Genetics and clinics: together to diagnose cardiomyopathies

Mario Urtis, Alessandro Di Toro, Roberto Osio, Lorenzo Giuliani, Alessandra Serio, Maurizia Grasso, Viola Fergnani, Alexandra Smirnova, Flaminia Aliberti, and Eloisa Arbustini

Transplant Research Area and Centre for Inherited Cardiovascular Diseases, Department of Medical Sciences and Infectious Diseases, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; and University of Texas at Austin, Austin, TX, USA

KEYWORDS
Cardiomyopathy; Genetics; Variant interpretation; ACMG classification

The diagnostic paths of hereditary cardiomyopathies (CMPs) include both clinical and molecular genetics. The first step is the clinical diagnosis that guides the decisions about treatments, monitoring, prognostic stratification, and prevention of major events. The type of CMP [hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy (ARVC)] is defined by the phenotype, and the genetic testing may identify the precise cause. Furthermore, genetic testing provides a pre-clinical diagnosis in unaffected family members and the basis for prenatal diagnosis. It can contribute to risk stratification (e.g. LMNA) and can be a major diagnostic criterion (e.g. ARVC). The test can be limited to a single gene when the pre-test diagnostic hypothesis is based on proven clinical evidence (e.g. GLA for Fabry disease). Alternatively, it can be expanded from a multigene panel to a whole exome or whole genome sequencing when the pre-test hypothesis is a genetically heterogeneous disease. In the last decade, the study of larger genomic targets led to the identification of numerous gene variants not only pathogenic (clinically actionable) but also of uncertain clinical significance (not actionable). For the latter, the pillar of the genetic diagnosis is the correct interpretation of the pathogenicity of genetic variants, which is evaluated using both bioinformatics and clinical-genetic criteria about the patient and family. In this context, cardiologists play a central role in the interpretation of genetic tests, performing the deep-phenotyping of variant carriers and establishing the co-segregation of the genotype with the phenotype in families.

© The Author(s) 2022. Published by Oxford University Press on behalf of the European Society of Cardiology. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
protocols have improved the clinical course of CMPs. Therefore, a patient who was originally non-hypertensive at the diagnosis may develop hypertension later on in life; this would not invalidate the original diagnosis but should be considered as comorbidity. A similar observation applies to the patency of the coronary tree: this criterion is systematically respected at baseline, but coronary atherosclerosis may occur, especially in the presence of risk factors such as hypercholesterolemia which does not exclude the diagnosis of CMP.

The ESC classification distinguishes dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and restrictive cardiomyopathy (RCM). Each type of CMP is then sub-grouped into genetic/familial and non-genetic/non-familial types. Mimics of primary CMPs may be acquired (phenocopies) or have genetic causes different from those typically causing the primary CMP (genocopies). In daily practice, cardiologists suspecting familial CMP first characterize the patient’s phenotype and then activate a path that includes genetic counseling and testing, clinical family screening, and multidisciplinary evaluations tailored to the different diseases, in particular syndromic CMPs.

**Genetics and cardiomyopathies**

DCM is characterized by LV dilatation and systolic dysfunction in the absence of other disorders sufficient to cause global systolic impairment. RV dilatation and dysfunction may be present but are not necessary for the diagnosis. The revised definition of DCM 2015, the ‘hypokinetic non-DCM’ is an ‘attempt to bridge the gap between recent understanding of the disease spectrum and its clinical presentation in relatives, which is key for early diagnosis and the institution of potential preventative measures’. This definition incorporates the diagnostic criteria in both probands and relatives and integrates the possible genetic cause. The causes of DCM are heterogeneous: genetic, primarily caused by defective genes that code for proteins expressed in cardiac myocytes; inflammatory; autoimmune; toxic. In genetic DCM, deep phenotyping and clinical family screening are uniquely useful to identify early instrumental markers of disease in asymptomatic family members and establish the mode of transmission. It can also show cardiac [e.g. atrioventricular (AV) block], extracardiac (e.g. neuromuscular involvement), or systemic manifestations (e.g. syndromic DCM) that may guide the search of the specific genetic causes.

Overall, the genetic test identifies the defect associated with the disease in up to 50% of cases. Although hundreds of genes have been reported as potentially associated with DCM, only a few of them are validated and curated; they should be systematically analyzed in DCM genetic testing (Table 1).

