Defining phenotypes and disease progression in sarcomeric cardiomyopathies: contemporary role of clinical investigations

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Mutations in cardiac sarcomere protein genes are associated with a variety of clinical phenotypes, including hypertrophic (HCM), dilated (DCM), and restrictive (RCM) cardiomyopathy as well as left ventricular non-compaction, with the overlap of morpho-functional manifestations in individual patients and families. Over time, initial phenotypes may undergo profound changes which determine clinical course and disease progression. Although genetic defects causing HCM and DCM have opposite effects at the myofilament level, a number of downstream maladaptive mechanisms, ranging from microvascular dysfunction and ischaemia to myocardial fibrosis and from diastolic dysfunction to abnormal sympathetic activation and arrhythmogenesis, seem to recur in sarcomeric cardiomyopathies, independent of the presenting phenotype. The extent and rate at which each of these features occur and evolve may be radically different in each form of cardiomyopathy, determining a clinical heterogeneity that is not only cross-sectional, but also longitudinal, i.e. time-related. Timely and sensitive detection of these long-term modifications in the clinical setting is a key to preventing advanced disease and identifying novel therapeutic targets. The present review evaluates the contribution of contemporary technology to pre-clinical diagnosis, characterization of phenotypes, and assessment of disease progression in sarcomere cardiomyopathies, including echocardiography, positron emission tomography, magnetic resonance, pathology, and circulating biomarkers.

Keywords
Cardiomyopathies • Sarcomere • MRI • PET

1. Introduction

Mutations in cardiac sarcomere protein genes are associated with a constellation of clinical phenotypes, whose heterogeneity contrasts sharply with a stereotyped molecular basis (Figure 1 and Table 1). The most common and best known form is represented by hypertrophic (HCM), dilated (DCM), and restrictive (RCM) cardiomyopathy as well as left ventricular non-compaction (LVNC) (Figure 1).1–3 Sarcomere mutations causing HCM and DCM have opposite functional effects at the myofilament level (i.e. enhanced as opposed to reduced calcium sensitivity and power output, respectively); RCM appears at the genetic and biophysical level to be within the spectrum of HCM, whereas LVNC is often associated with LV remodelling and dysfunction resembling DCM.4 Although each of these phenotypes is distinct and has different clinical implications, there is an ample overlap of morpho-functional manifestations occurring within individual patients and families, which ultimately reflect the common genetic background.5 Mixed phenotypes may occur because different features are present from the initial development of disease, as in the case of regional LVNC which may associate with virtually any other cardiomyopathy. Alternatively, they may represent the final result of long-term disease progression, as observed, for example, in patients with the overlap HCM/RCM phenotype caused by troponin I mutations, or in the rare end-stage HCM forms resembling DCM.

Over time, initial phenotypes undergo profound changes which determine clinical course and disease progression.5 A number of downstream maladaptive features, ranging from the prolongation of cardiomyocyte action potential to microvascular dysfunction, from intracellular calcium...
abnormalities to dysregulation of collagen turnover, and from energetic derangement to abnormal sympathetic activation, seem to recur in cardiomyopathies caused by sarcomere gene mutations, although with mechanisms which can be radically different based on the effects of the causing gene defect and the presenting phenotype. The extent and the rate at which each of these features occur and evolve are very variable in each form of cardiomyopathy—and within individual patients—determining a clinical heterogeneity that is not only cross-sectional, but also longitudinal, i.e. time-related. These manifestations suggest that sarcomere gene mutations may act as strong disease drivers or modifiers, but that their ultimate clinical expression is determined by a complex hierarchy of genetic, epigenetic, and environmental factors.

Understanding the long-term modifications of clinical phenotypes is a key for the identification of disease mechanisms and the prevention of disease progression. Constant advances in the epidemiology, clinical characterization, imaging, and laboratory work-up have considerably enriched our perception of cardiomyopathies, allowing increasing confidence in the diagnosis of atypical phenotypes and improving our acuity in detecting signs of deterioration. Aim of this work, largely focusing on HCM, is to review the contribution of contemporary technology to the characterization of phenotypes and the assessment of disease progression. A detailed review of differential diagnoses of cardiomyopathies is beyond the scope of our work and has been exhaustively addressed by recent consensus documents. Furthermore, indications, methodologies, and interpretation of genetic testing in HCM are reviewed in this issue by Ho et al. and in previous reviews on the subject and will not be addressed in the present work.

2. Overview of clinical phenotypes

2.1 Hypertrophic cardiomyopathy

HCM was the first disease linked to sarcomere gene mutations, in 1990, and has currently been associated with ~1500 mutations in 11 genes.
Table 1 Pattern of inheritance, diagnostic yield, and causative genes associated with the four cardiomyopathy phenotypes

