiology of cardiomyopathy genes in: o Large biobank cohorts including phenotyped participants o Cohorts of patients sharing common phenotypes
Advantages	• Fast (for small genes) and low cost • High diagnostic yield • Low number of variants of uncertain significance • Easy interpretation of results	• Covering all validated disease genes (actionable) • High diagnostic yield • Easy interpretation and explainable results • Low cost	• High number of genes tested • Large range of genetic diseases detectable with one test. • Possibility of discovering new candidate genes (research).	• Detection of copy number variations and deep intronic variants • Sequencing of all coding, intergenic, and intron regions. • Possibility of discovering new candidate genes (research).
Limits	• Risk of missing a second genetic disease • Missing copy number variations detection • Time-consuming depending from gene size	• Need of expanding gene panels when new disease genes are validated • Missing deep intronic variants (if not specifically targeted) • Difficult copy number variations detection.	• Higher costs compared with multigene panels • Very high number of variants of uncertain significance • Diagnostic yield similar to multigene Panels • Missing of deep intronic variants	• Very high sequencing costs • Very high number of variants of uncertain significance • Minimal improvement of diagnostic yield for cardiomyopathies
Issues	• Based on robust diagnostic hypothesis: o Pathology (e.g. GB3 accumulation in Fabry disease) o Imaging (scintigraphy in *TTR* amyloidosis) o Instrumental records	• Interpretation of novel, rare variants • Enrolment and testing strategies (familial cardiomyopathies vs. single index patients) can impact the diagnostic yield	• Unresolved results in many cases • Difficult variant interpretation • Management of secondary findings • Difficulties in Sanger confirmations • Non-actionability of variants in genes of uncertain significance	• Difficult data management and analysis • Difficult variant interpretation • Management of secondary findings • Difficulties in Sanger confirmations • Non-actionability of variants in genes of uncertain significance
	CLINICAL FIRST → GENOTYPE FIRST			

The table summarizes the main pros and cons of the different types of genetic tests. The strategy of testing depends on its scope: for clinical diagnostic applications, many centres test multigene panels that include all genes validated by ClinGen. While the use of WES and WGS is increasing, most primary CMP genes are now known and 'new' disease-related genes occur as rare conditions. Both WES and WGS, however, are powerful research tools for the discovery of rare cardiomyopathies.
GB3, globotriaosylceramide; CMP, cardiomyopathy

Table 2 Factors that may contribute to negative/inconclusive results of next-generation sequencing**-based genetic tests**

	Incomplete gene list	Type of variant			Technical sequencing issues[a]	Interpretation[b]
		Deep intronic	Large deletions or duplications	Large insertions, translocations, and inversions		
Multigene panel	X[c]	X	X	X	X	X
Clinical exome	X[c]	X	X	X	X	X
Whole exome		X	X	X	X	X
Whole genome				X[d]	X	X

Negative test results in patients with high pre-test probability can be explained by incomplete testing, acquired phenocopies, or variable coverage of specific genetic regions, typically deep intronic variants in WES, or technical sequencing issues (all tests). Misinterpretation of genetic variants may affect results of all tests.
[a]Targets with low sequencing quality or difficult to analyse.
[b]Interpretation issues include possible multigene defects effect.
[c]The multigene panel and clinical exome may not include all genes either provisionally associated with CMP or genes still unknown as disease genes.
[d]WGS detects large insertions, translocations, and inversion with limited accuracy usually identified using other methods such as array-Comparative Genomic Hybridization.