With the few exceptions of rare genetic auto-inflammatory diseases [e.g. familial Mediterranean fever (FMF), tumor necrosis factor receptor associated periodic syndrome (TRAPS), etc.], genetic testing is not indicated in ‘inflammatory’ DCM that is defined by the presence of inflammatory infiltrates in the myocardium and is associated with persistent LV dysfunction and ventricular remodelling. The genetic test should not be performed in patients with ‘toxic’ DCM that may follow the administration of different classes of drugs, in particular chemotherapeutic agents for cancer, or immunotherapies such as immune checkpoint inhibitors and cell therapies such as Chimeric Antigen Receptor T-Cell therapy. In addition, genetic testing is non-indicated in ‘alcoholic, cirrhotic, and liver transplantation-related cardiomyopathies’; however, TTN gene mutation carriers seem to be particularly predisposed to the risk of alcoholic CMP. Finally, genetic testing should be performed in patients with peripartum cardiomyopathy (PPCM)—a new-onset, potentially reversible LV systolic dysfunction that was absent before pregnancy—because PPCM can unmask a genetic DCM.

HCM, with a prevalence ranging between 0.02 and 0.23% in adults, is defined by the presence of an increased left ventricular (LV) wall thickness that is not solely explained by abnormal loading conditions. Clinical manifestations include arrhythmias, HF with preserved EF and, less commonly, evolution through HF with reduced EF, and thromboembolic complications. Symptoms may manifest years after the electrocardiogram or echocardiographic evidence of LVH. The clinical diagnostic path is well known and supported by risk stratification tools.

HCM is the most common cardiac genetic disease which is characterized by locus and allelic heterogeneity (defects in different genes may cause similar phenotypes) (Table 1). Genetic testing is recommended (Class I, Level B) when the disease cannot be explained solely by a non-genetic cause, whether clinical or genetic testing will be used to screen family members. In primary HCM, myocyte hypertrophy depends on defective structural or regulatory components of the sarcomere. At present, the disease gene and mutation do not influence the prescription of specific therapies. Many genocopies may currently benefit from target treatments, in particular, lysosomal storage diseases with intramyocyte accumulation of undigested substrates such as the type II glycogenosis, Anderson Fabry disease, or even genetic iron-storage diseases such as hemochromatosis, either HFE, which is the most common form in Europe, or the other less common forms associated with defects of HJV, SLC40A1, TFR2, FHT1, HAMP, and BMP2 genes. The timely and precise diagnoses provide the basis for a specific treatment that actually modifies the natural history of these diseases. Cardiac amyloidosis is a clinical mimic of HCM characterized by thickening of the cardiac walls and diastolic dysfunction without myocyte hypertrophy: the thickening is due to the extracellular deposition of amyloid fibrils. Genetic testing distinguishes genetic from non-genetic forms, providing the family with the key information for early diagnosis and possible early administration of novel targeted therapies.