<table>
<thead>
<tr>
<th>Clinical phenotypes</th>
<th>Inheritance</th>
<th>Genetic heterogeneity</th>
<th>Diagnostic yield</th>
<th>Causative genes</th>
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<tr>
<td>HCM</td>
<td>AD, AR, X-linked, mitochondrial (rare)</td>
<td>&gt;10 genes &gt;1000 variants</td>
<td>40–65%</td>
<td>Sarcomeric protein mutations: MYBPC3, MYH7, TNNT2, TNNI3, MYL2, TPM1, MYL3, ACTC1 Other: CSR3, CAV3, JPH2, OBSCN, ACTN2, MYH6, TTN, TNNC1, Glycogen and lysosomal storage diseases: PRKAG2, LAM2P, GLA, etc. Infiltrative disorders: familial amyloidosis (TTR, etc.) Mitochondrial DNA Syndromic HCM: PTPN11, FRDA, etc. Sarcomeric protein mutations: same as HCM Cytoskeletal genes: DMD, DES, VCL; Sarcomyosin complex: Epicardin EYA4 Z-band: MLCP, TAC Nuclear membrane: LMNA, EMD. Mitochondrial DNA Intercalated disc protein mutations: JUP, DSP, PKP2, DSG2, DSC2, RyR2; TGFb3 Other: TAZ, PSENI1–2, MYPN, NEXN, PLN</td>
</tr>
<tr>
<td>DCM</td>
<td>AD, AR, X-linked, mitochondrial (infrquent)</td>
<td>20–30%, including titin (higher in syndromic) &gt;30 genes &gt;400 variants</td>
<td>Sarcomeric protein mutations: same as HCM (especially TNNI3 and MYH7) Others: DES, TTR and APOE; HFE, DES Sarcomeric protein mutations: same as HCM Other: LDB3, DTNA, TAZ</td>
<td></td>
</tr>
<tr>
<td>RCM</td>
<td>AD</td>
<td>Genetic origin possible</td>
<td>Unknown</td>
<td>Sarcomeric protein mutations: same as HCM Other: LDB3, DTNA, TAZ</td>
</tr>
<tr>
<td>LVNC</td>
<td>AD, X-linked</td>
<td>&gt;7 genes &gt;20 variants</td>
<td>Low (15–25%)</td>
<td>Sarcomeric protein mutations: same as HCM Other: LDB3, DTNA, TAZ</td>
</tr>
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ACTC1 (actin); ACTN2 (α-actinin-2); APOE (apolipoprotein E); CSR3 (Cysteine And Glycine-Rich Protein 3 - Cardiac LIM Protein); CRYAB (Crystalin, Alpha B chain); CAV3 (caveolin 3); DMD (dystrophin); DES (desmin); DSG2–3 (desmogloin); DTNA (a-dystrobrevin); DSC2 (desmocollin2); DSP (desmoplakin); EMD (emerin); FRDA (frataxin); GLA (alpha galactosidase); HFE (haemochromatosis); JUP (plakoglobin); JPH-2 (junctophilin-2); LAMP2 (lysosome-associated membrane protein2); LMNA (lamin A/C); LDB3 (LIM domain binding 3); MYBPC3 (myosin-binding protein C); MYH7 (beta myosin heavy chain); MYL2 (ventricular myosin regulatory light chain); MYL3 (ventricular myosin essential light chain); MLP (muscle LIM protein); MYPN (myopalladin); MYH6 (α-myosin heavy chain); NEXN (nexilin).

encoding proteins of the thick and thin contractile myofilament components or the adjacent Z-disc. It is the most common genetic heart disease, characterized by complex pathophysiology, heterogeneous morphology, and variable clinical manifestations over time. HCM is defined by the presence of primary LV hypertrophy, not explained by other cardiac or systemic conditions. Hypertrophy is typically regional and asymmetric, usually involving the basal septum and anterior LV wall, but developing in virtually all imaginable patterns and occasionally involving the right ventricle and papillary muscles. Wall thickness may vary abruptly in adjacent regions of the LV, and non-contiguous hypertrophic segments separated by areas of normal thickness have been described. In addition, the HCM phenotype is characterized by the variable interplay of diastolic dysfunction, mitral valve and subvalvar abnormalities, dynamic LV outflow and mid-ventricular obstruction, right ventricular outflow obstruction, atrial remodelling, myocardial crypts, bridging of the epicardial coronary arteries, apical aneurysms, and autonomic nervous system abnormalities. Genetic testing for HCM has been available for over two decades and is now largely available following the introduction of next-generation sequencing. Despite early hopes that genotyping may prove useful for the prediction of cardiac morphology and long-term outcome, dedicated studies have failed to establish stringent relationships between specific sarcomere myofilament genes, phenotype, and outcome. Some generic genotype–phenotype correlations have been identified: these include a particularly high yield of genotype-positive individuals among patients with reverse septal curvature, elevated penetrance and marked hypertrophy associated with MYH7 mutations, mild hypertrophy but enhanced arrhythmic propensity in patients with TNNT2-related disease and early onset, marked diastolic dysfunction and atypical localization of hypertrophy in thin filament disease, severe phenotype and adverse outcome in patients with complex genotypes featuring multiple mutations. Nevertheless, all these principles are non-specific and have countless exceptions. In most patients, HCM affords normal longevity, with only mild symptoms and stable clinical course. However, a life-long remodelling process occurs within the myocardium which, in a substantial subset, leads to clinical progression and may culminate in the so-called end-stage phase in ~5–7%. Four stages of disease have been proposed for clinical and investigational purposes (Figure 2). (i) ‘Non-hypertrophic HCM’ is a state characterized by the absence of LV hypertrophy in individuals harbouring HCM-causing mutations, investigated in the course of systematic family screenings. Individuals in this stage are defined ‘genotype-positive/
Regrettably, subtle morphological abnormalities are often encountered in this group, consistent with subclinical phenotypic expression. (ii) 'Classic HCM phenotype' is defined as the phase in which the hypertrophy is fully developed and the LV is hyperdynamic—often promoting outflow obstruction—in the absence of significant fibrotic changes. (iii) 'Adverse remodelling', occurring in ~15% of patients, is characterized by unfavourable structural modifications, superimposed to the ‘classic’ phenotype, translating into extensive LV fibrosis and initial evidence of functional impairment (i.e. an LVEF in the low-normal range of 50–65%), with preserved clinical and hemodynamic balance. (iv) 'Overt dysfunction', occurring in ~5–7% of patients, characterized by severe functional deterioration of the LV (defined as overt systolic dysfunction and/or restrictive pathophysiology), subtended by extreme degrees of LV fibrosis and atrial dilatation, associated with haemodynamic decompensation and adverse outcome. Rather than being an ‘average’ process, progression of HCM is a selective pathway followed by a subset of HCM patients, which may occur at any age and lead to advanced heart failure over years or decades.6,19

