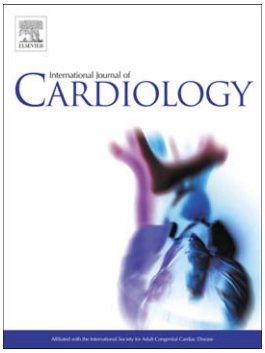

Familial dilated cardiomiopathy: A multidisciplinar entity, from basic screening to novel circulating biomarkers

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FAMILIAL DILATED CARDIOMIOPATHY: A MULTIDISCIPLINAR ENTITY, from basic screening to novel circulating biomarkers

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ABSTRACT

Idiopathic dilated cardiomyopathy has become one of the most prevalent inherited cardiomyopathies over the past decades. Genetic screening of first-degree relatives has revealed that 30-50% of the cases have a familial origin. Similar to other heart diseases, familial dilated cardiomyopathy is characterized by a high genetic heterogeneity that complicates family studies. Clinical screening, 12-lead electrocardiogram and transthoracic echocardiogram are recommended for patients and first-degree family members. Magnetic resonance also needs to be considered. Genetic technologies have become fundamental for the clinical management of this disease. New generation sequencing methods have made genetic testing feasible for extensive panels of genes related to the disease. Recently, new imaging modalities such as speckle-tracking, strain and strain rate, novel magnetic resonance parameters and circulating biomarkers such as non-coding RNAs, have emerged as potential strategies to help cardiologists in their clinical practice. Imaging, genetic and blood-based techniques should be considered together in the evaluation and testing of familial dilated cardiomyopathy. Here, we discuss the current procedures and novel approaches for the clinical management of familial dilated cardiomyopathy.

Keywords: familial dilated cardiomyopathy; genetics; echocardiography; magnetic resonance; non-coding RNAs; biomarkers

Dilated cardiomyopathy (DMC) is defined as left ventricular dilatation and dysfunction in the absence of abnormal loading conditions or coronary artery disease that is sufficient to cause global systolic impairment [1]. DMC is a leading cause of heart failure and sudden cardiac death. It is characterized by remodeling of the left ventricle and subsequent loss of its geometry as well as impaired ventricular function. The right ventricle may be involved, although this is not necessary for a diagnosis of DMC [2]. An idiopathic DMC diagnosis is based on the absence of secondary causes such as hypertension, coronary artery disease, valvular and congenital disease or active myocarditis. The estimated prevalence of DCM is at least 1 in 2500 people, of which 30–50% is familial DCM [1]. Clinically, there are no differences between familial and idiopathic DCM [3]. In cases of suspected familial DCM, clinical screening is recommended in first-degree relatives, as well as an imaging confirmation of an enlarged left ventricle with depressed ejection fraction [3]. Nonetheless, risk stratification criteria in familial DCM populations have not been defined and the extent to which genotype *per se* is a determinant of outcome has yet to be established.

DCM is considered familial when at least two members of the same family meet the diagnostic criteria, after excluding other causes of DCM [4]. A familial DMC diagnosis is assigned according to the phenotype and pedigree information and is not based on molecular genetic data [5]. This condition is usually characterized by incomplete and age-related penetrance and variable expression. Negative genetic testing in patients with familial DCM does not exclude a genetic cause because only 15–25% of familial DCM genetic causes have been described [6]. Although the prevalence of large genomic rearrangements remains unknown, titin (*TTN)* and lamin A/C (*LMNA)* mutations are the most frequently reported and are estimated to occur in 20-30% and 4-6% of patients with DCM, respectively. Other low-frequency variants of familial DCM have been linked to the sodium voltage-gated channel alpha subunit 5 *(SCN5A)* and desmin *(DES)* genes. The recent application of next-generation

sequencing (NGS) for the genetic screening of patients has led to the identification of a wide array of DCM-related genes including genes involved in muscle contraction and ion channels.

During the screening protocol, a comprehensive family and personal cardiovascular history is necessary. Sudden cardiac death or death under unusual circumstances has to be considered, as well as a family history of premature cardiovascular disease, cardiac transplantation, pacemakers or atrial fibrillation before the age of 40. Information regarding the family history enables physicians to confirm if the DMC is familial or not and also helps to determine the inheritance pattern and identify relatives who might be at risk [7]. However, even a detailed family history might be negative in some familial DCM cases because the asymptomatic period of the DCM-causal pathway can extend for years. Clinical screening, including imaging of the left ventricle to determine size and function, is essential to complete the family history. A diagnosis in early stages may be complex but necessary and vital for identifying and monitoring mutation carriers. The age for performing cardiac screening will depend on the age when cardiac complications are relevant to clinical management. In most cases, the phenotype begins to manifest during or after puberty [6].