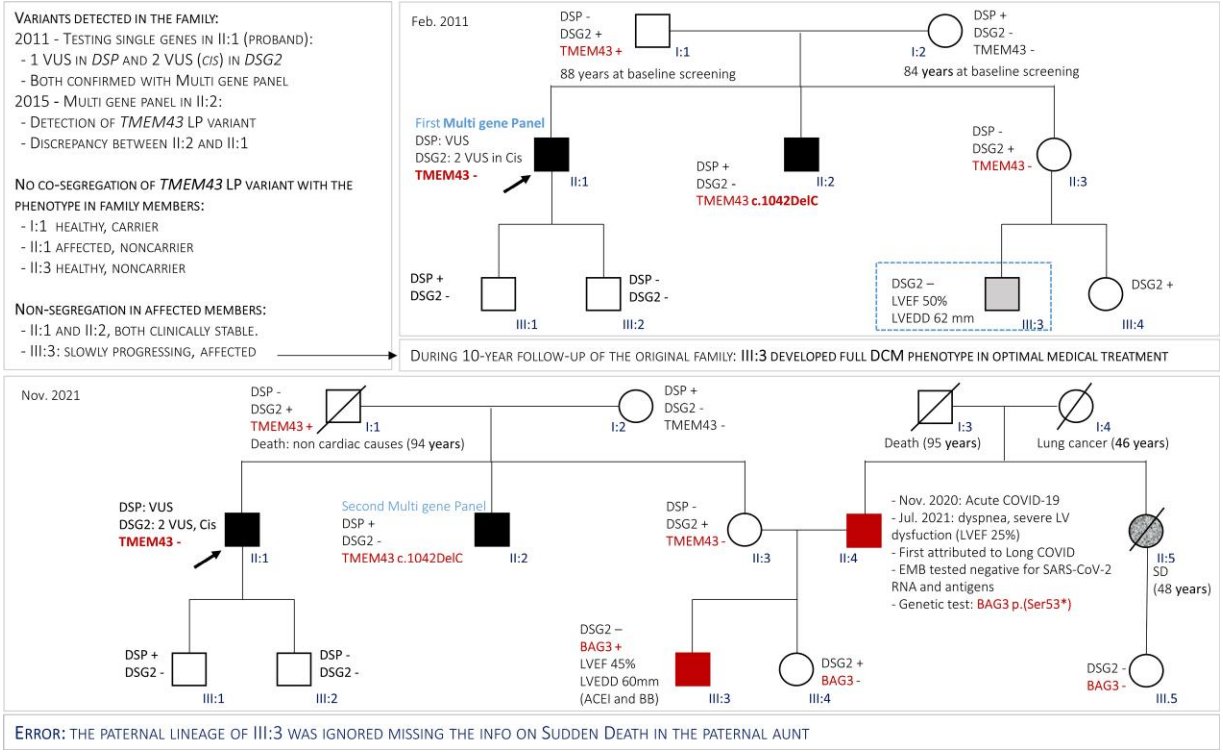


Figure 1 Example of family genotype–phenotype discordance and incomplete tests. The figure shows an example of non-segregation of a pathogenic variant in affected members of the family and the development over time of the phenotype in a young relative who does not carry the proband's pathogenic variant. Expanded family screening identifies two affected unrelated relatives and a second pathogenic variant in a different gene. The pedigree summarizes both genotype–phenotype discordance (II:1 and II:2) as well as incomplete genotyping in the same family (III:3). The genetic cause of the dilated cardiomyopathy in the young patient III:3 was identified after the acute onset of dilated cardiomyopathy in the father, who was first diagnosed with post-COVID acute heart failure, and then recognized to be affected by *BAG*-related cardiomyopathy. Complete clinical evaluation and genetic re-analysis are needed before closing the genetic diagnostic path. VUS, variant of uncertain significant; LP, likely pathogenic; Cis, a pair of variants that occurs in same copy of the gene; Yrs, years; +, positive for the variant, carrier; –, negative for the variant, non-carrier; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LV, left ventricular; EMB, endomyocardial biopsy; SD, sudden death; ACEI, ACE inhibitors; BB, beta blockers