In paediatric patients, HCM recognizes more heterogeneous and complex aetiologies compared with the adult population.20 In more than 800 newly diagnosed children from the Pediatric Cardiomyopathy Registry, HCM was associated with a malformation syndrome in 9%, inborn errors of metabolism in 9%, and neuromuscular disorders in 8%.21 An aetiological diagnosis is clinically relevant in paediatric patients, as outcome varies greatly by cause and age at diagnosis.22 Children with HCM associated with an error of metabolism or malformation syndrome, both of which present at a younger age, had low 5-year survival rates of 42 and 74%, respectively, when compared with 98% in those with neuromuscular disorders, which normally present at an older age. Among children with idiopathic HCM, 5-year survival was 94% for those diagnosed after 1 year of age but only 82% for those diagnosed before 1 year of age. Sudden death occurred in 44% of children diagnosed before 1 year of age but in all those diagnosed >1 year.21

### 2.2 Restrictive cardiomyopathy

In the spectrum of sarcomere diseases, a restrictive phenotype may represent the most advanced stage of disease progression in HCM patients carrying mutations in thin filament protein genes16 or complex phenotypes.7 However, a primary presentation as true RCM has been described associated with Troponin I mutations, characterized by a small LV with normal wall thickness, extreme diastolic dysfunction, and marked bi-atrial dilatation.23 Idiopathic RCM is a rare entity, which may present from infancy to late adulthood, often associated with severe functional limitation and progressive clinical course leading to heart failure-related death or cardiac transplantation.23–25 Outcome is poor, especially in children, with a 5-year survival rate from diagnosis around 65%.24–26 Endomyocardial biopsy demonstrates non-specific findings such as myocyte hypertrophy, interstitial fibrosis, and occasionally endocardial fibrosis. The differential diagnosis of idiopathic RCM includes infiltrative cardiomyopathies—particularly amyloidosis—endomyocardial fibrosis and constrictive pericarditis.24

### 2.3 Dilated cardiomyopathy

In 2000, Kamisago et al.26 for the first time showed cosegregation for mutations in β-myosin heavy chain and troponin T, in pedigrees with DCM. Subsequent studies have repeatedly identified sarcomere gene mutations in DCM families, as recently reviewed by McNally et al.27 Prior to the introduction of next-generation sequencing techniques, sarcomere mutations have been identified in 25% of idiopathic DCM cases26 and account for 10% of familial DCM.28 The most common sarcomere genes identified in familial DCM are β-myosin heavy chain (MYH7) (10%) and Troponin T (TNNT2) (3%), followed by low-prevalence genes such as α-tropomyosin (TPM1), troponin C (TNNC1), troponin I (TNNI3), and cardiac actin (ACTC).27,29 Autosomal recessive familial DCM has also been caused by mutations in cardiac troponin I (TNNI3).30 Recently, next-generation sequencing technology for the first time allowed to screen the giant sarcomeric gene coding for titin (TTN). As a result, disruptive TTN mutations were found in up to 25% of DCM index patients, changing this landscape significantly.31 However, it remains to be established how TTN mutations translate into disease, as many variants and even truncations may be found also in healthy controls.

Clinically, DCM caused by sarcomere gene mutations is indistinguishable from other forms of idiopathic DCM and lacks the ‘red flags’ found in laminopathies and dystrophin-related and mitochondrial disease, such as elevated CK levels, skeletal muscle involvement, conduction blocks, or hearing impairment.8 However, sarcomeric DCM may be suspected based on age at presentation and long-term course. Specifically, presentation early in life, from infancy to adolescence, is not uncommon and associated with severe outcome including sudden cardiac death and
refractory heart failure, although striking improvement may be seen following appropriate treatment. Conversely, adult-onset DCM of sarcomeric origin tends to have a mild course observed and little tendency towards progression. This clinical profile differs significantly from that seen in other genetic causes of DCM, such as that caused by lamin A/C or phospholamban mutations, which typically silent until adulthood but associate with progressive course after their onset. Of note, DCM associated with TNNC1 and TNNT2 mutations seems to be particularly severe and penetrant, a behaviour mirroring that of thin filament-associated HCM and RCM.

In subclinical DCM mutation carriers, subtle abnormalities in systolic function have been described, despite normal left ventricular size and systolic function. Conversely, mutations carriers in HCM families exhibit evidence of impaired relaxation, in the absence of LV hypertrophy. These findings support the theory that the mutation’s intrinsic impact on sarcomere function determines whether a dilated or hypertrophic phenotype will ultimately develop.

2.4 Left ventricular non-compaction

LVNC is characterized by prominent and diffuse trabeculation of the LV alternating with deep intertrabecular recesses. LVNC is often detected in individuals with an otherwise normal LV and seems to represent an accessory feature of all genetic cardiomyopathies and other conditions such as congenital heart disease. Thus, it is still debated whether it should be considered a separate clinical and genetic entity or a non-specific morphological trait. The debate has become exceedingly hard to solve, in view of the uncertainty surrounding diagnostic criteria for LVNC. In the absence of a widely accepted gold standard, a number of different criteria have been developed over the years, with little internal agreement. To date, there are four echocardiographic and two magnetic resonance imaging (MRI)-based approaches, which differ on the diagnostic ratio of compacted to non-compacted myocardium and the timing of measurement (end-systole or end-diastole). A consequence of this uncertainty is the.

**Figure 3** Different pathology findings of non-obstructive HCM. (A) Sudden death in a 15-year-old boy: short-axis view of the heart, showing massive LV hypertrophy and a large white scar in the posteroseptal area. An intramyocardial course of the left anterior descending coronary artery is also visible. (B) Heart transplant for end-stage systolic dysfunction. A 59-year-old male patient with a troponin T mutation: heart removed at transplantation with thinning of basal and mid-ventricular septum (12 mm) compared with distal LV. (C) Disarrayed cardiomyocytes associated with increased interstitial fibrosis, characterized by strands of collagen interlacing or enveloping myocardial fibres. (D) Replacement-type fibrosis with collagen substituting myocyte loss (asterisk) associated with medial remodelling and lumen narrowing of coronary arterioles (arrow). (Azan Mallory stain, original magnification 5×). (A) reprinted from Basso et al. Hum Pathol 2000 with permission. (B) reprinted from Melacini et al. EHJ 2010 with permission.
substantial risk of LVNC overdiagnosis in the general population, due to the high prevalence of LV trabeculation which, however, seems to have no clinical or prognostic relevance.42