ARVC is diagnosed when multiple and combined clinical, pathological and genetic criteria from 6 categories fulfill the requirements for (i) definite, (ii) borderline and (iii) possible diagnosis as per 2010 Task Force (TF) indications. The six categories consist of: (i) global or regional RV dysfunction (LV function and size can be normal), (ii) structural abnormalities (fibro-fatty replacement of myocytes), anomalies of (iii) repolarization or (iv) depolarization, (v) arrhythmias, and (vi) familial history and/or presence of pathogenic genetic variants.
| Definitive | DCM | HCM | ARVC | RCMa |
|-----------|-----|-----|------|------|
| LMNA (AD) | ACTC1 (AD) | DSC2 (AD) | TNNI3 (AD) |
| BAG3 (AD) | ALPK3 (AR) | DSG2 (AD) | DES (SD) |
| DES (AD) | MYBPC3 (AD) | DSP (AD) | BAG3 (AD) |
| FLNC (AD) | MYH7 (AD) | PKP2 (AD) | FLNC (AD) |
| MYH7 (AD) | MYL2 (AD) | TMEM43 (AD) | MYL2 (AD) |
| RBM20 (AD) | MYL2 (AD) | | |
| SCN5A (AD) | MYL3 (AD) | | |
| TNN1C1 (AD) | PRKAG2 (AD) | | |
| TNN1T2 (AD) | TNN13 (AD) | | |
| TPM1 (AD) | TTN2 (AD) | | |
| TPML (AD) | | | |
| Moderate | | | |
| ACTC1 (AD) | CSRP3 (AD) | DES (AD) | TNN1T2 (AD) |
| JPH2 (SD) | JPH2 (AD) | PLN (AD) | TNN1C1 (AD) |
| NEXN (AD) | TNN1C1 (AD) | | |
| TNN13 (AD) | | | |
| TPM1 (AD) | | | |
| VCL (AD) | | | |
| Limited | | | |
| ABCC9 (AD) | ANKRD1 (AD) | CDH2 (AD) |
| ANKRD1 (AD) | CALR3 (AD) | CTNA3 (AD) |
| CSRP3 (AD) | KLF10 (AD) | LMNA (AD) |
| CTF1 (AD) | MYH6 (AD) | MYBPC3 (AD) |
| DSG2 (AD) | MYLK2 (AD) | MYH7 (AD) |
| DTNA (AD) | MYOM1 (AD) | MYL3 (AD) |
| EYA4 (AD) | MYOZ2 (AD) | SCN5A (AD) |
| GATA1 (AR) | MYPN (AD) | TGF83 (AD) |
| ILK (AD) | NEXN (AD) | TJP1 (AD) |
| LAMA4 (AD) | PDLIM3 (AD) | TTN (AD) |
| LDB3 (AD) | RYR2 (AD) | | |
| MYBPC3 (AD) | TCAP (AD) | | |
| MYH6 (AD) | TRIM63 (AD) | | |
| MYL2 (AD) | TTN (AD) | | |
| MYPN (AD) | VCL (AD) | | |
| NEBL (AD) | | | |
| NKX2-5 (AD) | | | |
| OBSCN (AD) | | | |
| PLEKH2 (AR) | | | |
| PRDM16 (AD) | | | |
| PKHD1 (AD) | | | |
| Refuted | | | |
| TMPO (AD) | | | |
| No known disease relationship | | | |
| NPPA (AR) | ACTA1 (AD) | RYR2 (AD) |
| LRRC10 (AR) | TMPO (AD) | ACTC1 (AD) |
| MIB1 (AD) | TNN1C2 (AD) | MYL2 (AD) |
| TTN13 (AD) | | | |
| TNN1T2 (AD) | | | |
| TPM1 (AD) | | | |
| Dosage sensitivity | FLNC (HI) | | |
| DSP (HI) | | | |

AD, autosomal dominant; AR, autosomal recessive; HI, haploinsufficiency; SD, semi-dominant.

*ClinGen: RCM has not yet validated gene-disease correlations.
Pathologically, ARVC is defined by the presence of progressive replacement of RV myocardium with adipose and fibrous tissue often confined to a ‘triangle of dysplasia’ comprising the RV inflow, outflow, and apex.\textsuperscript{16} The disease is typically caused by defects in genes coding desmosome proteins (\textit{Table 1}). A new classification proposal distinguishes the classic ARVC, the biventricular and left-dominant variants; the three variants are unified under the term ‘arrhythmogenic cardiomyopathy’ (ACM).\textsuperscript{17} This term, however, could encompass all cardiomyopathies, as all cardiomyopathies are potentially arrhythmogenic. In ARVC, the main clinical issue is the risk of life-threatening arrhythmias. ARVC less commonly causes HF. However, right ventricular ejection fraction can be severely impaired and complicated by pulmonary thromboembolic events, with heart transplantation required even in patients with normal LV size and function. Genotypes include RVOT tachycardia, and Brugada Syndrome, while phenocopies include sarcoidosis, Chagas Disease, and left dominant myocarditis.\textsuperscript{16}