The primary problem with familial DCM is due to its clinical heterogeneity. Clinical cardiologists should approach diagnosis of DCM using different established imaging techniques and new biomarkers. Diagnosis and treatment of familial DCM requires a multidisciplinary approach. Here, we review the current methodologies and novel technologies for the clinical management of familial DCM **(Figure 1)**. First, we will focus on the cardiac imaging techniques, including transthoracic echocardiogram (TTE) and cardiac magnetic resonance (MR). Then, we will discuss genetic testing as a tool for diagnosis and characterization of the underlying pathophysiology. Finally, we will comment on the

potential role of novel biomarkers, such as circulating non-coding RNAs, as non-invasive biochemical indicators with clinical application.

1. IMAGING DIAGNOSIS

The most advantageous cardiovascular imaging tests for patient management are TTE and cardiac MR. TTE provides basic information for DCM such as left ventricle dimensions and function but also provides information about other chambers. Cardiac MR, with a higher spatial resolution, is considered a key imaging test for cardiomyopathies. A complete cardiac exam with MR imaging is entirely non-invasive and enables the assessment of measures with clinical interest in a single diagnostic test. In this section, we describe in detail the primary imaging procedures with clinical application for the management of familial DCM.

1.1 Transthoracic echocardiogram

Non-invasive imaging techniques such as TTE are useful for the screening of both patients and relatives affected by familial DCM. TTE allows cardiologists to diagnosis DCM, evaluate the patient's response to treatment and establish the patient's risk for sudden cardiac death. TTE is often the first imaging method used to complete a patient's clinical evaluation. The utility of this technique has been previously demonstrated. In a study performed by Michels *et al.*, 5% of familial DCM was determined using only the clinical family history, whereas echocardiographic screening detected 20% of familial DCM [8]. TTE allows cardiologists to assess the volume and function of the atria and ventricles in addition to pulmonary artery pressure and other parameters.

<u>Evaluation of the left ventricle</u>

The left ventricle is the primary chamber of interest according to the DCM definition [1]. Impairment of contractile performance and ejection fraction ≤ 45% are evidence of DCM. Global hypokinesia, thinned walls and chamber enlargement, accompanied by geometric changes in axial symmetry, as well as a uniform circumferential distribution, suggest heterogeneous changes in regional contractile function in subjects with idiopathic DCM [9]. Despite the small sample size, Henn *et al.* have recently shown that contractile alterations in this population are heterogeneous and are mainly located in a portion of the interventricular septum [10]. Clinically, these initial results infer the existence of contractile impairment that can be labeled as a sentinel region, with possible prognostic significance during post-treatment contractility and functional recovery. Assessment of left ventricular function in this population has diagnostic and prognostic implications. Furthermore, therapeutic decisions regarding the development of heart failure, defibrillator implantation and heart transplantation will be based on these data. Nevertheless, TTE has its limitations. Indeed, interobserver variability is approximately 10%. Various methods could be used to support the findings of the TTE including a two-dimensional Simpson's biplane method, three-dimensional echocardiography, global longitudinal strain using ST and dobutamine stress echocardiogram [11]. It has been recommended that the two-dimensional Simpson's biplane technique be used as the method of choice for quantification of the ejection fraction. A four-chamber view can be used when the two-chamber view is not sufficiently accurate; the forced inclusion of the two chambers can induce an error in up to 15% of patients [12].

To prevent a "lower limit of normal ejection fraction", particularly in young patients, the familial context must be taken into account. The cardiologist has to address a series of issues when considering the familial group to detect early DCM markers and to stratify the patient’s risk. The best markers for these “on-the-frontier" patients have been shown to be

simple M-mode left ventricle measurements (adjusted for age, height and weight) and fractional shortening [13]

There are some left ventricular measurements that can be used as a marker with prognostic implications such as the sphericity index. The sphericity index considers the ventricular remodeling that occurs in individuals with DMC. The left ventricular sphericity index can be defined as the ratio of the long-axis length divided by the left ventricular short-axis length [14] and predicts the functional capacity in patients with left ventricular dysfunction [15]. The left ventricle cavity becomes more spherical as the ratio approaches 1; ratio values greater than 0.7 are predictive of failure to repair functional mitral regurgitation [16, 17].

The left ventricle and novel non-invasive evaluation methods

New non-invasive methods for evaluating myocardial function allow the assessment of regional left ventricular myocardial function. Three-dimensional echocardiography, Doppler tissue imaging (DTI), strain imaging and two-dimensional ST, as well as stress echocardiography supplement the information obtained through traditional echocardiography.