3. Insights from pathology

Pathological studies on explanted and autopsy hearts have provided important insights on the mechanisms underlying the clinical features and natural history of cardiomyopathies and have provided the basis for validation of tissue characterization by contemporary imaging techniques. In clinical practice, pathological studies of myocardial tissue are limited to patients undergoing surgery or endomyocardial biopsy. Biopsies were routinely performed in the early days of cardiomyopathies, but their role in diagnosis and management has considerably diminished following the advent of genetic testing and multimodality imaging and is now largely confined to the differential diagnosis of rare conditions (e.g. storage disease), evaluation of inflammatory/autoimmune disease, or follow-up of transplanted patients.9

3.1 Hypertrophic and restrictive cardiomyopathy

The distinctive macroscopic and histopathological features of HCM have been thoroughly characterized (Figure 3). At the macroscopic level, HCM is characterized by LV and/or right ventricular hypertrophy that is usually, but not invariably, asymmetric and preferentially involves the interventricular septum. Microscopic analysis shows hypertrophy and spatial disorganization of myocardial fibres (‘disarray’) involving fibre bundles or single myocytes. This is usually associated with increased interstitial fibrosis, i.e. strands of collagen interlacing or enveloping myocardial fibres (Figure 3C). The degree of interstitial fibrosis in HCM is greater than in secondary LV hypertrophy and thus likely to represent a primary feature of disease.43 Furthermore, HCM is characterized by replacement-type fibrosis, i.e. collagen fibres substituting myocyte loss (Figure 3D), predominantly located in the mid-myocardium of the regions with maximum wall thickness, suggesting a direct influence of regional hypertrophy.44 Representation of replacement-type fibrosis can vary profoundly, ranging from patchy microscopic scars to larger spots, visible to the naked eye. Replacement-type fibrosis shows a spatial relationship with remodelling of coronary arterioles (diameter <100 μm), characterized by medial wall thickening due to smooth muscle hypertrophy and increased collagen deposition, with variable degrees of intimal thickening and perivascular fibrosis (Figure 3D), resulting in marked narrowing of the vessel lumen. This is in keeping with the concept of an ischaemic origin of myocyte loss in HCM, supported by clinical evidence of abnormal microvascular physiology.45 Alternative mechanisms advocated for myocyte injury in HCM are energy depletion leading to apoptosis and epicardial coronary artery disease, including myocardial bridging.46,47

Figure 4 MRI images showing rapid disease progression in a patient with HCM and complex genotype. In a 22-year-old male with HCM caused by double Myosin Binding Protein C3 mutation, MRI performed at the time of diagnosis in 2007 (A and C) and repeated after 2 years demonstrated marked increase in LGE over time. A and B: four-chamber views; C and D: corresponding short-axis views.
Figure 5  The broad spectrum of the echocardiographic phenotype. (A and B) Classic HCM phenotype. Parasternal long- (A) and short-axis (B) view showing asymmetrical HCM in a 35-year-old female carrying the c.2864_2865delCT (p.Pro955Argfs) mutation in MyBPC3. (C) Left ventricular non-compaction and intraventricular thrombosis. Apical three-chamber view showing LVNC (arrow) with a thrombus (star) in a 35-year-old male carrying the c.1633G>A (p.Asp545Asn) and the c.2863G>A (p.Asp955Asn) mutations in MYH7. (D–F) HCM progression to end-stage. (D and E): adverse remodelling in a patient with HCM carrying the c.3133C>T (p.Arg1045Cys) in MYH7. This patient with a ventricular septum of 33 mm at baseline (D) showed LV wall thinning and cavity dilatation during follow-up. At last evaluation (E), septal wall thickness was 17 mm, and end-diastolic diameter was 68 mm. (F): parasternal long-axis view in a 37-year-old male with DCM associated with the c.2713T>C (p.Cys905Arg) mutation in MYH7. (G and H) Obstructive HCM. Continuous wave Doppler interrogation of the left ventricular outflow tract showing the absence of obstruction in resting condition (peak gradient 12 mmHg; G) but severe obstruction after provocation with the Valsalva manoeuvre (85 mmHg, H). (I) Strain values per segment during the heart cycle in a normal heart and a patient with HCM. Ejection fraction was normal in both patients. The HCM patient showed regional decrease in peak systolic strain compared with the normal control, most evident at the basal and mid-wall septal segments (green and white lines).
Clinicopathological studies have identified the morphological substrates of disease progression in HCM. Patients with systolic dysfunction show extensive myocardial scarring, LV wall thinning, and chamber dilatation. In addition, heart failure may occur in the setting of severe diastolic impairment in patients with normal or mildly reduced ventricular cavities, leading to a restrictive evolution, also characterized by diffuse myocardial fibrosis. These pathological findings are also typical of RCM associated with sarcomere mutations. Of note, however, neither RCM nor restrictive HCM exhibit the extent of replacement-type fibrosis or microvascular remodelling seen in the hypokinetic-dilated form of HCM (Figure 3), suggesting a distinct pathogenetic pathway. Rarely, a restrictive pathophysiology in HCM may be due to massive LV hypertrophy leading to chamber obliteration, in the absence of fibrotic changes.

Electrical instability can cause sudden arrhythmic death at any stage of the natural history of cardiomyopathies. Pathology studies of young HCM subjects dying suddenly almost invariably showed spots of replacement-type fibrosis associated with acute and subacute myocardial ischaemia. Grossly visible septal scars were observed in more than half of these patients, in keeping with the hypothesis that myocardial scarring is a pathological substrate for anisotropic re-entry. These scars correlate with areas of late gadolinium enhancement (LGE) visualized in vivo by MRI (Figure 4). However, other arrhythmic substrates include the electrophysiological remodelling of the cardiomyocytes, extensive myocyte disarray, and in children, coronary myocardial bridging.

3.2 Other cardiomyopathies

The pathology of DCM and LVNC has been reviewed elsewhere. At present, distinctive pathological features are not recognized for DCM and LVNC of sarcomeric aetiology. This is due to the lack of studies specifically addressing the gross and microscopic findings of this small subset of disease.