\textbf{RCM} is characterized by ‘restrictive ventricular physiology in the presence of normal or reduced diastolic volumes of one or both ventricles, normal or reduced systolic volumes, and normal ventricular wall thickness’.\textsuperscript{1} Restrictive ventricular physiology occurs when increased myocardial stiffness causes ventricular pressure to rise precipitously with only a small increase in volume. Atrial enlargement is an early common marker and a useful predictor of atrial arrhythmias. Beyond RCM, restrictive ventricular physiology can be observed in hypertrophic and dilated cardiomyopathies, in endomyocardial diseases, as well as in epi-pericardial diseases.\textsuperscript{18} Primary RCM are rare genetic diseases caused by defects of genes encoding for sarcomeric structural and regulatory proteins, Z-disk proteins, and intermediate filaments (\textit{Table 1}). The most common disease gene is \textit{TNNI3} that encodes the thin filament Troponin I.\textsuperscript{19,20} Mutations in children can \textit{de novo} and be associated with severe phenotypes leading to early heart transplantation. Restrictive ‘cardio-myobibrillar diseases’ such as desmopathies caused by gain of function defects in \textit{DES} gene, typically show conduction disease requiring PM implantation, and myopathy, either subclinical or overt.\textsuperscript{20} Recently, RCM phenotypes have been associated with defects of \textit{BAG3}, \textit{FLNC}, and \textit{RBM20} genes.\textsuperscript{18} Genocopies, partly overlapping with HCM, include e.g. lysosomal or iron storage diseases, while phenocopies include drug toxicity e.g. hydroxychloroquine drug toxicity. Genetic testing should be performed in both primary RCM and genetic phenocopies, while toxic causes should be recognized and removed to restore myocardial function.

\section*{Genetic testing}

\textbf{Protagonist or contributor to the diagnosis}

The type of CMP is defined by its phenotype and not by its genetic cause. Regardless of the genetic test and its results, the CMP remains defined by its phenotype that guides the clinical decisions: treatments, monitoring, prognostic stratification, and prevention of major events. Genetic testing confirms clinical diagnoses; in families, it provides the pre-clinical diagnostic basis in still unaffected young members; for couples with procreative programmes, it provides the basis for prenatal diagnosis. Genetic test can contribute to risk stratification: some genes (typically LMNA) are clinically actionable contributors to primary SD prevention decisions.\textsuperscript{21} Finally, in ARVC, a positive genetic test (pathogenic variant in a validated disease gene) is a major diagnostic criterion.\textsuperscript{20}

Although many disease genes have been proposed in the last 20 years, relatively few are classified as ‘definitive’ (\textit{Table 1}). This does not diminish the importance of genes with moderate or limited evidence of association with CMP but rather calls for the implementation of careful analysis of phenotypes and their segregation with genotypes. In this regard, cardiologists have an essential role\textsuperscript{22} in the validation of the pathogenicity criteria, especially for variants of uncertain significance (VUS).\textsuperscript{23} Importantly, apparently sporadic phenotypes do not exclude a genetic cause that can be a \textit{de novo} gene defect\textsuperscript{22} and, in turn, can be transmitted to the progeny.

\section*{From single gene to whole genome sequencing}

The genetic test can be limited to a \textit{single gene} when the pre-test diagnostic hypothesis is based on proven clinical evidence; the test specifies the defect causing the disease. Typical examples are the X-linked Anderson Fabry Disease and the Autosomal Dominant \textit{TTR} cardiac amyloidosis. The former is caused by defects in the \textit{GLA} gene and is characterized by a combination of multi-organ/tissue traits induced by specific intracellular GB3 accumulation, which is easily detectable with simple skin punch biopsy in systemic forms or with endomyocardial biopsy in late-onset cardiac variants. In the latter, easily diagnosed with tissue biopsy, the genetic test distinguishes the \textit{ATTR}\textsuperscript{\textit{A}} from \textit{ATTR}\textsuperscript{\textit{C}} and then provides the basis for family care, early diagnoses, and timely treatments to prevent the progression of cardiac amyloid deposits.

