

# Interpretation and actionability of genetic variants in cardiomyopathies: a position statement from the European Society of Cardiology Council on cardiovascular genomics

Eloisa Arbustini <sup>1\*</sup>, Elijah R. Behr <sup>2</sup>, Lucie Carrier <sup>3,4</sup>, Cornelia van Duijn <sup>5</sup>, Paul Evans<sup>6</sup>, Valentina Favalli<sup>7</sup>, Pim van der Harst <sup>8</sup>, Kristina Hermann Haugaa <sup>9,10</sup>, Guillaume Jondeau <sup>11,12,13†</sup>, Stefan Kääh<sup>14,15</sup>, Juan Pablo Kaski <sup>16,17</sup>, Maryam Kavousi<sup>18</sup>, Bart Loeys <sup>19,20</sup>, Antonis Pantazis<sup>21</sup>, Yigal Pinto<sup>22</sup>, Heribert Schunkert<sup>23,24</sup>, Alessandro Di Toro <sup>1</sup>, Thomas Thum <sup>25,26</sup>, Mario Urtis <sup>1</sup>, Johannes Waltenberger <sup>27,28</sup>, and Perry Elliott <sup>29,30</sup>

<sup>1</sup>Transplant Research Area and Centre for Inherited Cardiovascular Diseases, Department of Medical Sciences and Infectious Diseases, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; <sup>2</sup>Cardiology Research Section and Cardiovascular Clinical Academic Group, Institute of Molecular and Clinical Sciences, St George's, University of London and St George's University Hospitals NHS Foundation Trust, London, UK; <sup>3</sup>Institute of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; <sup>4</sup>DZHK (German Centre for Cardiovascular Research), Partner Site Hamburg/Kiel/Lübeck, Hamburg, Germany; <sup>5</sup>Nuffield Department of Population Health, University of Oxford, Oxford, UK; <sup>6</sup>Department of Infection, Immunity and Cardiovascular Disease, and INSIGNEO Institute, University of Sheffield, Sheffield S10 2RX, UK; <sup>7</sup>Genetics and Bioinformatics, 4bases SA, Lugano, CH, Switzerland; <sup>8</sup>Department of Cardiology, Division of Heart and Lungs, University Medical Center Utrecht, Utrecht, The Netherlands; <sup>9</sup>ProCardio Center for Innovation, Department of Cardiology, Oslo University Hospital, Rikshospitalet, Postboks 4950 Nydalen, Oslo 0424, Norway; <sup>10</sup>University of Oslo, Boks 1072 Blindern, Oslo 0316, Norway; <sup>11</sup>CNMR Syndrome de Marfan et apparentés, Member of VASCERN, AP-HP Hôpital Bichat, Service de Cardiologie, 46 rue Henri Huchard, Paris 75018, France; <sup>12</sup>INSERM LVTS U1148, Paris 75018, France; <sup>13</sup>Université de Paris, Paris, France; <sup>14</sup>Medizinische Klinik und Poliklinik I, LMU University Hospital Munich, Munich, Germany; <sup>15</sup>German Center for Cardiovascular Research, Munich Heart Alliance, Munich, Germany; <sup>16</sup>Institute of Cardiovascular Science, University College London, London, UK; <sup>17</sup>Centre for Inherited Cardiovascular Diseases, Great Ormond Street Hospital, London, UK; <sup>18</sup>Department of Epidemiology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands; <sup>19</sup>Cardiogenomics, Center for Medical Genetics, Antwerp University Hospital/University of Antwerp, Antwerp, Belgium; <sup>20</sup>Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands; <sup>21</sup>The Royal Brompton and Harefield Hospitals, Part of Guy's and St Thomas' NHS Foundation Trust, London, UK; <sup>22</sup>Department of Experimental Cardiology, University of Amsterdam, Amsterdam University Medical Center, Meibergdreef 15, Amsterdam 1105 AZ, The Netherlands; <sup>23</sup>Department of Cardiology, Deutsches Herzzentrum München, Technische Universität München, München, Germany; <sup>24</sup>Deutsches Zentrum für Herz- und Kreislaufrorschung (DZHK), Munich Heart Alliance, Munich, Germany; <sup>25</sup>Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Hannover, Germany; <sup>26</sup>Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM), Hannover, Germany; <sup>27</sup>Department of Cardiology and Cardiovascular Medicine, Medical Faculty, University of Münster, Münster, Germany; <sup>28</sup>Cardiovascular Medicine, Hirslanden Klinik Im Park, Seestrasse 220, Zürich 8027, Switzerland; <sup>29</sup>Barts Heart Centre St Bartholomew's Hospital, London, UK; and <sup>30</sup>Institute for Cardiovascular Science, University College London, London, UK

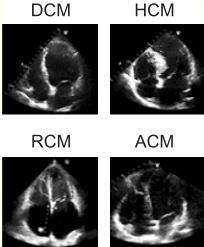
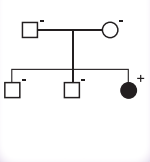
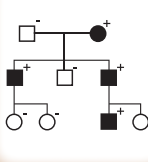

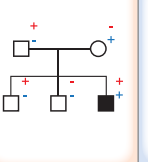
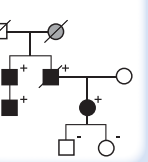
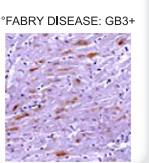
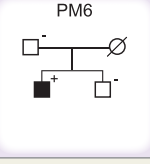
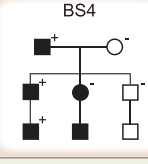
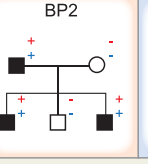
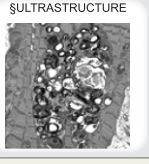
Received 22 June 2021; revised 3 December 2021; accepted 20 December 2021

\* Corresponding author. Tel: +39 0382501487, Fax: +39 0382501893, Email: [e.arbustini@smatteo.pv.it](mailto:e.arbustini@smatteo.pv.it)

† [www.marfan.fr](http://www.marfan.fr); [www.LVTS.fr](http://www.LVTS.fr).

© Crown copyright 2022.

This article contains public sector information licensed under the Open Government Licence v3.0 (<http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/>).

PHENOTYPING	GENETIC VARIANTS INTERPRETATION - ACMG CRITERIA THAT DEPEND ON CLINICAL CARDIOLOGY: 8 CRITERIA BASED ON CLINICAL EVALUATION AND 2 CRITERIA BASED ON FUNCTIONAL EVIDENCE					
-PROBANDS AND RELATIVES; -BASELINE AND FOLLOW-UP; -CARDIAC AND EXTRACARDIAC TRAITS; -NON-SYNDROMIC VS. SYNDROMIC CARDIOMYOPATHIES; -PHENOCOPIES.	<b>DE NOVO VARIANTS</b>  PS2: PROVEN  PM6: NOT PROVEN	<b>SEGREGATION:</b>  PP1: SEGREGATION  BS4: NON-SEGREGATION	<b>PRESENT IN HEALTHY CONTROLS FOR FULL-PENETRANT PHENOTYPES</b> BS2*	<b>TRANS OR CIS</b>  PM3: TRANS  BP2: CIS	<b>PERTINENCE OF THE PHENOTYPE TO THE AFFECTED GENE/S</b>  PP4	<b>FUNCTIONAL EVIDENCE</b>  PS3: EVIDENCE  BS3: NO EVIDENCE
 DCM    HCM RCM    ACM MAJOR CARDIOMYOPATHY PHENOTYPES	PS2 	PP1 	BS2 	PM3 	PP4 	PS3 *FABRY DISEASE: GB3+ 
	PM6 	BS4 	FULL PENETRANT PHENOTYPES  I.E. BARTH SYNDROME DANON SYNDROME	BP2 	HOLT-ORAM SYNDROME TBX5 p.Ala34Glyfs*27, ASD, VSD, SKELETAL ANOMALIES (LIMBS IN PARTICULAR, INCLUDING SYNDACTYLY), DCM, CONDUCTION DISEASE.	ULTRASTRUCTURE 

**Graphical Abstract** Impact of the cardiologic phenotyping of probands and relatives on ACMG criteria. The ideal 'drawing' of the family pedigree is complete and correct when all available family members have been clinically evaluated and, eventually, longitudinally monitored. \*Cardiologists and geneticists may add their own experience, data, and local population information. °Endomyocardial biopsy - anti-GB3 immuno-stain (positive brown); §Typical ultrastructural pattern. DCM = dilated cardiomyopathy; HCM = hypertrophic cardiomyopathy; RCM = restrictive cardiomyopathy; ACM = arrhythmogenic cardiomyopathy; ASD = atrial septal defect; VSD = ventricular septal defect; GB3 = globotriaosylceramide.

This document describes the contribution of clinical criteria to the interpretation of genetic variants using heritable Mendelian cardiomyopathies as an example. The aim is to assist cardiologists in defining the clinical contribution to a genetic diagnosis and the interpretation of molecular genetic reports. The identification of a genetic variant of unknown or uncertain significance is a limitation of genetic testing, but current guidelines for the interpretation of genetic variants include essential contributions from clinical family screening that can establish a de novo assignment of the variant or its segregation with the phenotype in the family. A partnership between clinicians and patients helps to solve major uncertainties and provides reliable and clinically actionable information.

**Keywords** Genetic variant • Pathogenicity • Interpretation • Cardiomyopathies • Variants of uncertain significance (VUS)

## Introduction

Following major successes in linking cancer genomics to treatment, cardiovascular disease is one of the next fields in which complex genetic data have the potential to transform clinical care. There are, however, many barriers delaying translation of new scientific discoveries into direct benefit for patients, including the need for translational research that bridges the gap between sequence information and treatment, and the development of a workforce with the skills to exploit new scientific opportunities. Clinical services for inherited cardiac conditions need to cater for the social, psychological, and medical needs of patients and relatives of all ages and throughout the life course. In particular, they should provide genetic counselling to all patients with potentially inheritable cardiovascular conditions by trained healthcare professionals working as part of a multidisciplinary team to help patients understand and manage the psychological, social, professional, ethical, and legal implications of a genetic disease. Molecular genetic testing is offered to patients with a heritable clinical phenotype and, when a definite causative genetic

mutation is identified in the index case, relatives can be offered predictive testing. Given the pivotal role that genetic diagnoses have in modern cardiological practice, it is timely and necessary for professional organizations like the European Society of Cardiology (ESC) to take the lead in promoting genetic and genomic literacy among healthcare professionals.