4. Insights from electrocardiography

Although not diagnostic per se, ECG abnormalities are very common in patients with cardiomyopathies and their families, often leading to the initial recognition of the disease, and may represent the only phenotype in individuals with sarcomere gene mutations.

4.1 Hypertrophic cardiomyopathy

In HCM cohorts, a normal ECG is present in less than 10% of patients, generally associated with mild phenotype and favourable outcome. About 40% exhibit abnormalities which may raise the suspicion of HCM, including Romhilt Estes point score ≥ 5, giant negative T wave (typically associated with apical HCM), or a pathological inferolateral Q wave, whereas over 50% have non-specific findings such as re-polarization or intraventricular conduction abnormalities, which merely represent ‘red flags’ for the identification of individuals deserving further investigation. In one study, both a Romhilt Estes score ≥ 5 and pathological Q waves were associated with a greater LV mass index, whereas greater LV wall thickness values and Q waves were associated with the increased prevalence of LGE. The presence of low voltages, pre-excitation, or atrioventricular blocks may suggest alternative diagnoses such as cardiac amyloidosis, mitochondrial, or storage disease. Rhythm disturbances such as atrial fibrillation and non-sustained ventricular tachycardia are common and have important prognostic implication, the former with regard to disease progression and heart failure and the latter for prediction of sudden death risk, particularly in the young.

4.2 Other cardiomyopathies

In sarcomere-related DCM patients, the ECG is generally non-specific, with progressive left bundle branch block as the most common pattern. The presence of distinct patterns such as posterior or postero-lateral infarct-like or progressive atrioventricular conduction delay point to alternative diagnoses such as distrophin-related disorders or laminopathies. In idiopathic RCM, the ECG is usually characterized by abnormalities reflecting bilateral atrial enlargement, such as prominent and biphasic P waves and atrial fibrillation. Non-specific findings resembling those of HCM are also common, including a strain ST–T pattern, pathological Q waves, and QT prolongation. However, unlike HCM, QRS duration is usually normal. Paediatric patients with RCM may exhibit conduction disturbances, such as PR prolongation and widening of the QRS, and are at risk of developing high-grade AV block, associated with sudden cardiovascular decompensation.

In LVNC, the presence of an entirely normal ECG is rare, although no specific ECG patterns have yet been identified. The most prominent features are marked biventricular hypertrophy, ST elevation and T wave abnormalities (isolated or diffuse T-wave inversion), intraventricular conduction delay, and re-polarization abnormalities. Notably, a Brugada-like pattern has also been reported as a frequent feature of patients with LVNC, although its prognostic implications are unresolved.

5. Insights from echocardiography

Transthoracic echocardiography (TTE) is a quintessential tool for the evaluation of patients with cardiomyopathies. Besides establishing the diagnosis, TTE provides key morpho-functional information for risk stratification, clinical decision-making, longitudinal follow-up, and family screening programs. With the use of recently developed techniques, assessing myocardial deformation, TTE constitutes a powerful research tool providing insight into the pathophysiology of sarcomere-related conditions (Figure 5).

5.1 Chamber morphology

End-diastolic LV cavity dimensions are generally normal or reduced in HCM and RCM, and—by definition—increased in DCM. LV geometry is altered in patients with cardiomyopathies, ranging from the spade-shaped cavity of apical HCM (Figure 5A and B) to the spherical shape of advanced DCM causing tethering of the mitral leaflets and functional regurgitation. In LVNC, the LV wall has a typical spongy appearance, with a thick non-compacted endocardial layer and a thin compacted epicardial layer, exceeding a 2:1 ratio (Figure 5C). Contrast echocardiography or MRI may be required to differentiate LVNC from apical HCM. Left atrial enlargement is common in all sarcomere-related phenotypes, although most striking in HCM and RCM. Antero-posterior LA diameter, indexed volumes, and LA fractional shortening provide important prognostic information in HCM, including risk of atrial fibrillation and long-term outcome.

5.2 LV hypertrophy

TTE is very accurate in measuring LV wall thickness, by virtue of its spatial resolution and the capability of distinguishing the septum from fibromuscular paraseptal structures. Patients with DCM or RCM present with normal LV wall thickness, whereas HCM is defined by values ≥ 15 mm (or equivalent indexed to body size in children) ranging to ≥ 30 mm (Figure 5A and B). Lesser values (12–14 mm) may be diagnostic in individuals with family history of HCM. In most patients, LV hypertrophy is
preferentially located at the basal septum and anterior wall, but different locations have been described such as the apex and lateral wall; in such instances, MRI may prove more accurate than echocardiography in defining LV morphology.13

5.3 LV function
Significant degrees of systolic dysfunction are associated with adverse prognosis in all sarcomeric cardiomyopathies. A reduction in LV ejection fraction (LVEF) is the hallmark of DCM, but is also a feature of LVNC and end-stage HCM (Figure 5D–F). When severe, it is always coupled with marked diastolic dysfunction.68 Conversely, LVEF is usually preserved in RCM and supernormal in classic HCM (with values >70%), although indexes of longitudinal LV systolic function are impaired. Assessing diastolic function in cardiomyopathies is challenging and, despite the wide array of indexes developed over time, no single measure is considered sufficiently accurate or comprehensive. The best estimate originates from a combination of mitral inflow velocities, tissue Doppler Imaging, pulmonary vein flow velocities, pulmonary artery systolic pressure, and LA size.62 RCM is defined by the presence of extreme diastolic impairment and severe outcome despite relatively preserved systolic function. In HCM and DCM, progression of diastolic dysfunction as part of adverse remodelling over time is also related to adverse outcome, particularly when a restrictive LV filling pattern becomes evident.5