Three-dimensional echocardiography is recommended for experienced laboratories that can provide optimal images. This method has been proven to be accurate and reproducible for calculating ventricular volumes with 5% variability. In patients with adequate acoustic windows, the accuracy of three-dimensional echocardiography is comparable to that of cardiac MR, although volumes tend to be lower on echocardiographies [18].

Furthermore, DTI show characteristic changes in patients with cardiomyopathy. DTI analyses have revealed subtle abnormalities in systolic and diastolic function that may help predict familial DCM in relatives who will later develop the disease. Peak longitudinal

systolic (S´) and early diastolic (E') tissue velocities are reduced in basal left ventricle segments of patients with DCM. These changes are detectable in genetically tested asymptomatic patients and in early stages of cardiomyopathy, where an impaired left ventricular ejection fraction or increased wall thickness are not yet present [4, 14].

The global longitudinal strain represents the average of the maximum systolic values of all of the segments, and a good correlation between the longitudinal strain and left ventricular function has been shown [19]. Furthermore, ST provides information on the extent and the effect of myocardial interstitial fibrosis on ventricular systolic and diastolic function. Thus, ST highlights heterogeneous contractile function in DCM. DCM patients have lower circumferential and longitudinal strain values compared to individuals with normal ventricular function. These values are further reduced after the increase in afterload using isometric handgrip stress and low-dose dobutamine. The ST detects subclinical patients with systolic dysfunction regardless of normalization of the ventricular function after optimal drug therapy [20]. Related to fibrosis, in a study performed to assess the degree of interstitial fibrosis in patients with idiopathic DCM, a significant correlation between the degree of fibrosis and longitudinal strain parameters was shown [21]. In patients with an *LMNA* mutation, ST has shown that an alteration in the mechanical dispersion leads to a higher risk of ventricular arrhythmias despite a ventricular function > 35%, which reflects myocardial heterogeneity [22].

Finally, stress echocardiography is a useful echocardiographic technique for the evaluation of this disease. It is well known that catecholamine sensitivity is reduced in patients with myocardial alteration due to abnormalities in β-adrenergic receptors [23]. Dobutamine stress echocardiograms allow for the identification of reduced contractile reserves in patients with idiopathic DCM who are asymptomatic or mildly asymptomatic in

functional class I or II, which provides screening information for subjects who are in preclinical stages of the disease [24].

In summary, left ventricle assessment plays a key role in the diagnosis and risk stratification of DCM. Traditional echocardiography could be supplemented with new diagnostic and prognostic tools to evaluate global and regional left ventricular function.

Evaluation of the right ventricle

In addition to the left ventricle, right ventricle dysfunction has been reported in approximately 65% of patients with DCM, suggesting that DCM is a biventricular condition. All of the previously mentioned echocardiographic tools should also be used to assess the right ventricle. Idiopathic DCM presents more frequently with right ventricular dysfunction than other causes. Patients with right ventricular involvement tend to have a worse prognosis [25]. The tricuspid annular plane systolic excursion (TAPSE) is a simple measurement that can be used on a daily basis. In patients with DCM, TAPSE values lower than 14 mm are associated with poor prognosis [26]. Therefore, the evaluation of the right ventricle using TAPSE or fractional area change, wave S´ by DTI and longitudinal deformation by ST are essential and should be routinely used. Right ventricular assessment also provides information on the risk stratification for heart transplant or ventricular assistance candidates [27, 28].

Systolic pulmonary artery pressure

The non-invasive systolic pulmonary artery pressure is derived from the sum of the central venous pressure and the peak pressure gradient across the tricuspid valve between the right atrium and the right ventricle during systole, which is obtained by spectral Doppler and calculated with the use of the simplified Bernoulli equation. Although this method is used the

most in daily practice, there are still some controversies regarding the confidence of this method [29]. Systolic pulmonary artery pressure increases the risk of mortality in patients with left ventricular systolic dysfunction [30]. DCM and pulmonary arterial hypertension lead to more severe symptoms, a greater increase in BNP levels, more severe mitral regurgitation and diastolic dysfunction. Refractory heart failure and a higher mortality rate have been proven in young patients with left ventricular dysfunction and increased systolic pulmonary artery pressure [31]. These results suggest that the evaluation of pulmonary artery pressure helps identify a subgroup of patients with high-risk DMC that should be carefully followed when timing non-pharmacological strategies, including heart transplant. Mild to moderate pulmonary hypertension increases the risk of developing pulmonary hypertension in the early phases after heart transplantation. An increase in systolic pulmonary artery pressure severity predicts a worse prognosis for this group of patients; therefore, it may be useful to consider systolic pulmonary artery pressure during the echocardiographic evaluation in patients with idiopathic DCM [32].