In this statement, we show how a structured and systematic approach to history taking and clinical phenotyping is central to the interpretation of genetic test results. In fact, genetic diagnosis is a fundamental component of precision medicine. Technological advances over the last 20 years have made it possible to move from tests that evaluate single genes to those that analyse multigene panels, whole exomes, and whole genomes.<sup>1</sup> Multigene panels and whole-exome sequencing (WES) or whole-genome sequencing (WGS) substantially increase the probability of identifying genetic variants associated with human diseases<sup>2</sup> but also pose challenges with respect to the interpretation. Guidelines from the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) provide criteria for

the assignment of pathogenicity to genetic variants and help to prevent false-positive interpretation.<sup>3</sup> They are underpinned by clinical data from patients and families and bioinformatic analyses that use population data (allele frequencies in populations such as 1000G, Exp, ExAC, gnomAD, computational predictors of functional damage, and *in silico* tools), interpretation of established data repositories (e.g. ClinVar, ClinGen, and LOVD3.0), and clinical databases (e.g. OMIM and MedGen).<sup>4,5</sup> When available, *in vitro* and *in vivo* functional data also contribute to the classification of variants.

The specific aim of this document from the ESC Council on Cardiovascular Genomics is to show how clinical and family data are essential for the correct interpretation of genetic variants. Indeed, broadening of the sequencing capacity of molecular genetic tests calls for an even more stringent clinical assessment (i.e. clinical phenotyping) to provide the correct interpretation of any finding. Mendelian cardiomyopathies are used as a reference to show how detailed, systematic, and iterative evaluation of patients and relatives cannot only establish the pathogenicity of genetic variants but also provide a basis for personalized medicine in heritable cardiovascular conditions.

## American College of Medical Genetics and Genomics/ Association for Molecular Pathology guidelines and variant classification, reinterpretation, and incidental findings

In 2013, a working group consisting of members of the ACMG, AMP, and College of American Pathologists set out to develop recommendations for a standard terminology for classifying sequence variants with the aim of reducing the substantial number of variants being reported as 'causative' of disease in the absence of sufficient supporting evidence. The terms 'mutation' and 'polymorphism' were replaced by the term 'variant' with modifiers: (i) benign, (ii) likely benign, (iii) uncertain significance, (iv) likely pathogenic, or (v) pathogenic.<sup>3</sup>

The system is applicable to variants in all Mendelian genes, whether identified by single-gene tests, multigene panels, exome sequencing, or genome sequencing. Pathogenicity is determined by the entire body of evidence in aggregate, converging on a single unequivocal interpretation.

### Variant classification

The ACMG/AMP guidelines for variant interpretation utilize 16 criteria that favour pathogenicity (P) and 12 criteria that support benign (B) interpretation.<sup>3</sup> They are based on clinical genetics and phenotype information, genetic epidemiology/population data, computational/predictive data, and the characteristics of the gene and mutation under examination. The strength of each contributor to the interpretation of pathogenicity is classified as very strong (PVS1), strong (PS1–4), moderate (PM1–6), or supporting (PP1–5). The evidence grade for each contributor to a benign interpretation ranges from stand-alone (BA1, the high

prevalence of the variant in general population is sufficient to consider it benign) to strong (BS1–4), or supporting (BP1–7). The ACMG guidelines provide a 'baseline' suggested strength (e.g. in PM1 the suggested strength is moderate) that can be, and often is, modified based on the available evidence. Some criteria have been originally formulated to be flexible (PP1); others have been adapted with subsequent studies (PVS1 and BS3).<sup>6,7</sup> Over time, these adaptations have become both gene-specific and disease-specific (e.g. MYH7).<sup>8</sup>

The general ACMG/AMP scheme is summarized in [Table 1](#). The combination of each contributor generates a score that corresponds to the five classes of variants: benign (B), likely benign (LB), variants of uncertain significance (VUS), likely pathogenic (LP), and pathogenic (P). In practice, the five classes are often simplified into three categories: benign (B and LB), VUS, and pathogenic (LP and P). For common genetic diseases such as hypertrophic cardiomyopathy (HCM), VUSs are relatively common. Variants are classified as VUS due to the lack of information and/or the presence of conflicting data on their role in a given phenotype.

### Variant reinterpretation

Variants classified as VUS or pathogenic at initial evaluation may be reclassified subsequently based on novel validated biomarkers, the observation of new cases/families confirming or excluding pathogenicity, new functional studies, or the identification of novel causative genes.<sup>14,15</sup> On the contrary, the probability that a variant originally interpreted as benign is reinterpreted as pathogenic is very low and may concern rare synonymous variants introducing alternative cryptic splice sites, subsequently identified in functional studies.

Between 2016 and 2019, of 4501 variants reclassified in ClinVar—the major archive of variant interpretation containing >1 million submissions—41 P variants (0.91%) and 165 LP variants (3.7%) were reclassified as VUSs while only 4 B variants (0.09%) were reclassified as P ( $n = 1$ ) and LP ( $n = 3$ ), respectively.<sup>16</sup> In children, 71 of 330 variants were reclassified (21.5%); 44 VUSs were reclassified, 9 as LP/P and 35 as LB/B, respectively; 25 of 71 (35.2%) were reclassified from LP/P to VUSs; 0 LB/B to LP/P.<sup>17</sup>

### Misinterpretation

The primary purposes of clinical genetic testing are to support the identification or confirmation of a disease, to guide individualized treatment decisions, and reliable cascade screening of families. Misinterpretation may negatively affect not only diagnostic tests but also—and even more importantly—predictive tests used in asymptomatic persons to predict future risk of disease.<sup>18</sup> Thus, the reliability of a test is fundamental to the actionability of any findings (positive or negative) in the clinic.

Inconclusive test results can create mistrust of genetic evaluation and cynicism about the opportunities for targeted management of heritable diseases.<sup>19–22</sup> This emphasizes the importance of clinicians in the assessment of clinical phenotypes and the framing of question to geneticists.

Continuous reappraisal of genetic data in the light of familial segregation and functional data is one of the methods by which uncertainty in the clinics can be reduced. A VUS, as long as it remains so classified, is not clinically actionable while a variant, previously

interpreted as a VUS, that is proven by new evidence to be pathogenic takes on new clinical significance with practical implications (e.g. monitoring of healthy carriers, early initiation of medications, concealed arrhythmogenic risk, and pre-natal/pre-implantation diagnosis).<sup>9,14,15,23–26</sup>

## Guidelines for contacting and informing variant carriers after reinterpretation

In the past, many genetic variants were discovered and classified in the context of research programmes rather than clinical genetic evaluation. The American Society of Human Genetics (ASHG) have provided guidelines on the contacting of research participants after reinterpretation of genetic and genomic research results<sup>27,28</sup> (see [Supplementary material online, Table S1](#)). As a general principle, it is reasonable to contact participants and offer updated results if the reinterpretation is consistent with the phenotype under study and is reasonably expected to affect a research participant's medical management. Information on a possible reinterpretation of genetic variants should be anticipated in the pre-test counselling phase, focusing on two issues: (i) diagnostic tests in affected carriers vs. risk-predicting tests in healthy carriers and (ii) levels of actionability of the reclassified variants. An LP/P variant downgraded to a VUS (common) makes the variant non-actionable clinically. Conversely, the implications of upgrading a VUS or a B/LB variant to an LP/P (rare) can be substantial but depend on the disease (e.g. severe cardiomyopathies such as Barth syndrome or Danon disease, pre-natal or pre-implantation diagnosis, potential malignancy: poly-ADP ribose polymerase inhibitors or risk-reducing surgery in hereditary breast and ovarian cancer). For children, a recent scientific statement from the American Heart Association highlighted the role of pre- and post-test counselling, balancing benefits and harms, but does not mention reclassification,<sup>29</sup> which occurred in around 10% of variants before the ACMG guidelines.<sup>17</sup> In the cardiomyopathy setting, guardians of healthy children, carriers of parental pathogenic variants, should be reassured that deep clinical phenotyping and systematic monitoring guarantee the best cardiologic care.

## Incidental or secondary findings

The use of clinical exome and WES/WGS in clinical practice has inevitably led to the identification of variants in genes unrelated to the primary medical reason for testing. These findings have been defined as incidental<sup>30</sup> or secondary.<sup>31</sup> The possibility of identifying secondary findings should be properly communicated before testing, and patients should also consent to the receipt of such results. The ACMG SF v3.0 has provided recommendations on the reporting of secondary findings of those pathogenic or likely pathogenic variants identified in the 73 actionable genes reported in [Table 1](#) of Miller et al.<sup>31</sup> This list of ACMG SF v3.0 genes includes 19 cardiomyopathy genes (*MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, *TPM1*, *MYL3*, *ACTC1*, *PRKAG2*, *GLA*, *GAA*, *MYL2*, *TTN*, *FLNC*, *LMNA*, *PKP2*, *DSP*, *DSC2*, *TMEM43*, and *DSG2*), as well as genes related to non-cardiomyopathy cardiovascular phenotypes, cancer, inborn errors of metabolism, and to miscellaneous phenotypes (see [Supplementary material online, Table S2](#)).<sup>10</sup> Variants in these genes have to be returned in genetic test performed for any

reason. These recommendations may not coincide with the national guidelines about the reporting of incidental and secondary findings.

## Variants in genes of uncertain clinical significance

According to the ACMG definition, a 'gene of uncertain significance' (GUS) corresponds to 'a gene without validated association with the patient's phenotype'.<sup>3</sup> A gene is a GUS when it has never been associated with any patient phenotype or it has been previously associated with a different phenotype from the one under consideration. When variants are identified in GUS, the guidelines recommend their report as 'variants in a gene of uncertain significance' that should always be classified as a VUS,<sup>3</sup> irrespective of fulfilling the ACMG criteria for pathogenicity (an LP/P variant in a GUS is a VUS).

A ClinGen initiative has recently comprehensively re-evaluated all genes involved in HCM, dilated cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy, and only a minority of them are now considered to have robust evidence for causal link with the disease/s. Currently, according to the ClinGen, genes that meet categories of 'limited or disputed evidence' are considered as the GUS (<https://clinicalgenome.org>).

## How clinicians contribute to implementation of the ACMG/AMP guidelines

The essential role of clinicians in variant interpretation is the gathering of coherent evidence that supports and reinforces classification of gene variants identified in a laboratory at their request. In fact, of the 16 ACMG/AMP criteria of pathogenicity, at least 8 are based upon clinical and phenotypic data and family segregation studies<sup>3</sup> ([Graphical Abstract](#)).