5.4 Outflow tract obstruction
Dynamic LV outflow obstruction at rest is found in ~25% of HCM patients.63–66 In an additional proportion close to 40%, a gradient ≥50 mmHg can be elicited with exercise. Thus, symptomatic HCM patients without obstruction at rest should be tested for dynamic obstruction using TTE during provocative manoeuvres such as Valsalva or, ideally, physiological exercise. Conversely, pharmacological provocation with dobutamine or amyl nitrite are not recommended due to high false-positive rates.66 LV outflow obstruction is a major determinant of symptoms and is associated with increased risk of heart failure-related complications and death.64 It is classically due to mitral systolic anterior motion (SAM) causing systolic septal contact and obliterating the outflow tract (Figure 5). SAM is produced by drag forces deriving from high velocity, abnormally directed anterograde systolic flow within the LV, and is responsible for loss of leaflet coaptation and mitral regurgitation associated with obstruction.65 Several anatomical and pathophysiological mechanisms predispose to SAM. Importantly, anomalies of the mitral valve and subvalvular apparatus, such as leaflet elongation, anterior displacement of the papillary muscles, and direct insertion of papillary muscles into the anterior leaflet, are frequent in HCM patients. Finally, HCM patients may be also characterized by LV mid-ventricular and right ventricular outflow obstruction.9

5.5 Myocardial deformation
Recently introduced, two-dimensional tissue tracking allows the measurement of myocardial velocity and the assessment of longitudinal, circumferential, and rotational myocardial displacement. In HCM, reduced longitudinal systolic strain and the strain rate are seen in hypertrrophic regions, even though global contraction is normal or even enhanced (Figure 5).67 This pattern seems to differ from that observed in other causes of myocardial hypertrophy and might be helpful in differential diagnosis. Echocardiographic analysis using tissue Doppler and strain rate imaging in G+/Ph− individuals revealed subclinical systolic dysfunction in DCM and subtle diastolic abnormalities in HCM.68

LV twist, defined as the wringing motion of the heart caused by the clockwise rotation of the base and the counterclockwise rotation of the apex, has an important role in ejection and filling. Twist results from the interaction of muscle fibres in the right-handed subendocardial and left-handed subepicardial helices. Peak systolic twist is increased in HCM patients, likely due to ischaemia of the subendocardial helix. Conversely, untwisting is delayed. In DCM, LV twist is reduced due to impaired apical counterclockwise rotation. In LVNC, the LV base and apex rotate in the same direction. This ‘solid body rotation’ behaviour is probably caused by incomplete formation of the subendocardial helix and is supportive of the diagnosis of LVNC.69

6. Insights from MRI
The value of MRI in the management of cardiomyopathies relies on detailed evaluation of heart morphology, function, perfusion, and characterisation of myocardial texture. The latter, unique to MRI, is based on the capability of identifying various pathological processes affecting the myocardium, including oedema (associated with acute myocardial injury and inflammation), fat, fibrosis, haemorrhage, and iron or protein deposition (Table 2). Magnetic resonance spectroscopy also provides unique information on cardiac metabolism, although currently restricted to research applications.70

6.1 Morphology
Morphological features including chamber volumes, ventricular mass, and spatial distribution of hypertrophy can be accurately assessed by MRI. The technique is more sensitive than echocardiography in detecting hypertrophy in the apical and lateral wall, thus increasing the diagnostic yield in patients with a suspicion of HCM.13 Furthermore, MRI has helped unravel additional features of the disease such as crypts, apical aneurysms, and mitral valve abnormalities. In HCM patients, anterior mitral valve leaflets are considerably longer than those of healthy controls, irrespective of age and extent of hypertrophy, predisposing to dynamic obstruction.71 In >80% of G+/Ph− individuals from HCM families, MRI identified profound crypts in the basal and mid-segments of the inferoseptal myocardium. These crypts may represent the precursor stage of a disease process ultimately leading to focal hypertrophy.72 The same considerations apply to LV apical-basal muscle bundles.73 Due to its high spatial resolution, MRI allows detailed visualization of LV trabeculations and represents the gold standard to the diagnosis of LVNC. However, as previously discussed, the proposed diagnostic criteria for LVNC lack specificity and are often met in patients with other cardiomyopathies or normal individuals.74

6.2 Function
MRI tomographically defines the LV and therefore provides actual quantification of volumes, relevant to the functional assessment of hypokinetic forms such as DCM, LVNC, and end-stage HCM, with greater accuracy compared with echocardiography. In addition, specific information on intramyocardial deformation can be obtained by MRI using tissue tagging.75 HCM is characterized by contractile heterogeneity of the LV and depressed systolic deformation, not only in regions with LGE reflecting scarring or excess interstitial collagen deposition, but also in the core of apparently healthy hypertrophic segments.75 This suggests different and as yet unknown mechanisms contributing to regional systolic dysfunction in HCM. Furthermore, tagging techniques allow the identification of subclinical LV dysfunction in G+/Ph−, such as subtle diastolic abnormalities and increased LV torsion.76 The latter thought
The characterization of myocardial tissue allowed by MRI has a significant value in determining disease progression in cardiomyopathies. In HCM patients, LGE techniques identify patchy mid-wall enhancement in 50–80%, correlating positively with regional LV systolic function; cine MRI is the gold standard for assessing myocardial wall motion and thickening patterns and has potential in the assessment of ventricular dyssynchrony.77,78

### 6.3 Tissue characterization

The characterization of myocardial tissue allowed by MRI has a significant value in determining disease progression in cardiomyopathies. In HCM patients, LGE techniques identify patchy mid-wall enhancement in 50–80%, correlating positively with regional LV systolic function; cine MRI is the gold standard for assessing myocardial wall motion and thickening patterns and has potential in the assessment of ventricular dyssynchrony.77,78

### 6.4 Myocardial perfusion

Myocardial perfusion by MRI is derived from first-pass contrast-enhanced signal intensity vs. time curves. The technique is semi-quantitative; however, quantitative MBF in ml/min/g can be calculated using deconvolution modelling. In HCM patients, adenosine-induced hyperaemic MBF is blunted in proportion to the magnitude of hypertrophy,83 thus reproducing the main findings of the earlier studies based on positron emission tomography (PET). Of note, reduced maximal coronary flow is associated with regional LV contractile abnormalities, as assessed by MRI tissue tagging, independent of LGE. However, in the presence of LGE, perfusion abnormalities and LV dysfunction are more severe.83