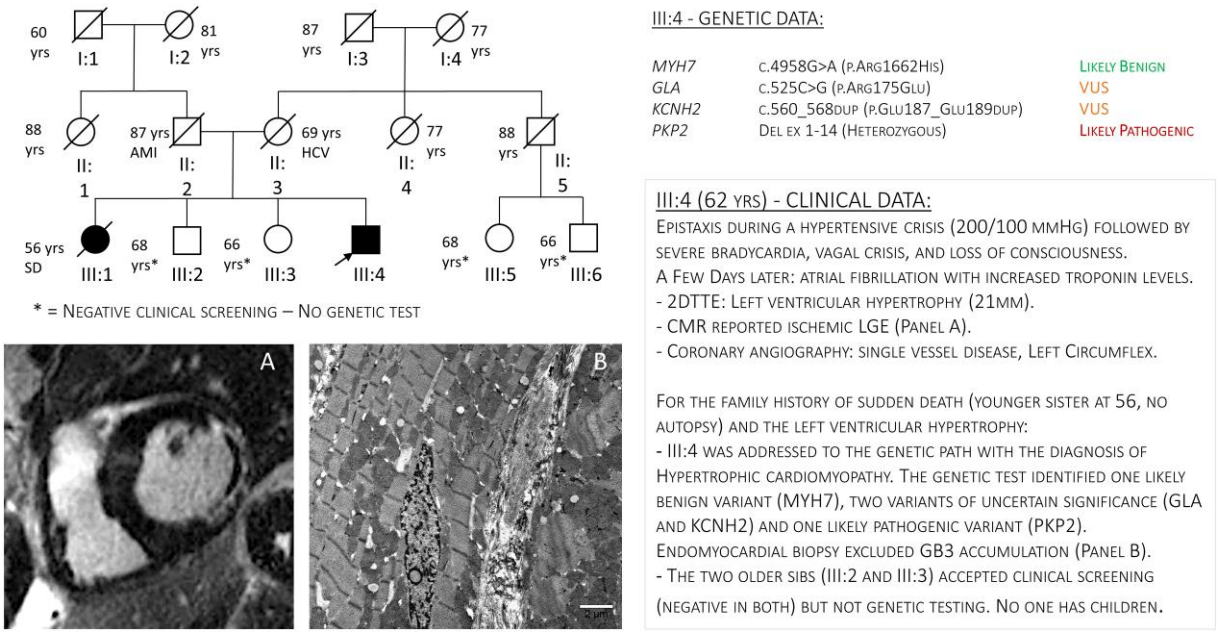


Figure 2 Example of genotype–phenotype discordance: appropriate testing? Genotype–phenotype discordance arises for different reasons: clinical and genetic screening may reveal the presence of multiple variants including variants of unknown significance and likely pathogenic–pathogenic variants; comorbidities can also confound interpretation of phenotypes. In the family shown in this figure, the proband underwent genetic testing following the clinical diagnosis of hypertrophic cardiomyopathy. The test provided inconclusive results for the phenotype because the identified likely pathogenic variant in *PKP2* gene is not associated with hypertrophic cardiomyopathy. The segregation study in the family was limited to clinical screening. Genetic testing was of limited utility. For instance, none of the four siblings have children. Yrs, years; AMI, acute myocardial infarction; HCV, hepatitis C virus; SD, sudden death; VUS, variant of uncertain significant; 2DTTE, two-dimensional transthoracic echocardiography; CMR, cardiac magnetic resonance; LGE, late gadolinium enhancement; GB3, globotriaosylceramide

disease phenotype different from that of the patient),[46] or the discovery of genetic variants that are predicted to be pathogenic on the basis of *in silico* and population data, but which do not segregate with the phenotype in families. Some examples of discordant and non-clinically actionable results are shown in *Figures 1* and *2*. These examples contrast the resolution of clinical discrepancies with family screening and prospective monitoring of relatives (*Figure 1*) with unresolved scenarios (*Figure 2*). Our key message is that clinical decision-making begins and ends with the clinical phenotype (in probands and relatives). Detection of a pathogenic gene variant confirms the clinical diagnosis and provides additional potentially actionable prognostic information that can have significant therapeutic consequences. Genetic testing can be harmful when discrepancies between phenotype and genotype are ignored or overlooked.

Interpretation of variant pathogenicity and role of bioinformatics

The ACMG/AMP classification provides a standardized approach for the interpretation of gene variants and has been refined by various initiatives (e.g. the US ClinGen Sequence Variant Interpretation Working Group).[47] However, contemporary bioinformatics tools that implement ACMG criteria for variant interpretation in the absence of clinical data rely on *a priori* criteria derived from population data, clinical genetic databases, and *in silico* prediction tools. Validated functional tests and family segregation data are often absent. When variant pathogenicity is assigned in this way, individual bioinformatics tools for variant interpretation can return different results for the same variant depending on the algorithms used by each software package (Supplementary data online, *Table S1*).

Interpretation of VUS and use of functional testing

Variants of unknown significance are rare (or unique) variants that do not fulfil *a priori* interpretation criteria for either B/LB or P/LP class or have conflicting ACMG classifications. The Association for Clinical Genomic Science Best Practice Guidelines for Variant Classification in Rare Disease 2020 currently rank VUS into six subclasses based on an estimated probability of their pathogenicity (*Figure 3*).[48] In this context, testing of a 'hot' VUS in a family may be justified when co-segregation of an appropriate phenotype adds the key contributor to the interpretation of pathogenicity. For most cardiomyopathies, the clinical risk of overestimating the significance of a VUS is less than that of underestimating a LP/P variant; this is in contrast, for example, to the consequences of overestimating a VUS in genes such as BRCA1/2 where the attribution of pathogenicity can lead to prophylactic breast and ovarian surgery.