Clinical data are informative not only at baseline evaluation but also during the follow-up of patients and families.<sup>11,12</sup> For most cardiomyopathy subtypes, clinical screening of relatives can be inconclusive at first evaluation due to age-related expression of disease. Depending upon the type of cardiomyopathy, the phenotype may fully manifest only in adult life (all cardiomyopathies) or less commonly in children (e.g. lethal restrictive troponinopathies, *TNNI3* gene).<sup>32</sup>

## De novo variants (PS2 and PM6 criteria)

While there are some pathogenic variants that typically occur *de novo* (an example is *DES* p.Arg454Trp), evaluation of both parents of an affected individual is required for confirmation (PS2 is the criterion used for proven *de novo* variants). In some cases, variants appear to be *de novo*, but paternity and/or maternity cannot be confirmed (PM6 is the criterion used for assumed but not proven *de novo* variants) ([Figure 1](#)). If paternity is not established, the Cardiomyopathy Expert Panel (CMP-EP) suggests upgrading of the PM6 to PS2 when at least three proven *de novo* occurrences have been reported, e.g. *MYH7* for cardiomyopathies.<sup>8</sup> For other conditions such as genetic hearing loss, the ClinGen variant

**Table 1** American College of Medical Genetics and Genomics/Association for Molecular Pathology criteria for interpretation of pathogenicity of genetic variants

Criterion	Description	Clinical role	Source of information	Notes
PVS1	Null variant (nonsense, frameshift, canonical $\pm 1$ or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease	This criterion does not depend upon clinical evaluation	Computational and predictive data	There are proven null pathogenic variants whose interpretation of pathogenicity stands alone. Vice versa, other predicted null variants are non-pathogenic [i.e. <i>DSC2</i> (p.Ala897LysfsTer4); <i>TRPM4</i> (p.Trp525*)] PVS1 strength can be downgraded if appropriate <sup>6</sup>
PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	This criterion does not depend upon clinical evaluation	Computational and predictive data	Interpretation may eventually benefit of segregation studies, i.e. a cryptic splice site is introduced
<b>PS2</b>	<b>De novo (both maternity and paternity confirmed) in a patient with the disease and no family history</b>	<b>Parents should demonstrate normal cardiac evaluation</b>	<b>Segregation data</b>	<b>If the same variant has been previously published as de novo in peer-reviewed journals, then it is standard practices to use this evidence</b>
PS3	Well-established <i>in vitro</i> or <i>in vivo</i> functional studies supportive of a damaging effect on the gene or gene product	This criterion does not depend upon direct clinical evaluation	Functional: either <i>in vivo</i> or <i>in vitro</i> studies	Tissue studies (i.e. tissue biopsy) in certain cardiomyopathies demonstrated the effects of the variants
<b>PS4</b>	<b>The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls</b>	<b>Clinical evaluation in a large at-risk population can support the real prevalence of the disease</b>	<b>Genetic epidemiology or population data</b>	—
PM1	Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation	This criterion does not depend upon clinical evaluation	Functional, protein modelling, computational, and predictive data Public databases (ClinVar, ClinGen, LOVD, HMD)	Hot spot and functional domain [i.e. <i>GLA</i> (p.Asn215Ser)]
PM2	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium	This criterion does not depend upon clinical evaluation	Prevalence in population databases	Activated when the variant is rare enough in population databases to be plausibly pathogenic
<b>PM3</b>	<b>For recessive disorders, detected in <i>trans</i> with a pathogenic variant</b>	<b>This criterion may benefit from clinical evaluation and inheritance model in family screening<sup>9–12</sup></b>	<b>Public databases (OMIM, MedGen)</b>	<b>Parents are healthy carriers with proven normal clinical evaluation. Allelic data from sample bioinformatic analysis and segregation data</b>
PM4	Protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants	This criterion does not depend upon clinical evaluation	Computational and predictive data. Predicted protein change	—
PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	This criterion does not depend upon clinical evaluation	Public databases (ClinVar, ClinGen, LOVD, HMD)	Different missense variants affecting the same residue. [i.e. <i>DES</i> (p.Arg454Trp) vs. <i>DES</i> (p.Arg454Gln) or <i>LMNA</i> (p.Arg189Trp), (p.Arg189Gln), and (p.Arg189Pro)]

Continued

**Table 1 Continued**

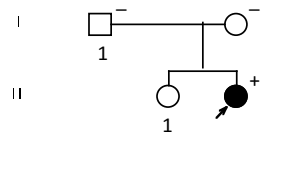
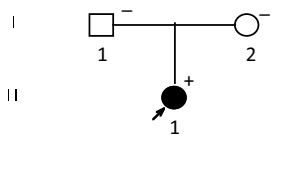
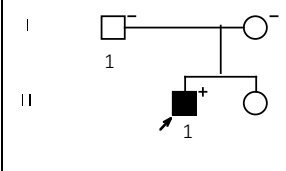
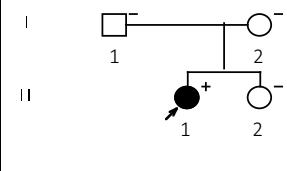
Criterion	Description	Clinical role	Source of information	Notes
PM6	Assumed <i>de novo</i> , but without confirmation of paternity and maternity	This criterion may benefit from clinical evaluation and family screening <sup>9-12</sup>	Segregation data	<b>De novo not proven: one parent is not available for testing. However, if the same variant has been previously published as <i>de novo</i> in peer-reviewed journals, then it is standard practice to use this evidence</b>
PP1	Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease	Clinical evaluation, genetic counselling, and family screening <sup>9-12</sup>	Segregation data	<b>This criterion is met when the family members, both affected and non-affected, had clinical and genetic screening. Deductions from pedigrees risk misinterpretation of pathogenicity. However, if extensive segregation data for the same variant have been previously published in peer-reviewed journals, then it is standard practice to use this evidence</b>
PP2	Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease	This criterion does not depend on clinical evaluation	Computational and predictive data	—
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)	This criterion does not depend on clinical evaluation	Computational and predictive data	—
PP4	<b>Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology</b>	<b>Geno-phenotype correlation</b>	<b>Genetic counselling, clinical evaluation of the patient</b>	<b>This criterion strongly relies on the deep phenotyping of proband and relatives, particularly for cardiomyopathies with red flags (cardiac and extra-cardiac) characterizing the phenotype</b>
PP5	Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation	This criterion does not depend on clinical evaluation	Public databases (ClinVar, ClinGen, LOVD, HMD) PubMed	To be considered with caution: many variants classified as 'disease causing' in the past, are now being shifted to non-pathogenic class and vice versa. The strength of the criterion may increase with the number and the reliability of sources. Recently, the experts of the ClinGen Sequence Variant Interpretation Working Group proposed that laboratories discontinue the use of criteria PP5 and BP6 as soon as that is practically achievable <sup>13</sup>
BA1	Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium	This criterion does not depend on clinical evaluation	Genetic epidemiology. Population genetic databases	—

Continued

**Table 1** Continued

Criterion	Description	Clinical role	Source of information	Notes
BS1	Allele frequency is greater than expected for disorder	This criterion does not depend on clinical evaluation	Genetic epidemiology. Population genetic databases Public databases (ClinVar, ClinGen, LOVD, HMD)	—
<b>BS2</b>	<b>Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age</b>	<b>The clinical screening and deep phenotyping of family members is essential for this criterion<sup>9–12</sup></b>	<b>Genetic epidemiology. Population genetic databases Public databases (ClinVar, ClinGen, LOVD, HMD)</b>	<b>To be considered with caution: for most CMP genes penetrance can be variable, incomplete and age-dependent. BS2 can be activated for conditions such as XLR Danon disease in male patients</b>
BS3	Well-established <i>in vitro</i> or <i>in vivo</i> functional studies show no damaging effect on protein function or splicing	This criterion does not depend on clinical evaluation	Functional studies	Functional studies or pathological studies do not show markers that are specifically linked with the cardiomyopathy
<b>BS4</b>	<b>Lack of segregation in affected members of a family</b>	<b>The clinical screening and deep phenotyping of family members is essential for this criterion.<sup>9–12</sup></b>	<b>Only from segregation data</b>	<b>This criterion largely depends upon the number of relatives available for clinical and genetic screening, and their age. Regular clinical monitoring of relatives may modify this criterion</b>
BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease	<i>This criterion does not depend on clinical evaluation</i>	Computational and predictive data	The typical example is provided by most missense <i>TTN</i> gene variants
BP2	Observed in <i>trans</i> with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in <i>cis</i> with a pathogenic variant in any inheritance pattern	This criterion does not depend on clinical evaluation	Public databases (ClinVar, ClinGen, LOVD, HMD)	Allelic data from bioinformatic analysis and segregation studies
BP3	In-frame deletions/insertions in a repetitive region without a known function	This criterion does not depend on clinical evaluation	Computational and predictive data	—
BP4	Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	This criterion does not depend on clinical evaluation	Computational and predictive data	—
BP5	Variant found in a case with an alternate molecular basis for disease	This criterion does not depend on clinical evaluation	Public databases (ClinVar, ClinGen, LOVD, HMD)	This criterion has limited value for most cardiomyopathies that are the paradigm of genetically heterogeneous diseases
BP6	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	This criterion does not depend on clinical evaluation	Public databases (ClinVar, ClinGen, LOVD, HMD) PubMed	The impact of the criterion may increase with the number and the reliability of sources
BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site and the nucleotide is not highly conserved	This criterion does not depend on clinical evaluation	Computational and predictive data. When possible, RNA-based functional analysis	—

Criteria based upon clinical evaluation are in bold.

A	B	C	D
Proband: onset at 6 yrs Phenotype: RCM, HTx at 12 <i>TNNT2</i> (p.Phe97Cys)	Proband: onset at 8 yrs Phenotype: RCM, HTx at 14 <i>MYL2</i> (p.Gly162Arg)	Proband: onset at 17 yrs Phenotype: HCM, ICD at 25 <i>MYBPC3</i> c.2737+5G>T	Proband: onset at 26 yrs Phenotype: DCM + AVB <i>LMNA</i> (p.Leu183Pro)
ACMG Likely Pathogenic (PM1, PM2, PP2, PP3)	ACMG Likely Pathogenic (PM1, PM2, PM5, PP2, PP3)	ACMG VUS (PM2, BP4)	ACMG Likely Pathogenic (PM1, PM2, PP2, PP3)
The addition of the ACMG criterion PS2 ( <i>Proven de novo</i> ) shifts the class.			
ACMG Likely Pathogenic + PS2 -> Pathogenic	ACMG Likely Pathogenic + PS2 -> Pathogenic	ACMG VUS + PS2 -> Likely Pathogenic	ACMG Likely Pathogenic + PS2 -> Pathogenic
			
○□ Unaffected female/male   ●■ Affected female/male   + Variant Carrier   - Non carrier   ➔ Proband   / Deceased			
Same variants: the addition of the ACMG criterion PM6 (assumed <i>de novo</i> but without confirmation of maternity and paternity) does not shift the class			
ACMG Likely Pathogenic + PM6 -> Likely Pathogenic	ACMG Likely Pathogenic + PM6 -> Likely Pathogenic	ACMG VUS + PM6 -> VUS	ACMG Likely Pathogenic + PM6 -> Likely Pathogenic
HTx= Heart transplantation; HCM= Hypertrophic Cardiomyopathy; RCM= Restrictive cardiomyopathy; DCM= Dilated Cardiomyopathy; AVB= Atrioventricular block; ICD= Implantable Cardioverter Defibrillator			

**Figure 1** Four examples of proven *de novo* variants—American College of Medical Genetics and Genomics criterion PS2 contributes to shift the American College of Medical Genetics and Genomics class from variant of uncertain significance to likely pathogenic.

curation SOP Committee proposed a point-based system for modified strength levels of PS2 and PM6 criteria, based on the parental confirmation, phenotypic consistency, and number of the *de novo* observations.<sup>33,34</sup>

Therefore, confidence that a *de novo* variant is pathogenic is increased by documentation of multiple individual occurrences associated with the expected phenotype. Nevertheless, a high frequency of the given *de novo* variant in population databases should raise doubts about pathogenicity, irrespective of the *de novo* status of the variant.<sup>35,36</sup> For cases with an apparent germinal mosaicism (e.g. affected sibs, offspring of negative parents), paternity/maternity must be proven for *de novo* criteria to be applied.