### 7. Insights from PET

Imaging with PET offers unique opportunities for research into tissue perfusion, biochemical pathways, and pharmacological mechanisms in vivo. This is made possible by the high sensitivity of PET imaging, enabling the measurement of radiolabelled tracers in concentrations sufficiently low so as not to disturb the processes under study, and the ability of current scanners to perform rapid dynamic imaging and provide good temporal resolution.84

#### 7.1 Myocardial blood flow

PET allows reproducible measurements of regional MBF (i.e. tissue perfusion in ml/min/g of tissue) providing pathophysiological and diagnostic information on the function of the coronary macro- and microcirculation. Oxygen-15 labelled water, nitrogen-13 labelled ammonia, and, more recently, generator-produced rubidium-82 are the tracers that have been validated in animals against the radiolabelled microsphere method.84 In the past two decades, studies where coronary flow reserve was measured in patients with cardiomyopathies and normal
coronary angiograms have led to the concept of coronary microvascular dysfunction.85 Table 3.

In HCM patients, microvascular function and coronary reserve are blunted diffusely, i.e. both in the hypertrophied and non-hypertrophied regions of the LV. Furthermore, HCM patients with sarcormae mutations are characterized by greater impairment of flow and prevalence of LGE, compared with genotype-negative patients, suggesting the genetic regulation of arteriolar remodelling.85,86 This finding is consistent with the higher prevalence of end-stage progression among genotyped individuals.87 Coronary microvascular dysfunction has been demonstrated also in patients with other cardiomyopathies, including DCM, LVNC, and Anderson Fabry disease. Both in HCM and DCM, the degree of microvascular dysfunction is an independent predictor of cardiac events and progression of heart failure.88,89 These findings support the hypothesis that microvascular ischaemia might represent a common pathway leading to disease progression in different cardiomyopathies and support the use of non-invasive imaging with PET for risk stratification. Finally, microvascular dysfunction represents an obvious therapeutic target in patients with cardiomyopathies, aimed at improving perfusion and preventing adverse LV remodelling.90 Because a blunted perfusion reserve precedes the development of myocardial fibrosis and systolic dysfunction by years, early identification of microvascular flow abnormalities represents an opportunity for pharmacological prevention of disease progression.

7.2 Autonomic dysfunction

In studies using 11C-CGP 12177, a non-selective β-adrenoceptor antagonist enabling the study of the functional receptor pool on the cell surface, patients with HCM have shown diffuse down-regulation of myocardial β-adrenoceptors, correlated to the degree of LV dysfunction.91 Furthermore, a PET study with 11C-labelled hydroxyephedrine has demonstrated significant impairment of transporter-mediated neuronal uptake at the pre-synaptic level in HCM. The net effect is an increased concentration of catecholamine in the synaptic cleft which in turn might contribute to β-adrenoceptor down-regulation.91 Of note, these abnormalities may actually precede the onset of LV dysfunction and thus play a role in its genesis, rather than represent an epiphenomenon. Further studies are warranted to understand whether this mechanism may represent a viable therapeutic target.91

8. Role of circulating biomarkers

Biomarkers provide clinically useful information, complementary to imaging, aiding clinicians in the evaluation of haemodynamic compensation, microvascular ischaemia, inflammatory status, and collagen turnover. In addition, biomarkers may predict disease progression and outcome in a variety of cardiac conditions including cardiomyopathies.

8.1 Hypertrophic cardiomyopathy

8.1.1 Troponins and brain natriuretic peptides

In 2003, Sato et al.73 provided the first report of increased cardiac troponin T (cTnT) levels in HCM patients, in whom reduced fractional shortening was noted during follow-up. Similar findings were later described for cardiac troponin I (cTnI) and high-sensitivity cardiac troponin T (hs-cTnT), supporting the idea that acute subclinical ischaemia is relevant to adverse LV remodelling and dysfunction. Indeed, hs-cTnT levels > 14 ng/L represented strong and independent predictors of cardiovascular events in HCM patients in one study.91 Furthermore, increased hs-cTnT values were associated with the presence of replacement fibrosis assessed by LGE.93 Overall, these results suggest a potential utility of troponin dosage in the evaluation of disease progression and response to treatment. Adverse LV remodelling is coupled with changes in ventricular stretch, accounting for increased circulating levels of the N-terminal fragment of pro-brain natriuretic peptide (NT-proBNP) in HCM patients, in whom a correlation has been reported between plasma NT-proBNP values and several indexes of LV remodelling and dysfunction. A prospective study enrolled 183 stable outpatients with HCM, largely asymptomatic or mildly symptomatic, followed-up for 4 years: plasma levels of NT-proBNP were found to be powerful and independent predictors of heart failure-related events.94 Similar results were reported by Coats et al.,95 who evaluated 847 HCM patients for an average of 3.5 years: baseline NT-proBNP values were associated with symptomatic progression, reduced LVEF and all-cause mortality or cardiac transplantation. Of note, the additive and independent prognostic relevance of hs-cTnT and NT-proBNP is emerging. If confirmed by larger studies, these findings will have direct applications in current clinical practice for risk stratification and therapeutic decision-making in HCM patients.94,95

8.1.2 Novel biomarkers

HCM patients exhibit higher levels of interleukin-6, compared with controls, even when inflammatory conditions are excluded,96 possibly as a response to abnormal LV contraction. A vicious circle has therefore been proposed in HCM pathogenesis, by which LV hypertrophy promotes interleukin-6 release, in turn promoting further hypertrophy. Similarly, increased levels of pro-apoptotic molecules such as tumour necrosis-α and soluble Fas ligand have generated the hypothesis that cardiomyocyte apoptosis might play a decisive role in promoting fibrosis and disease progression in HCM patients.96 Indeed, marked increases in tumour necrosis-α have been reported in the end-stage phase of HCM.97 Both interleukin-6 and tumour necrosis-α promote the expression of matrix metalloproteinases (MMPs), which have been related to cardiac remodelling and fibrosis in heart disease, including DCM.98 Several MMPs are increased in the serum of HCM patients, such as MMP-9 and MMP-3, respectively associated with LGE and increased arrhythmic risk.98 Aldosterone, known to promote MMP expression, is frequently elevated in HCM patients and has been repeatedly identified as a potential therapeutic target. Finally, the role of micro-RNAs as key regulators of gene expression is receiving increasing attention in cardiovascular disease.99 In HCM patients, of several up-regulated micro-RNAs, only the circulating levels of miR-29a were found to correlate with both myocardial hypertrophy and fibrosis, suggesting common molecular pathways for these two features.99