Our key message is that, whenever possible, interpretation of VUS should go beyond computational and predetermined statistical

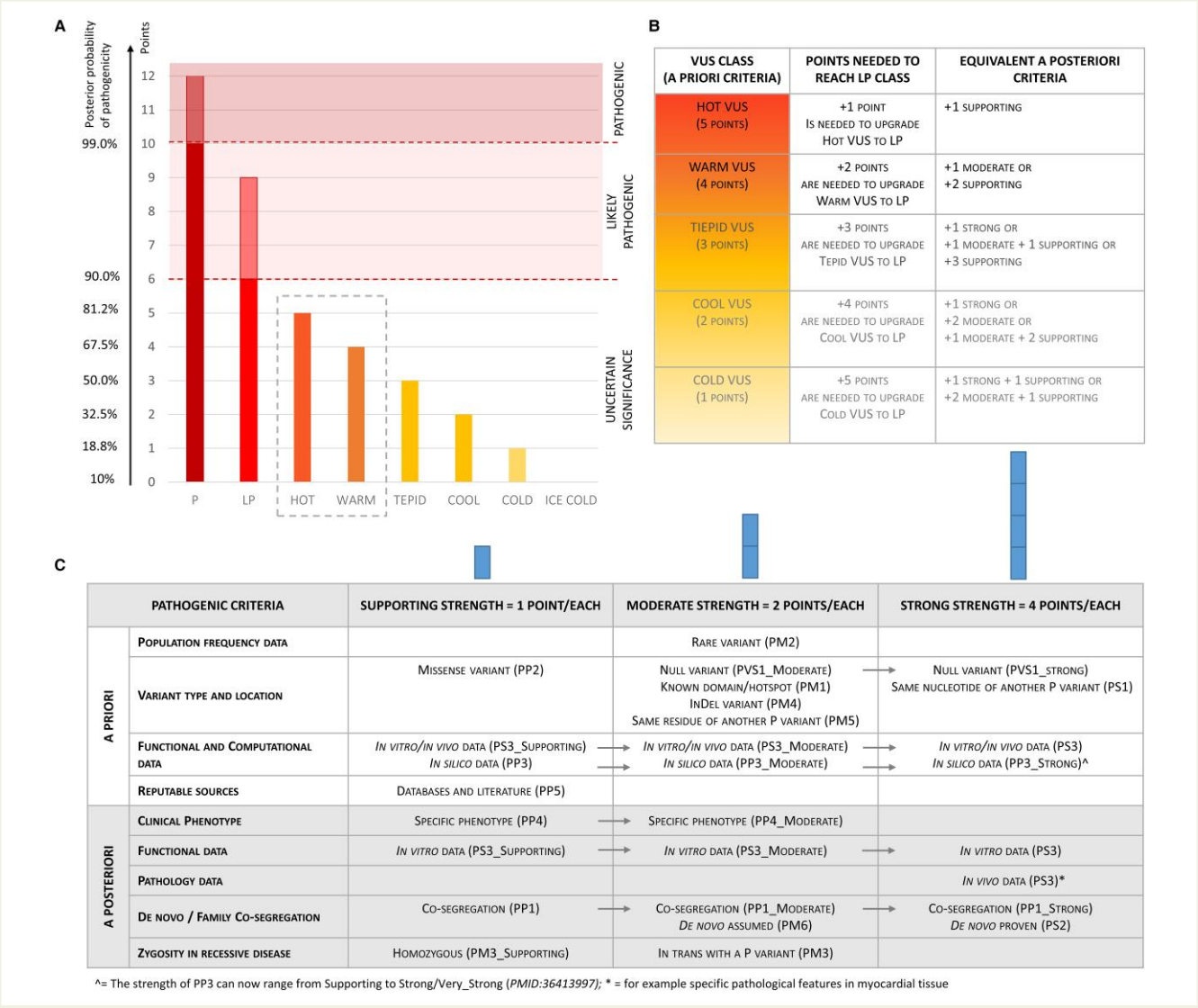


Figure 3 Variants of unknown significance subclasses. (*A*) The graph shows the pathogenicity classes (from variants of unknown significance to pathogenic) according to the scoring system and pathogenicity probabilities calculated using the Bayesian model of the American College of Medical Genetics guidelines. The threshold of six points, corresponding to the 90% probability of pathogenicity, is the minimum score to classify a variant as likely pathogenic. The subclasses of variants of unknown significance are represented according to the Association for Clinical Genomic Science model where the 'temperature' is proportional to the pathogenicity score. The dotted rectangle shows the hot variants of unknown significance and the warm variants of unknown significance which should always be taken into consideration for further impact assessment studies. (*B*) The table shows the subclasses of variants of unknown significance according to the Association for Clinical Genomic Science model, the points needed to reach the threshold of the likely pathogenic class, and the combinations of equivalent criteria. (*C*) After becoming familiar with the system, the cardiologist can verify the assignment of scores that define hot and warm variants of unknown significance, and consider carriers