### Co-segregation (PP1) and non-segregation (BS2 and BS4) criteria

For cardiomyopathies, confirmation of pathogenicity is greatly enhanced by evidence of co-segregation in families. Ideally, as many relatives as possible should be evaluated using deep phenotyping (defined as the precise and comprehensive analysis of phenotypic abnormalities in which the individual components of the phenotype are observed and described)<sup>11,37–39</sup> and documentation of disease in deceased individuals. Post-mortem genetic tests can be performed on a case-by-case basis; most reported studies address the search for the cause of sudden cardiac death more than segregation studies. However, post-mortem testing can contribute to variant classification and can be performed when retained biological samples of deceased individuals are available and are used within an appropriate ethical/regulatory framework with

an informed consent by entitled relatives. Given the age-related penetrance of most cardiomyopathies, co-segregation of a VUS (so defined at first detection) in a family may require repeat evaluations in multiple individuals over some years (Figure 2). Therefore, interpretation of a VUS may not be possible at baseline family screening but only after family monitoring in the mid or long term. The evaluation of the descendants of deceased patients may prove the obligate carrier status of affected family members who died at a young age (see Supplementary material online, Figure S1).

The segregation PP1 criterion applies when a variant occurs in a gene definitively known to cause the disease and co-segregates in multiple affected family members (see Supplementary material online, Figure S2). A quantitative definition of segregation has been proposed for the PP1 criterion. The computation of segregation evidence proposes cut-off values for three levels of evidence in single family or >1 family: strong, moderate, and supporting.<sup>16</sup> Supplementary material online, Figure S3 shows an example of how to evaluate the three levels of evidence. Co-segregation studies may shift the ACMG class from uncertain to likely pathogenic or pathogenic (see Supplementary material online, Figure S4).

The non-segregation BS4 criterion applies when one family member is affected but is a non-carrier of the genetic variant interpreted as disease-causing in the family. However, a digenic contribution to the phenotype or coexistent phenocopies should be excluded (Figure 3) and the stringency of the phenotypic traits of each cardiomyopathy (diagnostic criteria) can influence the reliability of the segregation study.<sup>8</sup>



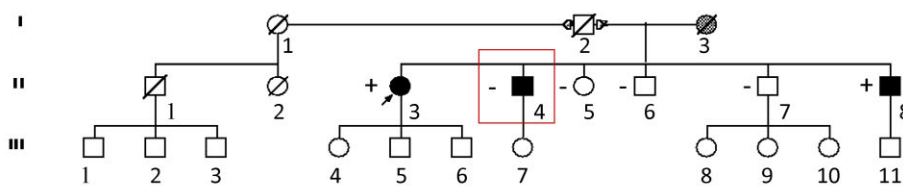
PEDIGREE BASED ON CLINICAL SCREENING AND MONITORING OF FAMILY MEMBERS	CLINICAL DATA
	<p>2000: Apparently sporadic DCM I:1, death, 70 yrs I:2, death, 90 yrs II:1, 65 yrs, healthy II:2, death, 52 yrs: HF, DCM</p>
	<p>2006: clinical family screening III:2, <b>Proband</b>, 46 yrs, professional athlete, handball. LVEDD 51 mm; LVEF 46,5%; (bisoprolol) III:1, LVEDD 57 mm; LVEF 50%; Carvedilol, Ramipril IV:1, normal 2DTE IV:2, normal 2DTE</p>
<p>SEGREGATION: not feasible before genetic testing.</p>	
	<p>2012: genetic testing <i>TNNT2</i> (p.Gly279Arg): ACMG VUS (PM2, PP2) II:1, death IHD (^) II:3, Breast cancer, chemotherapy (^) III:1, LVEF 50%; LVEDD 54 mm (*) III:2, <b>Proband</b>, LVEF 45%; LVEDD 57mm IV:1, military visit: non eligible; 2DTE: LVEDD 63 mm; LVEF 55% (^) IV:2, Normal LV size and function; (^)</p>
<p>SEGREGATION <math>N = (1/2)^1</math> affected and mutated relative, excluding the proband) = 1/2. This value corresponds to non-segregation.</p>	
	<p>2019 <i>TNNT2</i> (p.Gly279Arg): ACMG: Prior VUS (PM2, PP2) + PP1, strong → <b>Likely Pathogenic</b> II:3, 87 yrs, genetic test negative; hypertension; borderline LV dysfunction; AF (*) III:1, NYHA I; LVEF 55%; LVEDD 52 mm (*) III:2, <b>Proband</b>, NYHA II; LVEF 48%; LVEDD 54 mm (*) IV:1, LVEF 40-45%; LVEDD 72 mm (*) IV:2, LVEF 48%; LVEDD 53 mm (*)</p>
<p>○ □ Unaffected female/male   ● ■ Affected female/male   ◻ &gt;LVEDD male   ⊙ Unknown phenotype female   + Variant carrier   - Non carrier   ➔ Proband   / Deceased</p>	
<p>SEGREGATION <math>N = (1/2)^3</math> affected and mutated first degree relatives, excluding the proband x <math>(1/4)^1</math> affected and mutated second degree relative x <math>(1-1/2)^1</math> non affected and non-mutated relative) &lt; 1/32. This value corresponds to strong segregation.</p>	

§ POSSIBLE METHOD, IN "Jarvik GP, Browning BL. Consideration of Cosegregation in the Pathogenicity Classification of Genomic Variants. Am J Hum Genet. 2016;98(6):1077-1081".

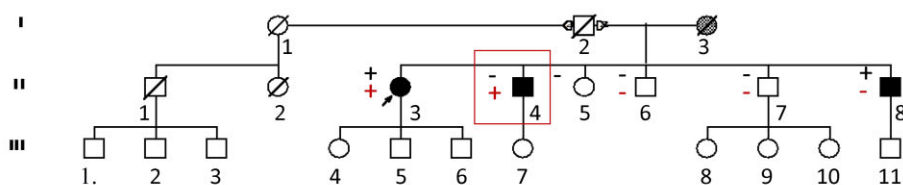
(\*) Usable for computation of segregation; (^) Not usable for computation of segregation; HF= Heart Failure; DCM= Dilated Cardiomyopathy; AF= atrial fibrillation; IHD= Ischemic heart disease; DCM= Dilated Cardiomyopathy; LV= Left ventricular; LVEDD= Left ventricular end-diastolic diameter; LVEF= Left ventricular ejection fraction, NYHA= New York Heart Association, VUS=variant of uncertain significance; 2DTE=two-dimensional transthoracic echocardiography

**Figure 2** The 15-year evolution of medical history; segregation is calculated after 15 years monitoring—American College of Medical Genetics and Genomics criterion PP1.

*TNNT2* (p.Arg278Cys). The variant meets the ACMG pathogenic criteria (PM5, PP2, PP3, PP5) and the benign criteria BS1 (allele frequency is greater than expected for disorder). ClinVar reports conflicting interpretations (Last evaluation: May 28, 2019). Segregation data in family include two affected carriers (II:3 and II:8), three non-affected non-carriers (II:5; II:6, and II:7) and one affected (the one with the most severe and typical phenotype) but non carrier II:4 (red square) who tested negative (BS4). ACMG Class LB (PM5, PP2, PP3, PP5, BS1) + BS4 -> VUS



II:4 -> Further deep phenotyping -> We considered the possibility of a phenocopy in II:4, who showed severe progression of LV thickness from 17 mm at 56 yrs -> 26 mm at 68 yrs. EMB demonstrated unequivocal GB3 accumulation and anti-GB3 specific immune reaction of intramyocyte accumulation bodies -> Genetic testing identified the GLA (p.Arg301Gly) (symbols in red in the pedigree below). The GLA variant meets 5 pathogenicity criteria (PM1, PM2, PM5, PP2, PP3) and is predicted to be LP. The proven intramyocyte accumulation adds the criterion (PS3\_Very Strong) that shifts the ACMG class from LP to P.



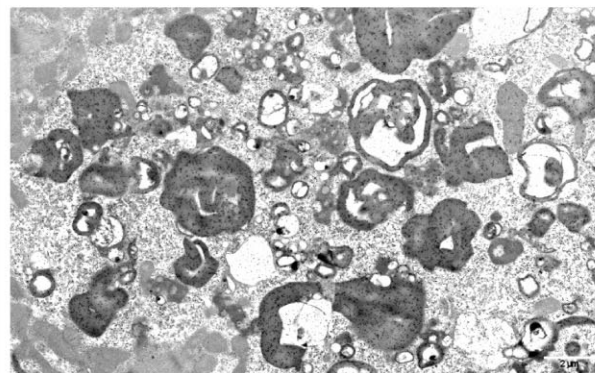
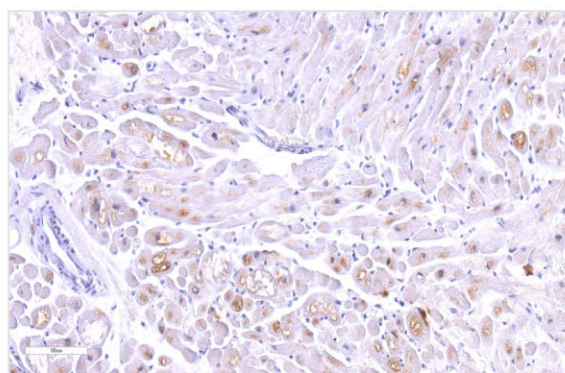
○ □ Unaffected female/male   ● ■ Affected female/male   ● Unknown phenotype female   + Variant carrier   - Non carrier   ➔ Proband   / Deceased

I:1, death, unknown cause  
 I:2, death, 64 yrs for cancer  
 I:3, death, 92 yrs, stroke; unspecified cardiac disease  
 II:1, SD, 58 yrs  
 II:2, death, 72 yrs, AR, COPD  
 II:3, **Proband**. Arterial hypertension since the age of 50; chest pain at 54 yrs; coronary angiography: patent coronary arteries. At 64 yrs: LV thickness = 13 mm; addressed for HCM. Non progression of LVH in 10 yrs (2010-2019). Current age 73 yrs. Recent diagnosis of Parkinson disease.  
 II:4, Arterial hypertension since the age of 44; diagnosis of concentric LVH at 50 (17 mm); progression of LVH up to 26 mm at 69 yrs.