8.2 Dilated cardiomyopathy

Increased hs-cTnT levels represent independent predictors of cardiac death in DCM. In addition, increased NT-proBNP levels are associated with adverse LV remodelling and functional deterioration and predict adverse outcome in children with DCM.100 In contrast, lower plasma levels of BNP predict reverse remodelling in response to treatment.101 Galectin-3 levels have not yet been shown to have a predictive role in DCM patients.102 However, both galectin-3 and ST2 are emerging as biomarkers of myocardial fibrosis, and strong outcome predictors in chronic heart failure patients, and deserve to be properly evaluated in DCM. Finally, creatine kinase should routinely be evaluated in DCM patients in relation to its diagnostic utility for...
lamin A/C cardiomyopathy and other forms of DCM with skeletal muscle involvement.8

9. Investigations and follow-up of phenotype-negative individuals

The availability of genetic testing in clinical practice has led to the identification of large G+/-Ph - cohorts in families with HCM, DCM, and LVNC. The exact proportion of G+/-Ph - subjects that develop overt disease is currently unknown. Penetrance of fully expressed phenotype increases with age, but is incomplete, in both children and adults, and an evident phenotype may be absent even at an advanced age.81 In HCM, the reported risk of adverse cardiac events occurring very early during disease development.105

In HCM, the reported risk of adverse cardiac events in G+/-Ph - is very low, and no sudden deaths were reported in the largest cohort reported to date.102 According to current HCM guidelines, G+/-Ph - children and adolescents should be evaluated by ECG and TTE at intervals of 1–2, whereas adults only every 2–5 years.9 There are no specific guidelines for the other sarcormeric cardiomyopathies, although there is consensus that all G+/-Ph - should be evaluated at regular intervals.

In HCM, ECG abnormalities can be present before LV hypertrophy develops, and their severity is related to the degree of LV hypertrophy and LGE on MRI.54 The ECG is therefore a sensitive screening tool, and the one with the highest cost–benefit ratio. In G+/-Ph - subjects, subtle changes can be found by TTE, not fulfilling diagnostic criteria for a specific cardiomyopathy. Criteria combining ECG and TTE data, including mitral valve or papillary muscle abnormalities, have been proposed to diagnose HCM. Tissue Doppler imaging studies in G+/- LVH - subjects have consistently revealed subtle evidence of diastolic dysfunction.103 In G+/-Ph - subjects from DCM families, mild- LV enlargement and subtle abnormalities in systolic function are common, representing markers of pre-clinical disease. MRI is a valuable adjunct in the cardiac evaluation of G+/-Ph - subjects, especially when TTE images are suboptimal. As an example, MRI can detect mild-LV hypertrophy in up to 10% of mutation carriers with negative TTE.73 Myocardial crypts, diastolic abnormalities, and increased LV torsion have been identified by MRI in G+/-Ph - subjects and represent pre-phenotypic features of HCM.104 Although LGE is extremely rare in G+/-Ph - subjects, they often exhibit increased extracellular volume and circulating end-products of collagen synthesis, such as the C-terminal propeptide of type I procollagen, supporting the concept of fibrotic remodelling occurring very early during disease development.105

10. Conclusions

The long-term care of patients with sarcomeric cardiomyopathies is largely based on a ‘low-tech, high-touch’ approach aimed at providing clinical stability over decades and preserving quality of life. Technological innovations such as the implantable defibrillator, contemporary surgical techniques, ventricular assist devices, and transplantation are reserved to a minority of patients, whereas standard medical therapy and lifestyle modifications are often sufficient in most. Irrespective of clinical severity, evaluation and follow-up by a dedicated, multidisciplinary team is of critical importance in order to establish the correct diagnosis, provide reassurance when appropriate, understand causes of deterioration, and identify high-risk patients. Instrumental evaluations lose their meaning unless they are oriented towards and followed by a comprehensive, integrated interpretation of findings in the hands of clinicians. Ongoing research is pursuing the ambitious aim of developing disease-specific therapies targeting pathophysiological aspects of sarcomere-related disease: a state-of-the-art multimodality assessment offers the best opportunities to achieve such aim, by identifying of clinical targets, characterizing patient subsets for randomized trials, defining viable study end-points, and evaluating the effects of treatment over time.

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References


| Table 3 Evidence of coronary microvascular dysfunction in cardiomyopathies |
|-----------------|-----------------|-----------------|-----------------|
|                  | Number of cases | Basal flow (mL/g/min) | Hyperemic flow (mL/g/min) | Coronary flow reserve |
| Healthy controls | Gould et al. J Am Coll Cardiol 2013;62:1639–1653 | 3484 | 0.82 ± 0.06 | 2.86 ± 1.29 | 3.55 ± 1.36 |
| HCM              | Gould et al. J Am Coll Cardiol 2013;62:1639–1653 | 345 | 0.90 ± 0.10 | 1.57 ± 0.33 | 1.84 ± 0.36 |
| DCM              | Neglia et al. | 22 | 0.80 ± 0.25 | 1.91 ± 0.76 | 2.38 ± 0.50 |
| LVNC             | Jenni et al. J Am Coll Cardiol 2002;39:450–454 | 9 | 0.86 ± 0.30 | 1.72 ± 0.75 | 2.13 ± 0.84 |
Clinical investigations in sarcomeric cardiomyopathies


Clinical investigations in sarcomeric cardiomyopathies