When the pre-test hypothesis is a genetically heterogeneous disease, the genetic test analyzes all validated and candidate disease genes with \textbf{multigene panels} using NGS technologies. The number of genes analyzed in these tests varies from a few tens to hundreds, depending on the choice of labs that can use pre-defined commercially available or customized multigene panels, which are built, based on the experience of the individual labs. These tests are largely used for cardiomyopathies, aneurysmal diseases, neuromuscular, renal, rheumatologic, auto-inflammatory diseases, and malignancies. When the test is hypothesis-free or the aim of the test is searching for novel candidate genes, the genetic test is performed with NGS of the whole \textbf{exome} (WES) or the \textbf{whole genome} (WGS). The former test provides information only on the coding regions of all known genes, and selectively analyzes a wide spectrum of candidate genes, validated and provisional. WES can be performed in singletons, although it should ideally analyze trios (Trio-WES analysis: usually, parents and an affected child, or other combinations of living affected
and non-affected members of the family, depending on suitable family members). The latter test analyzes both coding and non-coding regions of the human genome, thus providing the sequence of the entire genome of a subject, including variants occurring in deep intronic regions that are not captured by exome sequencing. WES and WGS are on the border between diagnostics and research, more commonly addressing research queries and therefore supported by dedicated funds as they increase the costs in face of non-predictable diagnostic benefits with respect to all multigene panels targeted to the given disease.

### Interpretation of genetic variants

#### The ACMG criteria

The correct interpretation of the pathogenicity of genetic variants is the pillar of genetic diagnosis. Compared to the past, however, the identification of a rare gene variant does not prove its pathogenicity and the causal relationship with the phenotype observed in the individual patient and in the families. The interpretation path can be difficult in the absence of precise interpretation criteria. For this reason, in 2015, the ACMG, in collaboration with the AMP, published guidelines for the

| Table 2 Integrated information contributing to the interpretation of pathogenicity of genetic variants in cardiomyopathies |
| --- |
| **Table 2 Integrated information contributing to the interpretation of pathogenicity of genetic variants in cardiomyopathies** |
| **Genetic visit (proband and relatives)** | **Genetic counselling** | **Genetic testing** |
| Anthropometric data; traits suggesting syndromic CMPs; examination of clinical reports | Collection of clinical data from the family; Pedigree construction; Mendelian or matrilineal inheritance | After informed and signed consent: genetic test, single gene or multigene panel or WES or WGS. Cascade genetic testing in relatives: preclinical diagnosis and segregation studies |
| Clinical screening of relatives, irrespective of genetic testing: Early clinical diagnosis | Same information provided to probands: to be given to relatives |

#### The gene

The defect affects a gene known as associated with the observed phenotype

#### The phenotype

The phenotype is characterized by traits typically associated with defects of the gene

#### The family

The family studies concordantly support the cause/effect impact of the variant

#### The type of genetic variant

The type of gene defect predicts the effect on the protein

### Types of gene defects and predicted effects on the protein amount, structure, and function

| Types of gene defects and predicted effects on the protein amount, structure, and function |
| --- |
| **Single nucleotide defect** | **Ins/Del of one or more nucleotides** | **Copy number variation -> up to whole gene (or >)** |
| Missense: most common in CMPs; nonsense: truncating effect | Out-of-frame: loss of triplet reading, predicted truncation | Segments of the gene are duplicated or deleted |
| Splice sites: canonical or cryptic splice sites | In frame: preserved reading frame splicing defects: possible |
| Synonymous: silent or rarely affecting crypt splice sites |

### In vivo functional evidence of pathogenicity of genetic variants (patient samples)

| **Direct functional evidence: biomarkers** |
| Disease-specific biomarkers unique to the disease (e.g. enzyme activity in lysosomal diseases) |
| **Indirect functional evidence:** |
| The involvement of non-cardiac organs and tissues: skeletal muscle, liver, kidney, metabolism, etc. |
| **Direct pathological evidence:** |
| Myocardial samples in diseases whose defects have corresponding pathologic markers |

### Animal models and in vitro functional evidence of pathogenicity of genetic variants

| **Knock-in animal model: variant induced by CRISP-CAS methods** |
| Heart phenotype studies (e.g. cardiac hypertrophy in MYH7 defect mouse); tissue pathophysiology studies; pathophysiological evidences; survival studies (e.g. HF in mice) |
| **Knock-in cellular model: variant induced by CRISP-CAS methods** |
| Electrophysiology studies (e.g. contraction capacity); morphologic studies; pathological evidences (e.g. lysosomal accumulations in GLA positive cells); survival studies |
| **Patient-derived cellular models** |
| Structural studies (e.g. crystallography); mechanical studies (e.g. motility assays in MYH7); biochemical studies (e.g. protein degradation rate or enzymatic product quantification); expression studies (IGC, ICC, etc.) |

---

*E.g. MYBPc3 deep intronic splice variants.