II:5, Arterial hypertension; asymptomatic; GLA not yet screened.  
 II:6, COPD  
 II:7, Clinical screening negative  
 II:8, HCM, Max LV thickness 13 mm, non-obstructive  
 III:1-3, Clinical screening negative  
 III:4-7, Clinical screening negative  
 III:7, 46 yrs, Clinical screening negative; LV thickness 8 mm; GLA test positive (OC)  
 III:11, Clinical screening negative  
 III:4, III:5, III:6, III:11 did not have *TNNT2* testing.  
 Vice versa, II:5, III:4, III:5, III:6 are undergoing GLA test.

II:4 Endomyocardial biopsy – Anti-GB3 immunostain

II:4 Immuno-electron microscopy with anti GB3-antibodies



(OC) Obligate carrier; EMB= Endomyocardial biopsy; GB3= Globotriaosylceramide; COPD= Chronic obstructive pulmonary disease; LV= Left ventricular; HCM= Hypertrophic Cardiomyopathy; LVH= Left ventricular hypertrophy; VUS= variant of uncertain significance; LB=likely benign; LP= likely pathogenic; P= pathogenic

**Figure 3** An example of non-segregation and non-pathogenic *TNNT2* variant in the two sibs II:3 and II:8; both remain genetically uncharacterized. The apparently non-segregating sib (II:4) is affected by Fabry disease; his daughter is obligate carrier. None of the members of the third generation had *TNNT2* test.

The non-segregation BS2 criterion applies when one family member is a VUS carrier but not affected (Supplementary material online, Figure S5). The criterion is for adult or older healthy carriers. Although variable expressivity is often reported in cardiomyopathies, this criterion is relevant for Mendelian monogenic diseases. However, BS2 is reported with low prevalence (0.37%) among variants classified as pathogenic and likely pathogenic.<sup>40</sup>

### Variants in *cis* or *trans* (PM3 and BP2 criteria)

*Cis* variants (two or more) occur in the same copy of the gene or in two adjacent genes that are jointly inherited (Figure 4A). *Trans* variants are in different copies of the gene or in two different genes and are independently transmitted (Figure 4B). The key for interpreting the *Cis* and *Trans* rules is the inheritance.<sup>3,34</sup> For autosomal recessive (AR) conditions, knowing the allelic configuration of genetic variants helps in their interpretation, particularly in recessive single-gene disorders. Indeed, in this circumstance, when two heterozygous variants are detected in the same gene [e.g. *GAA* (Figure 4B), or in rare neonatal dilated cardiomyopathy,<sup>41</sup> or in rare adult HCM]<sup>42</sup> and one is known as pathogenic, the determination of the *trans* status of the second variant (e.g. novel and mis-sense) can add 'evidence for pathogenicity' to the second variant (PM3). Otherwise, if the two variants are *in cis* and one is known as pathogenic, the interpretation of the second variant shifts to benign (BP2). For autosomal dominant (AD) conditions, the application of *Cis* and *Trans* rules depends upon the effect of homozygosity on the phenotype (embryonic lethality or very severe disease phenotypes) and is gene-dependent.<sup>34</sup>

In the case of homozygous variants, a family study confirms the heterozygous status of the parent (Figure 5A). Typical examples in the field of cardiomyopathies are desminopathies that are transmitted both in AR and AD ways, depending on the variant in the *DES* gene (Figure 5).

A peculiar case is represented by the *trans* status of a deleted allele associated with a pathogenic variant in the other allele of a recessive cardiomyopathy gene. Deletion of one copy of a gene region (hemizyosity) may mimic homozygosity in sequencing-based tests; however, the completion of the test with copy number variation analysis demonstrates the deletion. When one of the parents is unavailable for testing, his/her relatives may contribute (maternal and paternal sibs and related offspring) if they are carriers of the given variants. Finally, a patient can be affected by two different genetic diseases, a condition whose correct detection should be based on precise phenotypic characterization. An illustrative example is represented by sarcomeric HCM and Duchenne muscular dystrophy (Figure 6).

### Pertinence of the phenotype to the affected gene/s (the PP4 criterion)

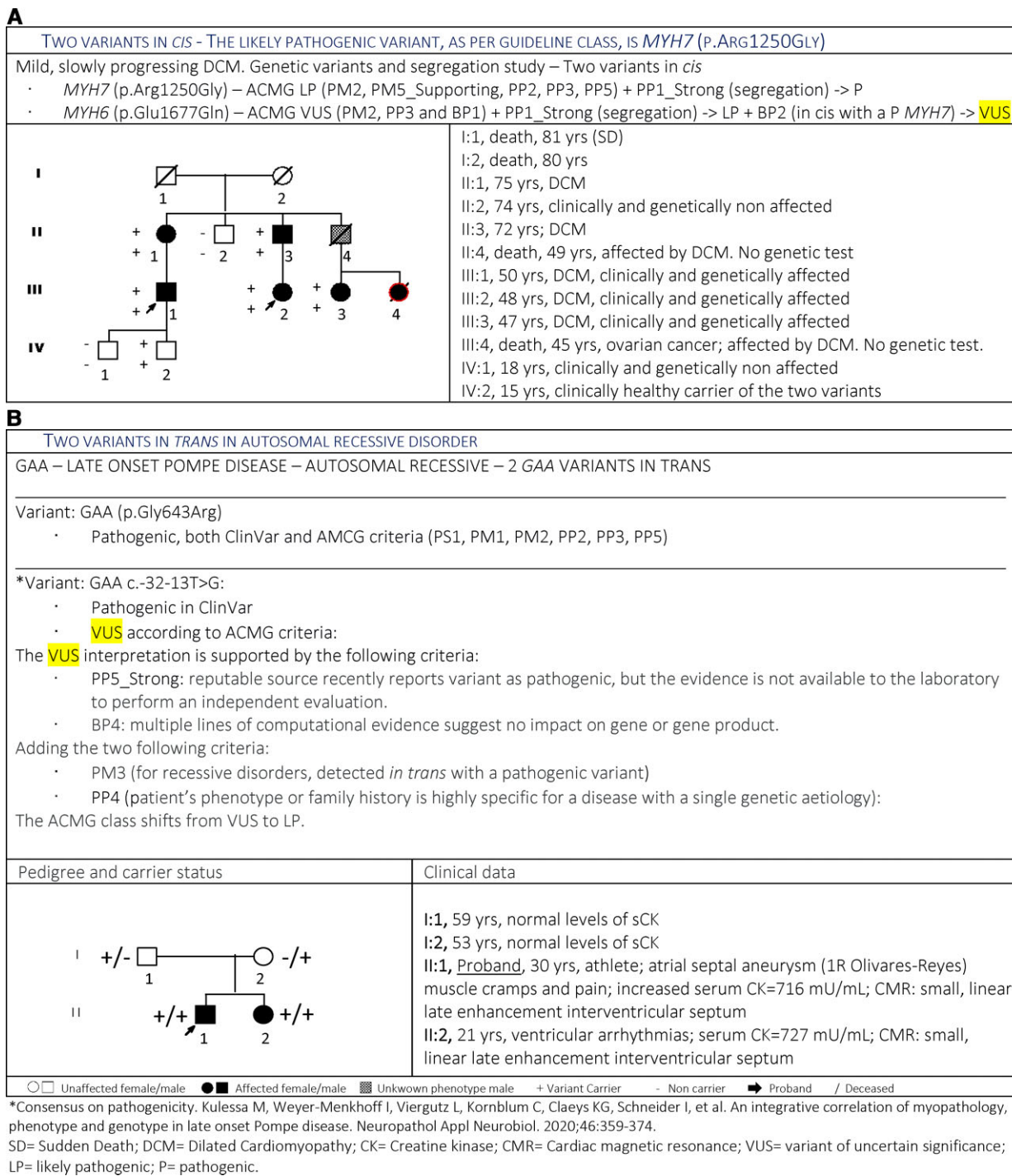
When evaluating a genetic variant, it is important to consider whether the phenotype in question is highly specific for a unique genetic aetiology (for example, *LAMP2* in Danon disease). This is rarely the case in cardiomyopathies as most are characterized by genetic heterogeneity and the frequent occurrence of phenotypes

such as mild left ventricular hypertrophy for which there may be other explanations such as hypertension, obesity, or athleticism. However, the coexistence of additional disease phenotypes does not exclude the pathogenicity of a variant (e.g. a *TTN* stop codon may predispose a person with alcohol abuse to develop cardiomyopathy).<sup>43</sup> Clinical markers (or diagnostic 'red flags')<sup>38</sup> may be of assistance in identifying specific disease phenotypes but need to be interpreted carefully. For example, a short PR interval occurs in the early phases of storage diseases such as Danon disease and, if associated with other traits such as HCM, skeletal myopathy and cognitive impairment, supports the diagnosis. Other examples include the combination of cardiomyopathy with left ventricular non-compaction, myopathy, leukopenia, skeletal abnormalities, methylglutaconic aciduria in the X-linked recessive Barth syndrome associated with pathogenic variants in *TAZ* gene;<sup>44</sup> atrial and ventricular septal defects, dilated cardiomyopathy, conduction disease skeletal anomalies, limbs in particular—syndactyly, thumb, carpal, radial, ulnar, vertebral, and chest anomalies—in the AD Holt–Oram syndrome associated with pathogenic variants in the *TBX5* gene;<sup>45</sup> symmetrical HCM, cornea verticillata, angiokeratomas, renal, brain, and peripheral nervous system involvement in the X-linked Fabry disease caused by pathogenic variants in the *GLA* gene.<sup>46</sup>

### Functional criteria (PS3 and BS3): can pathology and disease-specific biomarkers contribute to variant interpretation?