deserving of clinical attention in a similar way as carriers of likely pathogenic variants. The PP5 criterion can be critically revised by cardiologists who are now aware that many genetic variants classified as pathogenic in the past early descriptions are now being reclassified. VUS, variant of uncertain significance; LP, likely pathogenic; P, pathogenic

approaches by employing genotype–phenotype correlation studies, segregation in families, and, when feasible, pathological and functional studies.[1]

Functional assessment test *in vivo*

In circumstances where the interpretation of VUS has consequences for the management of patients and families, a functional assessment may be necessary. In other disciplines, most notably neurology, this often involves analyses of tissue samples, but this is less frequently considered in cardiology practice. In the future, it is possible that myocardial tissue analysis, including endomyocardial biopsies or opportunistically gathered tissue samples, will serve a similar purpose.

RNA-based tests

In addition to the enormous knowledge that transcriptomic tests provide in the understanding of the pathology of genetic defects associated

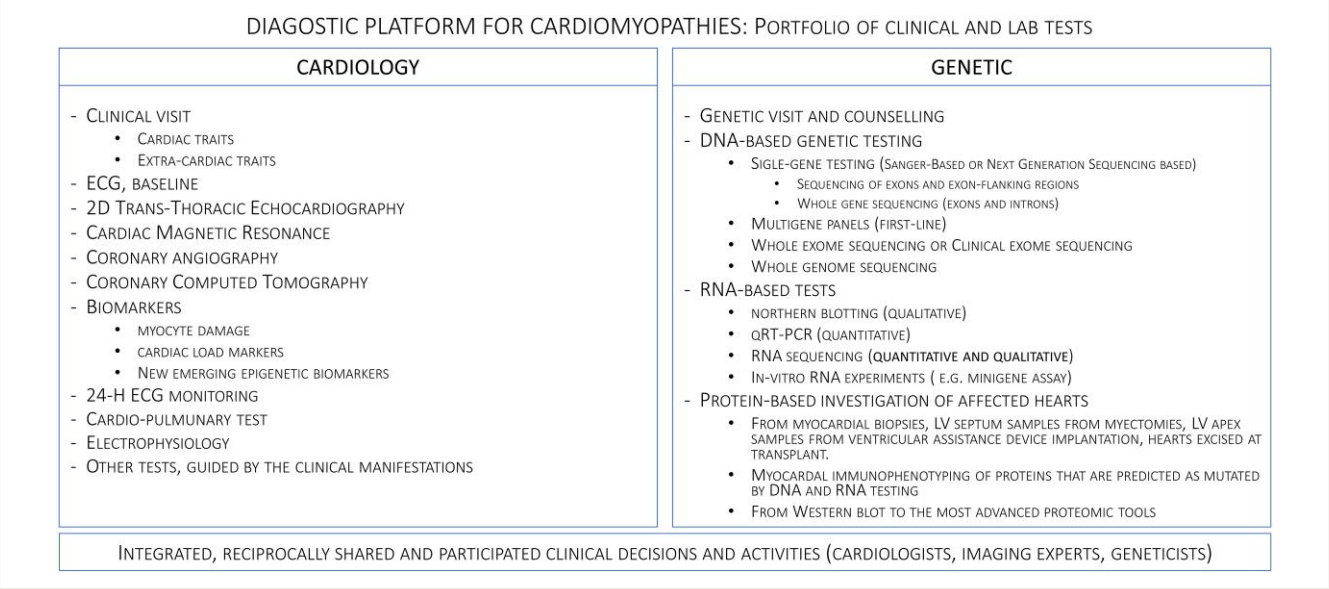


Figure 4 Diagnostic platform for cardiomyopathies. The figure summarizes the portfolio of tests and studies that can aid genetic interpretation. ECG, electrocardiogram; qRT-PCR, quantitative real-time polymerase chain reaction