Functional mitral insufficiency

Functional mitral regurgitation secondary to DCM has been extensively studied. The primary pathophysiological mechanism of ventricular remodeling is the apical and lateral displacement of the papillary muscles, which leads to tension on the chordae tendineae (heart strings). This restricts the movement of the leaflets causing tenting, annular dilatation and ventricular dyssynchrony, which eventually leads to incomplete closure of the mitral valve [33]. The presence of a secondary mitral regurgitation predicts a poor outcome. A mitral valve tenting area $\geq$ 6 cm^2 is generally associated with severe mitral insufficiency. Furthermore, three-dimensional echocardiography provides advantages over two-dimensional

echocardiography because it considers leaflet and chord tensile geometrical components as well as changes along the regurgitant orifice during systole [34].

Diastolic function

Diastolic dysfunction contributes to symptoms of heart failure and provides important diagnostic and prognostic information. Young patients with preclinical familial DCM can show normal stroke volume, cardiac output and grade I diastolic dysfunction [13]. However, as the disease progresses, the diastolic pattern may evolve to pseudonormal or restrictive, which worsens the prognosis [35]. The restrictive pattern indicates severe hemodynamic impairment, advanced symptoms and poor prognosis [36]. Shortening of the diastolic filling period, which could be a sign of atrioventricular dyssynchrony, is associated with reduced stroke volume and increased left atrial pressure [37].

Furthermore, previous studies have shown a relationship between the degree of fibrosis and diastolic dysfunction [36, 38, 39]. Fibrosis is a common endpoint in a variety of pathological myocardial processes that can alter the properties of compliance, relaxation and ventricular filling. Moreo *et al*. [40] showed that the degree of diastolic dysfunction was associated with a greater degree of myocardial fibrosis, which is better quantified through delayed enhancement cardiac MR imaging. These findings provide clinical information to detect early changes in diastolic function in the early stages of DCM development.

Thus, TTE is the first imaging method used to complete the clinical evaluation. It helps to assess the left ventricle and other hemodynamic characteristics used in the diagnostic and prognostic risk stratification of familial DMC patients.

The influence of genetic background

Concerning these echocardiographic tools, it should be noted that familial DCM shows different patterns depending on the affected gene, although these patterns are not as well-defined as those of hypertrophic cardiomyopathy. Individuals with *TTN* mutations have clinically and statistically larger left ventricular end-systolic diameters but lower mortality, fewer pacemakers, resuscitation and atrial fibrillation compared with *LMNA* mutation carriers [41]. Regarding these patients, Yoskovitz G. *et al*. [42] showed a late-onset presentation and clinical characteristics such as left axis deviation, poor R progression and complete left bundle branch block. Echocardiographic examinations revealed enlarged volumes, impaired systolic function and end-systolic dimension in these patients, while there was no observed impaired diastolic function or tissue Doppler measurements. Right ventricular systolic function was also affected. No differences in wall thickness were found. Pérez-Serra *et al*. [43] described a temporal pathological sequence in symptomatic patients with *LMNA* cardiomyopathy in which an electrical alteration is first seen in the ECG followed by mechanical cardiac impairments detected by TTE. Echocardiographies in these patients showed a mildly dilated and rounded left ventricle with a moderately depressed ejection fraction. However, in the absence of an established DMC, the diastolic impairment pattern was determined with DTI [43]. As suggested by Volpi *et al.*, early recognition of young patients carrying a pathogenic mutation in the *LMNA* gene may retard the rate of progression of the cardiomyopathy [44]. Although it is a relatively rare disease, awareness is increasing among clinical cardiologists regarding *LMNA* cardiomyopathy because of its aggressive course compared to most other inherited cardiomyopathies. This condition has been linked to an increased risk of sudden cardiac death related to severe ventricular failure. There is a 46% risk of sudden cardiac death and a 64% risk of heart failure secondary to left ventricular systolic dysfunction after the age of 50. Therefore, it is fundamental to the analysis of the

echocardiographic criteria for each specific genetic mutation related to familial DCM. Future studies should focus on this key issue for the management of patients with familial DCM.