*In vivo*, *ex vivo*, or *in vitro* functional studies are complex, time-consuming, and may require samples from affected tissues. The PS3 and BS3 criteria address the evidence functional studies supporting (PS3) or excluding (BS3) damaging effects of the mutated gene. The Sequence Variant Interpretation Working Group (SVI-WG) recommends a four-step framework to determine the strength of evidence of functional studies: (i) define the disease mechanism, (ii) evaluate the applicability of general classes of assays used in the field, (iii) evaluate the validity of specific instances of assays, and (iv) apply evidence to individual variant interpretation.<sup>7</sup> With new next-generation sequencing technologies, the detection rate of genetic variants is so fast that functional studies for each variant deemed potentially pathogenic is beyond the diagnostic capacity of most genetic labs. However, some cardiomyopathies are characterized by specific pathological findings that can be more informative than *in vitro* studies. Examples include Danon disease (loss of *LAMP2*) (Figure 7), Fabry disease (substrate accumulation)<sup>46</sup> (Figure 3), dystrophinopathies (focal or extensive loss of dystrophin expression of the cardiomyocyte membrane and skeletal muscle),<sup>47</sup> myofibrillar cardio-desminopathies (intracellular deposits of osmiophilic granulo-filamentous material that is immunoreactive with anti-desmin-antibodies in myocardium and skeletal muscle)<sup>48</sup> (Figure 5B), and laminopathies (severe nuclear damage and loss of myocyte nuclear immunostaining).<sup>49,50</sup>

In most cardiomyopathies, gene-specific biomarkers do not exist, and commonly tested biomarkers inform about myocyte



**Figure 4** (A) Two variants in cis—the likely pathogenic variant is in MYH7 (p.Arg1250Gly). (B) Late-onset Pompe disease. Two variants in trans—the second variant does not meet American College of Medical Genetics and Genomics pathogenicity criteria, but is validated as pathogenic in ClinVar.

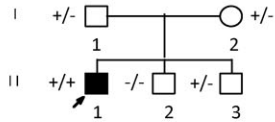
damage, or involvement of extra-cardiac organs and tissues but not on their cause. Functional effects of genetic variants may be obtained in the case of lysosomal diseases involving the heart (e.g. GAA and GLA)<sup>46,51</sup> by measuring blood enzyme level. New emerging biomarkers to be validated and confirmed will help

increase the spectrum of functional tests to be explored to strengthen the role of functional tests in the interpretation of variants.

Finally, future progress in variant interpretation will benefit from new technologies such as single-cell RNA sequencing

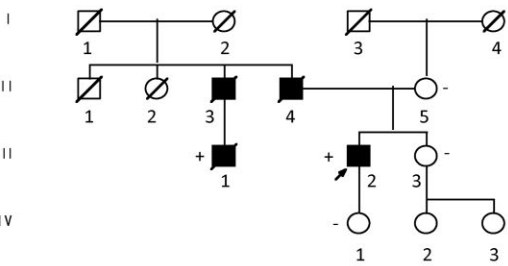
**A**

**AUTOSOMAL RECESSIVE RESTRICTIVE CARDIODESMINOPATHY – DESMINOPATHIES: AUTOSOMAL DOMINANT OR RECESSIVE**

DES (p.Arg16Cys) Homozygous	Family Data
	I:1, 61 yrs, Healthy heterozygous carrier I:2, 59 yrs, Healthy heterozygous carrier II:1, <b>Proband</b> , Homozygous, affected, RCM with AVB; onset at 17; desmin accumulation in the EMB. HTx at 26 yrs. II:2, 25 yrs at baseline screening. Healthy non-carrier II:3, 23 yrs at baseline screening. Healthy heterozygous carrier
DES Variant: Class ACMG Likely pathogenic (PM1, PM2, PP2, PP3, PP5) Addition of PS3 (well-established in vivo functional studies demonstrating the damaging effect on the gene product): desmin accumulation -> Final ACMG Class: <b>Pathogenic</b>	

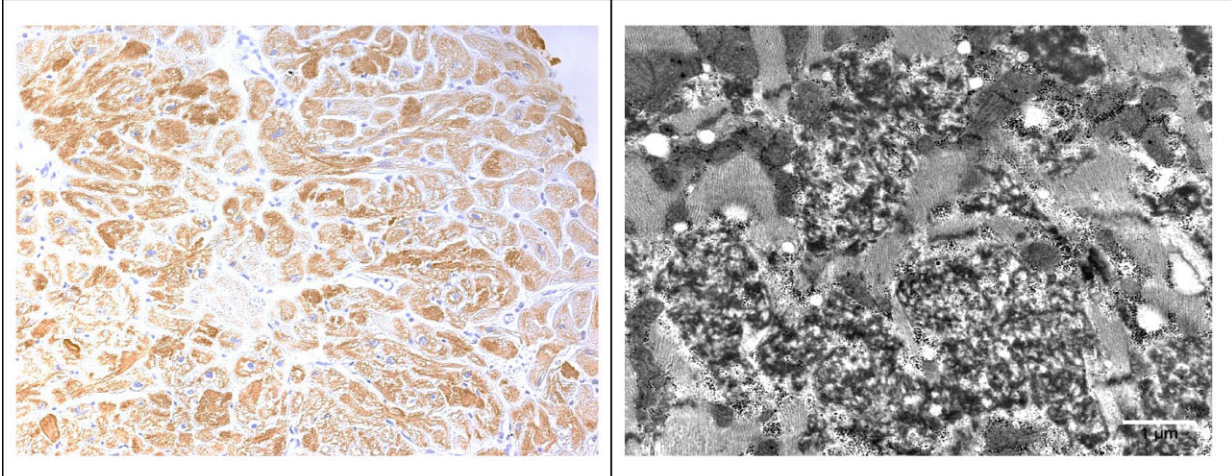
**B**

**AUTOSOMAL DOMINANT RESTRICTIVE CARDIODESMINOPATHY**

c.1398_1399DelGlnsTT (p.Gln466_His467delinsHisTyr) -> Class ACMG VUS (PM1, PM2, PP3). Adding <ul style="list-style-type: none"> <li>PP4 (patient's phenotype or family history is highly specific for a disease with a single genetic aetiology)</li> <li>PS3 (well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product; in this case, desmin accumulation)</li> <li>PP1 (Co-segregation with disease in multiple affected family members: II:3 and II:4 are obligate carriers)</li> </ul> the ACMG class of the c.1398_1399DelGlnsTT (p.Gln466_His467delinsHisTyr) shifts from VUS to P	
	I:1, death, 68; I:2, death, 85; I:3, death, 72; I:4, death, 80. II:1, death, 90 yrs, Alzheimer; II:2, death, 83 yrs II:3, death, 30 yrs, cardiomyopathy with PM. II:4, death, 63 yrs, cardiomyopathy onset at 40 yrs, PM at 43 II:5, 85 yrs, CRC at 80, senile hearing loss III:1, death at 33 yrs, after HTx: RCM, AVB and PM III:2, <b>Proband</b> , AVB at 29 yrs; PM at 30 yrs, AF, RCM evolving through LV dysfunction, 55 yrs: last LVEF 40%, EMB: intramyocyte granulo-filamentous material immuno-reacting with anti-desmin antibodies. Max sCK = 917U/L III:3, Clinical and genetic screening negative IV:1, 23 yrs, LVEDD 52 mm; LVEF 52%, LVT 9 mm; PQ interval 160 ms. Clinical and genetic screening: negative
□ Unaffected female/male   ■ Affected male   + Variant carrier   - Non carrier   ➔ Proband   / Deceased	

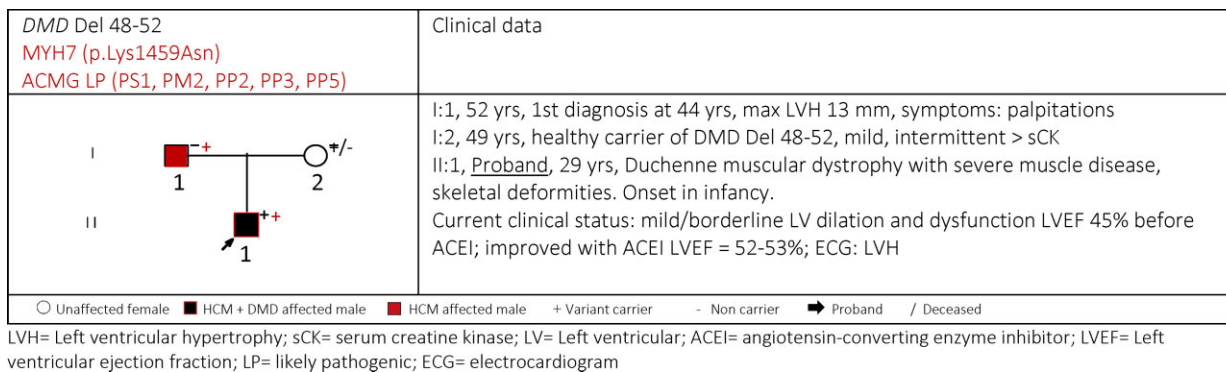
EMB confirmed desmin accumulation.

- Light microscopy (left panel) does not, by itself, confirm intramyocyte accumulation of desmin in EMB sample
- Electron microscopy demonstrate the typical accumulation of osmiophilic granulo-filamentous material (left panel)



RCM= Restrictive cardiomyopathy; AVB= Atrioventricular block; EMB= Endomyocardial biopsy; HTx= Heart transplantation; PM= Pacemaker; sCK= serum creatine kinase; LV= Left ventricular; LVEDD= Left ventricular end-diastolic diameter; LVEF= Left ventricular ejection fraction; LVT= Left ventricular thickness; VUS= variant of uncertain significance; P=pathogenic

**Figure 5** (A) Autosomal recessive desminopathies—parents are from the same small village. (B) Autosomal dominant restrictive cardiodesminopathy—endomyocardial biopsy shows the typical intramyocyte accumulation of granulo-filamentous osmiophilic material.



**Figure 6** X-linked recessive Duchenne muscular dystrophy and MYH7 likely pathogenic variant inherited from the father who only shows mild left ventricular hypertrophy.

(RNAseq), assays for transposase-accessible chromatin sequencing (ATACseq, exploring open regions of chromatin on a genome-wide scale), integration of ATACseq and RNAseq with epigenomics as well as integration of large data sets collecting genetic information, and the contribution of artificial intelligence (e.g. machine learning) tools.<sup>52,53</sup>