*E.g. Dystrophinopathies, laminopathies, Danon Disease, Anderson Fabry Disease, Pompe disease, myofibrillar desminopathies, amyloidosis, iron storage diseases, etc.*
interpretation of the genetic variants to generate a robust and reproducible model for the classification of variants’ pathogenicity. The ACMG system introduced five pathogenicity classes: pathogenic (P), likely pathogenic (LP), variable of uncertain significance (VUS), likely benign (LB), and benign (B) achieved through the combination of 28 criteria, 16 in favour of a pathogenic role and 12 in favour of a benign role. VUS and LB-B variants are not clinically actionable. Each criterion was assigned an associated strength that reflects the level of the evidence supporting the classification. The ACMG criteria can be grouped into bioinformatics criteria and clinical-genetic criteria. The former criteria evaluates the type of the mutated variant (truncation-predicting, missense, indel, intronic or synonymous variants), the gene locus (mutational hot-spots or homopolymer zones), or proteins (e.g. enzymatic sites, sites of protein interaction, etc.), the frequency of the variant in the healthy population, previous reputable information on the pathogenicity of the given or similar variants and the effect predicted by in silico tools. The activation of bioinformatics criteria can be carried out automatically by algorithms based on rules and thresholds that grade the strength of the different criteria; a gene-specific adaptation of ACMG rules is ongoing to optimize the classification system. The latter criteria evaluates the phenotype and genotype of carriers of the variant, the segregation of the variant into families, and the result of functional in-vitro, in-vivo, and ex-vivo studies (Table 2).

The role of cardiologists in contributing to the interpretation of variants pathogenicity
At least 8 of the 16 ACMG/AMP criteria of pathogenicity are based upon clinical/phenotypic data and family segregation studies. This data is only correctly gathered by clinicians looking for coherent evidence supporting and reinforcing the classification of gene variants identified in a laboratory at their request. In the case of suspected de novo variants in an affected individual, the ACMG PS2 criterion can be activated only after the evaluation of both parents (proven de novo variants). Vice versa, the PM6 criterion applies when the variant appears to be de novo but paternity and/or maternity cannot be confirmed (assumed but not proven de novo variants). The evidence of co-segregation of a variant in families can greatly support its pathogenicity. As many relatives as possible should be deep-phenotyped, due to the age-related penetrance of most cardiomyopathies. Also, the co-segregation of a VUS (so defined at first evaluation) in a family may require longitudinal follow-up of multiple family members. When a variant occurs in a gene that is definitely known to cause the disease and co-segregates in multiple affected family members, the PP1 criterion applies. Conversely, in case a family member is affected being a non-carrier of the genetic variant interpreted as disease causing in the family, the non-segregation BS4 applies. In the case of healthy adults and older healthy carriers of a VUS, the BS2 (non-segregation) criterion applies. Finally, the PP4 criterion is applied when the phenotype of the variant carrier is highly specific for a unique genetic aetiology. This rarely happens in cardiomyopathies, being most of them are characterized by genetic heterogeneity and the frequent occurrence of non-specific phenotypes. The characterization of gene-specific phenotypes may be supported by clinical red flags when present.

Conclusions
The genetic tests are an integral part of the diagnostic path of cardiomyopathies which, in different proportions for the various cardiomyopathies (HCM, DCM, RCM, ARVC), are caused by defects of genes encoding structural or regulatory proteins of the myocytes. Each sub-type of CMP has corresponding genocopies and phenocopies, both with possible target treatments that depend on the identification of the precise cause.

The recent expansion of NGS-based genetic tests has led to the identification of numerous gene variants not only pathogenic (clinically actionable) but also of uncertain clinical significance (not actionable) whose interpretation largely depends on the contribution of the clinical cardiologist with both deep-phenotyping of probands and segregation studies in families.

Funding
Studies on cardiomyopathies are supported by continuous RC funds on heritable cardiomyopathies from the Ministry of Health to the Fondazione IRCCS Policlinico San Matteo: SOMICS (888-rcr2017i-71); iPS cell modelling for cardiac genetic diseases and drug screening (961-rcr2019i2-71).

Conflict of interest: None declared.