with cardiomyopathies (beyond the scope of this document),[49] RNA sequencing or quantitative RT-PCR targeting single genes can aid the interpretation of variant pathogenicity. For example, RNA sequencing can demonstrate the damaging effect of classes of genetic defects such as synonymous variants introducing or abolishing splice sites, variants affecting regulatory regions (3′ UTR and 5′ UTR variants), and variants in non-canonical splice sites near exon–intron junctions or in deep intronic regions.[50–53] According to the official nomenclature of genetic variants, canonical splice sites at the intron–exon junction have GT at the donor site and AG at the acceptor site (±1/2 intronic nucleotide positions). Non-canonical splice sites include all splice variants that affect non-GT donor and non-AC acceptor sites.[54] Cardiologists should be aware that genetic reports describing non-canonical splice defects predicted by DNA-based testing require further confirmation.

RNA-based tests are essential to genetic diagnosis when DNA-based tests do not provide conclusive results, the target gene is consistent with the phenotype, and the observed VUS deserves further investigation for correct interpretation. Although many computational tools predict splice-modifying variants, the *in silico* results cannot be considered conclusive, and variant effects should ideally be tested with RNA sequencing and confirmed with studies examining the expression of the mutated protein. When *in silico* tools predict a splicing alteration effect of a VUS and the phenotype in probands and relatives is consistent with the presence of defects in the given gene, RNA studies can validate the pathogenicity, especially in the absence of pathogenic variants in other disease-related genes.

Simple examples are represented by rare synonymous variants in the *LMNA* gene, introducing either novel cryptic splice sites (e.g. c.768G>A, p.Val256Val), or abolishing canonical splice sites (e.g. c.513G>A, p.Lys171Lys)[52,53] (Supplementary data online, *Figure S2*) or by intronic variants computationally predicted as VUS but actually affecting splicing, such as the c.2905+5G>T in the *MYBPC3* gene that causes the loss of a donor site with a damaging effect on the protein.[53] Recent studies have shown that deep intronic variants with splicing effects are more common in *MYBPC3* causing HCM than in *BAG3*-, *DSP*-, *FLNC*-, and *LMNA*-related DCM: in the latter genes, analysis of entire intronic sequences would not significantly improve the efficiency of molecular diagnosis of DCM probands.[53,55,56]

For genes such as GLA where the pathogenicity and type of variants are essential for therapeutic decisions (e.g. enzyme replacement therapy or chaperone treatment), RNA-based tests such as *in vitro* mini-gene splicing assays may substantially contribute to establishing the pathogenicity of uncertain non-canonical and deep intronic variants.[57]

Although a precise estimate of the clinical need of RNA testing is difficult to provide, numerous cardiomyopathy genes show VUS the interpretation of which may depend upon RNA testing. This need is set to increase given that, even with comprehensive genetic testing such as exome sequencing, about 50% of patients with suspected Mendelian conditions remain undiagnosed.[58]

Protein-based tests

Immediately downstream of RNA-based tests, tissue studies that explore the expression of mutated and non-mutated proteins can be very helpful in the diagnosis of genetic diseases. Contemporary methods include multi-tool protein expression using western blotting, immunohistochemistry with light, electron, and laser scanning confocal microscopy, and protein mass spectrometry, the latter being increasingly implemented, for example, in amyloidogenic protein characterization.[59] Close dialogue is required between clinical and laboratory teams to decide whether information beyond DNA testing is required to achieve a precise diagnosis (*Figure 4*).

For several reasons (time, cost, complexity, technologies, and expertise), protein-based studies are rarely used in routine clinical practice. Nevertheless, recent studies, mostly of single cases and small series, demonstrate that tissue tests addressing the effects of the DNA variant on the defective cellular structure or function provide confirmation of pathogenicity and contribute to a greater understanding of the basic molecular mechanisms of disease.

Conclusions

Although cardiologists are not traditionally involved in laboratory activities, the governance and application of genetic testing remains their clinical responsibility. To fully exploit the possibilities of genomic medicine in cardiological practice, clinicians need to be active participants in the pathway from clinical assessment to laboratory analysis. The development of a new genetic literate workforce is a key priority for the ESC Council.

Supplementary Data

Supplementary data are available at European Heart Journal online.

Declarations

Disclosure of Interest

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