## From the clinic to the laboratory and back to the clinic

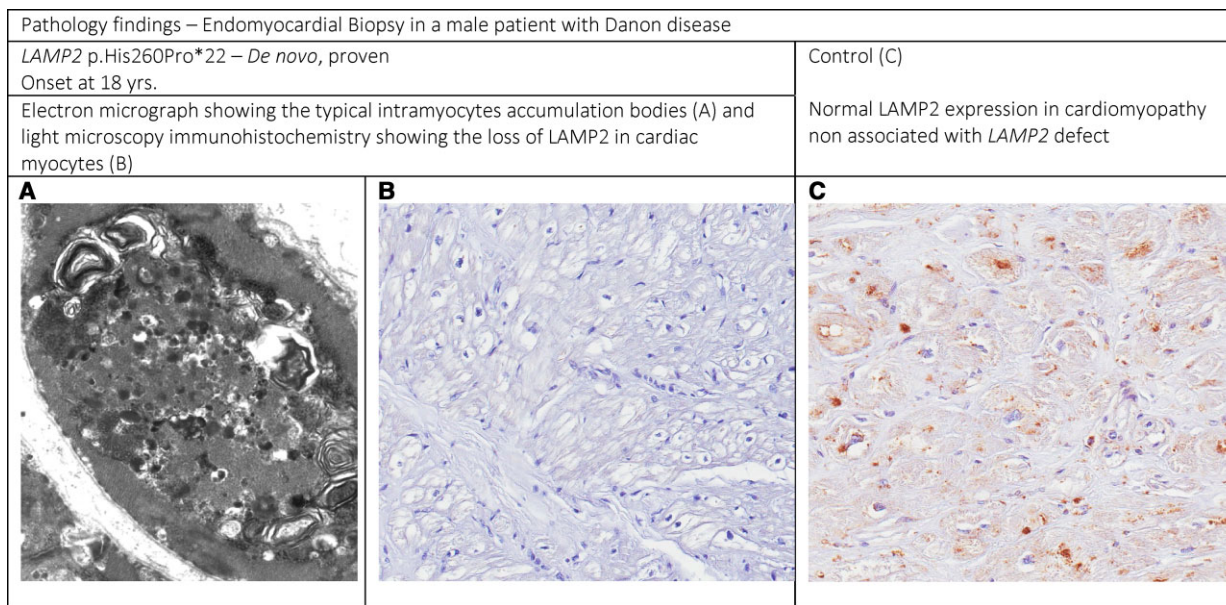
### How to inform patients and families

The owners of clinical genetic test results are patients and the transmission of information to other family members is their

responsibility. The need to extend genetic testing to family members should be anticipated in the pre-test counselling, when the reasons and significance of the test as well as the role of the family study should be explained to the patient.<sup>26</sup>

When a test is concluded, the patient may receive three types of information:

- (1) The genetic test is positive. This result provides the opportunity to positively impact both patient and family's health.<sup>47,54</sup>
- (2) The genetic test is negative. Patients should be informed about the limits of the test (number of genes analysed and completeness of the investigation). In the case of a negative test but clinical evidence of familial cardiomyopathy, clinical monitoring of at-risk relatives should be maintained, and efforts should be



**Figure 7** Pathological findings in endomyocardial biopsy of a male patient with Danon disease. Endomyocardial biopsy shows the loss of expression of the LAMP2 protein.

made to continue with the search for the causative genetic mechanism as well as for novel disease- or modifier-genes.

- (3) The possibility of finding a VUS should be discussed with the proband in the pre-test counselling. Informing the patient of the result of a test that has identified pathogenic (positive test) or benign (negative test for the analysed genes) variants is easier than communicating tests that have identified one or more VUSs (inconclusive test). Unlike many routine tests, there is no 'normal range' for a VUS.<sup>37,55,56</sup> The patient should be helped to understand that the relative importance of some criteria depends on the study of his/her family and that family-based data are uniquely useful for the correct interpretation of the variant.

## Guidelines for contacting variant carriers after reinterpretation

In the past, many genetic variants were discovered and classified in the context of research programmes rather than clinical genetic evaluation. The ASHG has provided guidelines on the contacting of research participants after reinterpretation of genetic and genomic research results<sup>28</sup> (see [Supplementary material online, Table S1](#)). As a general principle, it is reasonable to contact participants and offer updated results if the reinterpretation is consistent with the phenotype under study and is reasonably expected to affect a research participant's medical management.

## Release of genetic reports

Concluding a genetic workup with laboratory information alone can generate serious consequences for both individual patients and their families. A dynamic bidirectional exchange of information between laboratory and clinical teams is preferable before releasing diagnostic reports. Moreover, analysis of multigene panels targeting cardiomyopathies often identifies more than one genetic variant potentially associated with the phenotype and the need to issue a formal report within a pre-defined 'turnaround' period means that incomplete interpretation, especially when informative segregation data are missing, is frequent. In such circumstances, completion of the clinical and genetic screening of families (where both carriers and non-carriers of given variants contribute to the computation of the segregation) is essential.<sup>16</sup> Engagement with patients and their families in this endeavour assists in the completion of the segregation study and improves the likelihood of correct interpretation. Clinical screening in the family is generally accepted by relatives, who, regardless of the result of the test, can benefit from either clinical exclusion of the disease or early diagnosis or unexpected diagnosis.

## Cardiomyopathies and levels of actionability of genetic variants

Variants proven to be pathogenic are clinically actionable. For cardiomyopathies, clinical scenarios currently influenced by genetic test results include:

- Clinical monitoring according to the phenotype and genotype:<sup>26</sup> The monitoring of affected and healthy carriers (e.g. children or

young relatives of the proband) should be scheduled according to the age, baseline tests, symptoms, and other non-cardiac traits in the case of syndromic cardiomyopathies.

- Pre-clinical diagnosis: A gene variant recognized as pathogenic and with eventual complete penetrance should be given special attention from the moment of its identification. Very early clinical manifestations can be subtle and recorded only with serial monitoring (e.g. progressive prolongation of the ECG PQ interval, even within normal ranges).<sup>26</sup>
- Pre-symptomatic diagnosis: Early markers of disease or extra-cardiac traits in the case of syndromic cardiomyopathies may be detected in asymptomatic family members.<sup>57–59</sup>
- Early therapy: Administration of treatment to healthy carriers of pathogenic variants is evolving. For cardiomyopathies associated with ventricular or atrial remodelling (dilation, hypertrophy), medications are usually administered when there is an instrumental evidence for disease. This may change in the future with the advent of disease-modifying medication. A recent example is mavacamten in obstructive HCM.<sup>60</sup> Other examples include enzyme replacement therapy in some storage disorders or mitogen-activated protein/extracellular signal-regulated kinase inhibitors (i.e. selumetinib) for RASopathies, including neurofibromatosis type 1, Noonan syndrome, cardiofaciocutaneous syndrome, Costello syndrome, and others.<sup>61,62</sup>
- Personalized treatments: At present, few therapies (in cardiomyopathies) are based on genetic data with the exception of primary prophylactic implantation of cardioverter-defibrillators. However, ongoing trials of new therapeutic strategies, including small molecules and gene therapies, may transform this landscape.<sup>60</sup>
- Lifestyle adjustment: Advice on daily activities and recreational sport activity remains uncertain in healthy carriers<sup>63</sup> but are clear in individuals with evidence for disease expression.
- Pre-natal diagnosis and pre-implantation diagnoses: Identification of a pathogenic variant provides the opportunity for pre-implantation genetic testing.

## Conclusions

Correct clinical interpretation of genetic variants will be one of the key contributors to precision cardiology of the future but requires effective partnerships between clinicians, patients, scientists, and industry to maximize the benefits of genetic knowledge. Importantly, a genetic test supports and confirms, but does not substitute for a clinical diagnosis.

**Conflict of interest:** T.T. declares lecture fees from Takeda, Amicus Therapeutics, and Sanofi-Genzyme. T.T. also reports to be the committee member for the ESC Council for Cardiovascular Genomics, and founder and shareholder of Cardior Pharmaceuticals. Y.P. declares a contract with Roche Diagnostics, consulting fees from Forbion BV Netherlands and Daiichi Sankyo, two patents planned on treatment of cardiomyopathy, and a minor stock (<5%) in University Spin-off. J.W. declares consulting fees from Boehringer Ingelheim and Siemens Healthineers, and honoraria from Daiichi Sankyo. P.E. reports consulting fees from Sarepta, Pfizer, DinaQor, Bristol Myers Squibb (BMS), and Astra Zeneca; honoraria for lectures from Pfizer and BMS. P.E. is the Chairman of the ESC Council for Cardiovascular

Genomics, chair for the ESC Academy, and has participated in the INDORSIA modify ID-069A301 trial (IDMC). A.P. declares payments for advisory board meetings from Bristol Myers Squibb. J.K. reports a grant from the Medical Research Council and consulting fees from DiNAqor and Cytokinetics. K.H.H. declares honoraria from Boehringer Ingelheim.