References
1. Elliott P, Andersson B, Arbustini E et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology working group on myocardial and pericardial diseases. Eur Heart J 2008;29:270-276.
2. Repetto A, Dal Bello B, Pasotti M et al. Coronary atherosclerosis in end-stage idiopathic dilated cardiomyopathy: an innocent bystander? Eur Heart J 2005;26:1519-1527.
3. Rapeschi C, Arbustini E, Cafforio AL 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.
4. Charron P, Arad M, Arbustini E et al. European Society of Cardiology working group on myocardial and pericardial diseases. 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.
5. Pinto YM, Elliott PM, Arbustini E 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.
6. Favalli V, Serio A, Grasso M, Arbustini E. Genetic causes of dilated cardiomyopathy. Heart 2016;102:2004-2014.
Arbustini E, Di Toro A, Giuliani L et al. Cardiac phenotypes in hereditary muscle disorders: JACC state-of-the-art review. J Am Coll Cardiol 2018;72:2485-2506.

Caforio AL, Pankuweit S, Arbustini E et al. European Society of Cardiology working group on myocardial and pericardial diseases. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology working group on myocardial and pericardial diseases. Eur Heart J 2013;34:2636-2648.

de Boer RA, Hulot JS, Tocchetti CG et al. Common mechanistic pathways in cancer and heart failure. A scientific roadmap on behalf of the translational research committee of the heart failure association (HFA) of the European Society of Cardiology (ESC). Eur J Heart Fail 2020;22:2272-2289.

Ware JS, Amor-Salamanca A, Tayal U et al. Genetic etiology for alcohol-induced cardiac toxicity. J Am Coll Cardiol 2018;71:2293-2302.

Ware JS, Li J, Mazaika E, Yasso CM et al. IMAC-2 and IPAC investigators. Shared genetic predisposition in peripartum and dilated cardiomyopathies. N Engl J Med 2016;374:233-241.

Elliott PM, Anastasakis A, Borger MA et al. 2014 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.

O’Mahony C, Jichi F, Ommen SR et al. International external validation study of the 2014 European society of cardiology guidelines on sudden cardiac death prevention in hypertrophic cardiomyopathy (EVIDENCE-HCM). Circulation 2018;137:1015-1023.

Olivotto I, Orefziak A, Barriales-Villa R et al. EXPLORER-HCM study investigators. Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2020;396:759-769.

Garcia-Pavia P, Rapezzi C, Adler Y. Diagnosis and treatment of cardiac amyloidosis: a position statement of the ESC working group on myocardial and pericardial diseases. Eur Heart J 2021;42:1554-1568.

Marcus FI, McKenna WJ, Sherrill D et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. Eur Heart J 2010;31:806-814.

Corrado D, van Tintelen PJ, Mckenna WJ; international experts. Arrhythmogenic right ventricular cardiomyopathy: evaluation of the current diagnostic criteria and differential diagnosis. Eur Heart J 2020;41:1414-1429.

Arbustini E, Di Toro A, Giuliani L. Restrictive heart diseases. In: Fuster V, Narula J, Vaishnava P, Leon MB, Callans DJ, Rumsfeld J, Poppas A. eds. Fuster and Hurst’s the Heart, 15e. McGraw Hill; 2022.

Mogensen J, Hey T, Lambrecht S. A systematic review of phenotypic features associated with cardiac troponin I mutations in hereditary cardiomyopathies. Can J Cardiol 2015;31:1377-1385.

Arbustini E, Pasotti M, Pilotto A. Desmin accumulation restrictive cardiomyopathy and atrioventricular block associated with desmin gene defects. Eur J Heart Fail 2006;8:477-483.

Priori SG, Blomström-Lundqvist C, Nazzanti A, ESC Scientific Document Group. 2015 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: the task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC), endorsed by: association for European Paediatric and Congenital Cardiology (AEPc). Eur Heart J 2015;36:2793-2867.

Arbustini E, Behr ER, Carrier L. Interpretation and actionability of genetic variants in cardiomyopathies: a position statement from the European society of cardiology council on cardiovascular genomics. Eur Heart J 2022;43:1901-1916.

Richards S, Aziz N, Bale S. Bick D; ACMG laboratory quality assurance committee. 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.