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

## References

- Prokop JW, May T, Strong K, Bilinovich SM, Bupp C, Rajasekaran S, et al. Genome sequencing in the clinic: the past, present, and future of genomic medicine. *Physiol Genomics* 2018;**50**:563–579.
- Posey JE, O'Donnell-Luria AH, Chong JX, Harel T, Jhangiani SN, Coban Akdemir ZH, et al. Insights into genetics, human biology and disease gleaned from family based genomic studies. *Genet Med* 2019;**21**:798–812.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;**17**:405–424.
- Niehaus A, Azzariti DR, Harrison SM, DiStefano MT, Hemphill SE, Senol-Cosar O, et al. A survey assessing adoption of the ACMG-AMP guidelines for interpreting sequence variants and identification of areas for continued improvement. *Genet Med* 2019;**21**:1699–1701.
- Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;**536**:285–291.
- Abou Tayoun AN, Pesaran T, DiStefano MT, Oza A, Rehm HL, Biesecker LG, et al. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. *Hum Mutat* 2018;**39**:1517–1524.
- Brnich SE, Abou Tayoun AN, Couch FJ, Cutting GR, Greenblatt MS, Heinen CD, et al. Recommendations for application of the functional evidence PVS1/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *Genome Med* 2019;**12**:3.
- Kelly MA, Caleshu C, Morales A, Buchan J, Wolf Z, Harrison SM, et al. Adaptation and validation of the ACMG/AMP variant classification framework for MYH7-associated inherited cardiomyopathies: recommendations by ClinGen's Inherited Cardiomyopathy Expert Panel. *Genet Med* 2018;**20**:351–359.
- Walsh R, Mazzarotto F, Whiffin N, Buchan R, Midwinter W, Wilk A, et al. Quantitative approaches to variant classification increase the yield and precision of genetic testing in Mendelian diseases: the case of hypertrophic cardiomyopathy. *Genome Med* 2019;**11**:5.
- Miller DT, Lee K, Chung WK, Gordon AS, Herman GE, Klein TE, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2021;**23**:1381–1390.
- Favalli V, Serio A, Grasso M, Arbustini E. Genetic causes of dilated cardiomyopathy. *Heart* 2016;**102**:2004–2014.
- Arbustini E, Giuliani L, Di Toro A, Favalli V. Inherited cardiac muscle disease: dilated cardiomyopathy. In Kumar D, Elliott P, eds. *Cardiovascular Genetics and Genomics*. © Springer International Publishing AG 2018, p319–366.
- Biesecker Leslie G, Harrison Steven M. The ACMG/AMP reputable source criteria for the interpretation of sequence variants. *Genet Med* 2018;**20**:1687–1688.
- El Mecky J, Johansson L, Plantinga M, Fenwick A, Lucassen A, Dijkhuizen T, et al. Reinterpretation, reclassification, and its downstream effects: challenges for clinical laboratory geneticists. *BMC Med Genomics* 2019;**12**:170.
- Walsh R, Thomson KL, Ware JS, Funke BH, Woodley J, McGuire KJ, et al. Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. *Genet Med* 2017;**19**:192–203.
- Harrison SM, Rehm HL. Is 'likely pathogenic' really 90% likely? Reclassification data in ClinVar. *Genome Med* 2019;**11**:72.
- Cherny S, Olson R, Chiodo K, Balmert LC, Webster G. Changes in genetic variant results over time in pediatric cardiomyopathy and electrophysiology. *J Genet Couns* 2021;**30**:229–236.
- Evans JP, Skrzynia C, Burke W. The complexities of predictive genetic testing. *BMJ* 2001;**322**:1052–1056.
- Thomson KL, Ormondroyd E, Harper AR, Dent T, McGuire K, Baksi J, et al. Analysis of 51 proposed hypertrophic cardiomyopathy genes from genome sequencing data in sarcomere negative cases has negligible diagnostic yield. *Genet Med* 2019;**21**:1576–1584.
- Ouellette AC, Mathew J, Manickaraj AK, Manase G, Zahavich L, Wilson J, et al. Clinical genetic testing in pediatric cardiomyopathy: is bigger better? *Clin Genet* 2018;**93**:33–40.
- Lawal TA, Lewis KL, Johnston JJ, Heidlebaugh AR, Ng D, Gaston-Johansson FG, et al. Disclosure of cardiac variants of uncertain significance results in an exome cohort. *Clin Genet* 2018;**93**:1022–1029.
- Mazzarotto F, Olivetto I, Walsh R. Advantages and perils of clinical whole-exome and whole-genome sequencing in cardiomyopathy. *Cardiovasc Drugs Ther* 2020;**34**:241–253.
- Bennett JS, Bernhardt M, McBride KL, Reshmi SC, Zmuda E, Kertesz NJ, et al. Reclassification of variants of uncertain significance in children with inherited arrhythmia syndromes is predicted by clinical factors. *Pediatr Cardiol* 2019;**40**:1679–1687.
- Tsai GJ, Rañola JMO, Smith C, Garrett LT, Bergquist T, Casadei S, et al. Outcomes of 92 patient-driven family studies for reclassification of variants of uncertain significance. *Genet Med* 2019;**21**:1435–1442.
- Cuenca S, Ruiz-Cano MJ, Gimeno-Blanes JR, Jurado A, Salas C, Gomez-Diaz I, et al. Genetic basis of familial dilated cardiomyopathy patients undergoing heart transplantation. *J Heart Lung Transplant* 2016;**35**:625–635.
- Years DF, Niemiec E, Howard HC, Borry P. Analysis of VUS reporting, variant reinterpretation and recontact policies in clinical genomic sequencing consent forms. *Eur J Hum Genet* 2018;**26**:1743–1751.
- David KL, Best RG, Brenman LM, Bush L, Deignan JL, Flannery D, et al. Patient recontact after revision of genomic test results: points to consider—a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2019;**21**:769–771.
- Bombard Y, Brothers KB, Fitzgerald-Butt S, Garrison NA, Jamal L, James CA, et al. The responsibility to recontact research participants after reinterpretation of genetic and genomic research results. *Am J Hum Genet* 2019;**104**:578–595.
- Landstrom AP, Kim JJ, Gelb BD, Helm BM, Kannankeril PJ, Semsarian C, et al. Genetic testing for heritable cardiovascular diseases in pediatric patients: a scientific statement from the American Heart Association. *Circ Genom Precis Med* 2021;**14**:e000086.
- Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med* 2013;**15**:565–574.
- Miller DT, Lee K, Gordon AS, Amendola LM, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2021;**23**:1391–1398.
- Mogensen J, Kubo T, Duque M, Uribe W, Shaw A, Murphy R, et al. Idiopathic restrictive cardiomyopathy is part of the clinical expression of cardiac troponin I mutations. *J Clin Invest* 2003;**111**:209–216.
- Oza AM, DiStefano MT, Hemphill SE, Cushman BJ, Grant AR, Siegert RK, et al. Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss. *Hum Mutat* 2018;**39**:1593–1613.
- The Clinical Genome Resource ClinGen Variant Curation SOP Committee. ClinGen general sequence variant curation process standard operating procedure. Version 2.0, January 2021. [https://clinicalgenome.org/site/assets/files/5933/variant\\_curation\\_sop\\_2\\_0\\_jan\\_2021.pdf](https://clinicalgenome.org/site/assets/files/5933/variant_curation_sop_2_0_jan_2021.pdf)
- Paludan-Müller C, Ahlberg G, Ghose J, Svendsen JH, Haunsø S, Olesen MS. Analysis of 60 706 exomes questions the role of de novo variants previously implicated in cardiac disease. *Circ Cardiovasc Genet* 2017;**10**:e001878.
- Mani A. Pathogenicity of de novo rare variants: challenges and opportunities. *Circ Cardiovasc Genet* 2017;**10**:e002013.
- Charron P, Arad M, Arbustini E, Basso C, Bilinska Z, Elliott P, et al. Genetic counselling and testing in cardiomyopathies: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J* 2010;**31**:2715–2726.
- Rapezzi C, Arbustini E, Caforio AL, Charron P, Gimeno-Blanes J, Heliö T, et al. Diagnostic work-up in cardiomyopathies: bridging the gap between clinical phenotypes and final diagnosis. A position statement from the ESC Working Group on Myocardial and Pericardial Diseases. *Eur Heart J* 2013;**34**:1448–1458.
- Robinson PN. Deep phenotyping for precision medicine. *Hum Mutat* 2012;**33**:777–780.
- Qu HQ, Wang X, Tian L, Hakonarson H. Application of ACMG criteria to classify variants in the human gene mutation database. *J Hum Genet* 2019;**64**:1091–1095.
- Ganapathi M, Argyriou L, Martínez-Azorín F, Morlot S, Yigit G, Lee TM, et al. Bi-allelic missense disease-causing variants in RPL3L associate neonatal dilated cardiomyopathy with muscle-specific ribosome biogenesis. *Hum Genet* 2020;**139**:1443–1454.



42. Salazar-Mendiguchía J, Ochoa JP, Palomino-Doza J, Domínguez F, Díez-López C, Akhtar M, et al. Mutations in *TRIM63* cause an autosomal-recessive form of hypertrophic cardiomyopathy. *Heart* 2020;**106**:1342–1348.
43. Ware JS, Amor-Salamanca A, Tayal U, Govind R, Serrano I, Salazar-Mendiguchía J, et al. Genetic etiology for alcohol-induced cardiac toxicity. *J Am Coll Cardiol* 2018;**71**:2293–2302.
44. Ferreira C, Pierre G, Thompson R, Vernon H. Barth syndrome. 9 October 2014 (updated 9 July 2020). In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, Amemiya A, eds. *GeneReviews*® [Internet]. Seattle, WA: University of Washington; 1993–2021.
45. Patterson J, Coats C, McGowan R. Familial dilated cardiomyopathy associated with pathogenic *TBX5* variants: expanding the cardiac phenotype associated with Holt–Oram syndrome. *Am J Med Genet A* 2020;**182**:1725–1734.
46. Favalli V, Disabella E, Molinaro M, Tagliani M, Scarabotto A, Serio A, et al. Genetic screening of Anderson-Fabry disease in probands referred from multispecialty clinics. *J Am Coll Cardiol* 2016;**68**:1037–1050.
47. Diegoli M, Grasso M, Favalli V, Serio A, Gambarin FI, Klersy C, et al. Diagnostic work-up and risk stratification in X-linked dilated cardiomyopathies caused by dystrophin defects. *J Am Coll Cardiol* 2011;**58**:925–934.
48. Arbustini E, Pasotti M, Pilotto A, Pellegrini C, Grasso M, Previtali S, et al. Desmin accumulation restrictive cardiomyopathy and atrioventricular block associated with desmin gene defects. *Eur J Heart Fail* 2006;**8**:477–483.
49. van Tienen FHJ, Lindsey PJ, Kamps MAF, Krapels IP, Ramaekers FCS, Brunner HG, et al. Assessment of fibroblast nuclear morphology aids interpretation of LMNA variants. *Eur J Hum Genet* 2019;**27**:389–399.
50. Narula N, Favalli V, Tarantino P, Grasso M, Pilotto A, Bellazzi R, et al. Quantitative expression of the mutated lamin A/C gene in patients with cardiomyopathy. *J Am Coll Cardiol* 2012;**60**:1916–1920.
51. Pompe Disease Diagnostic Working Group, Winchester B, Bali D, Bodamer OA, Caillaud C, Christensen E, et al. Methods for a prompt and reliable laboratory diagnosis of Pompe disease: report from an international consensus meeting. *Mol Genet Metab*. 2008;**93**:275–281.
52. Suwinski P, Ong C, Ling MHT, Poh YM, Khan AM, Ong HS. Advancing personalized medicine through the application of whole exome sequencing and big data analytics. *Front Genet* 2019;**10**:49.
53. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011;**43**:491–498.
54. Pasotti M, Klersy C, Pilotto A, Marziliano N, Rapezzi C, Serio A, et al. Long-term outcome and risk stratification in dilated cardiomyopathies. *J Am Coll Cardiol* 2008;**52**:1250–1260.
55. Macklin SK, Jackson JL, Atwal PS, Hines SL. Physician interpretation of variants of uncertain significance. *Fam Cancer* 2019;**18**:121–126.
56. Clift K, Macklin S, Halverson C, McCormick JB, Abu Dabrh AM, Hines S. Patients' views on variants of uncertain significance across indications. *J Community Genet* 2020;**11**:139–145.
57. Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, et al. ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). *Eur Heart J* 2014;**35**:2733–2779.
58. Pinto YM, Elliott PM, Arbustini E, Adler Y, Anastasakis A, Böhm M, et al. Proposal for a revised definition of dilated cardiomyopathy, hypokinetic non-dilated cardiomyopathy, and its implications for clinical practice: a position statement of the ESC working group on myocardial and pericardial diseases. *Eur Heart J* 2016;**37**:1850–1858.
59. Corrado D, van Tintelen PJ, McKenna WJ, Hauer RNW, Anastasakis A, Asimaki A, et al. Arrhythmogenic right ventricular cardiomyopathy: evaluation of the current diagnostic criteria and differential diagnosis. *Eur Heart J* 2020;**41**:1414–1429.
60. Olivetto I, Oreziak A, Barriaes-Villa R, Abraham TP, Masri A, Garcia-Pavia P, et al. Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2020;**396**:759–769.
61. Bergqvist C, Wolkenstein P. MEK inhibitors in RASopathies. *Curr Opin Oncol* 2021;**33**:110–119.
62. Gross AM, Frone M, Gripp KW, Gelb BD, Schoyer L, Schill L, et al. Advancing RAS/RASopathy therapies: an NCI-sponsored intramural and extramural collaboration for the study of RASopathies. *Am J Med Genet A* 2020;**182**:866–876.
63. Pelliccia A, Sharma S, Gati S, Bäck M, Börjesson M, Caselli S, et al. 2020 ESC guidelines on sports cardiology and exercise in patients with cardiovascular disease. *Eur Heart J* 2021;**42**:17–96.