1.2 Magnetic resonance

MR is the gold-standard test for the diagnosis cardiomyopathies because it provides essential anatomical and functional information. Some different tools are used in the evaluation of a patient with DCM to uncover the global, regional and tissue myocardial characteristics **(Table 1).** Cine cardiac MR using steady–state free precession (SSFP) sequences provides a functional analysis of the heart that allows for the evaluation of not only systolic and diastolic function but also global and regional contractility. In fact, this technique is considered the gold standard for evaluating ventricular function and mass with excellent interobserver agreement [45]. For that reason, they are essential sequences in the evaluation of DCM. Although the imaging protocol should be adapted regarding the clinical scenario, the protocol should at least include horizontal and vertical long axis and short axis views, the last with full biventricular coverage for measuring volumes, mass and function [46]. In these sequences, DCM typically shows increased ventricular mass with larger trabeculae, end–diastolic and end–systolic volumes, reduced stroke volume and ejection fraction. Hypokinetic wall function could also be observed [47]. In advanced stages, there is myocardial wall thinning (< 5.5 mm) [48]. Moreover, valvular insufficiency can be determined by visualizing valve regurgitant jet in SSFP sequences **(Figure 2)**. Quantifications can also be carried out through phase contrast sequences with an estimation of the regurgitant volume and fraction [46].

One of the advantages of cardiac MR among other imaging techniques is the capacity to characterize normal and pathological myocardium by detecting the presence of intramyocardial fat, edema, focal (replacement) or diffuse (interstitial) fibrosis. This is done

by means of inherent myocardial T1 and T2 properties and may help in making an adequate differential diagnosis in DCM and identifying secondary causes.

T2-weighted short-tau inversion recovery image sequences (T2–STIR) are used to detect increases in myocardial free water content, which is indicative of myocardial edema of ischemic, inflammatory or infectious origins (acute or chronic myocarditis, cardiac allograft rejection, etc.). Although it reflects various pathologic conditions, edema is usually associated with reversible myocardial injury. T2–STIR sequences' main limitations include a) signal intensity variability caused by phased array coils; b) slow–flow artifacts; c) motion artifacts; and d) the subjective nature of image interpretation. With conventional T2–weighted images, diffuse myocardial edema is difficult to detect [49].

T2 mapping techniques have been developed to directly evaluate the T2 values of the myocardial tissue based on a parametrical map [50]. T2 mapping is preferred because of the absence of the limitations described for T2–STIR sequences. Regarding the T2 mapping sequences, the amount of water content is significantly higher compared to normal controls and progresses as left ventricular dysfunction increases [49]. Additionally, having been correlated with alterations in intramyocardial movement, decreases in circumferential strain measured by myocardial tagging were correlated with regions of myocardial inflammation [51].

Conversely, myocardial fibrosis could occur in response to the loss of myocytes (focal or replacement fibrosis) as a reparative method to preserve myocardial structural integrity or as a "reactive"/"interstitial" process secondary to a variety of insults (mechanical, toxic, infective or autoimmune), which are distributed throughout the myocardial tissue [50]. Late gadolinium enhancement is an effective and reproducible method for demonstrating macroscopic (replacement) fibrosis, which is not seen with other techniques, by using T1-weighted imaging sequences with an inversion time pulse for nulling normal myocardial

tissue 10-20 minutes after the infusion of an extracellular gadolinium-based contrast media. Delayed enhancement is seen in up to 20-40% of patients with idiopathic DCM [52].

Depending on the myocardial enhancement pattern, a differential diagnosis can be established confirming idiopathic versus secondary DCM. The enhancement pattern associated with idiopathic DCM appears as a thin band in the intramyocardial septum, patched or subepicardial in the inferolateral wall, without vascular territory distribution **(Figure 3)**. There is not a consensus on the meaning of the regions of intramyocardial fibrosis or on the identification of areas of myocardial injury in a chronic inflammatory process. Transmural or subendocardial enhancement in DCM is highly suggestive of an ischemic event, even in the absence of significant coronary lesions, which is justified by spontaneous recanalization after embolization or occlusion or minimal stenosis from unstable plaques [53].

T1 mapping allows direct quantification of the T1 signal intensity of the myocardium before (T1 native) or after administration of gadolinium-based contrast media at different time points (post–contrast T1 maps) with extracellular volume calculation. The T1 value of the myocardium is prolonged in myocardial edema, interstitial fibrosis and amyloid deposits and shortened in lipid or iron accumulation [54]. Native T1 is significantly lower in patients with DCM than in controls and performed better than post-contrast T1 mapping-derived parameters [55]. This information may enable important risk stratification in patients with DCM [56]. Myocardial fibrosis is a major independent predictor of adverse clinical outcomes and, possibly, is an important parameter for device implantation. Moreover, extracellular volume fraction allows for differentiating earlier than “overt” DCM and is considered a significant prognostic predictor of cardiovascular death, heart failure and malignant arrhythmias [57, 58].

MR spectroscopy (MRS) was recently suggested as a non-invasive modality for studying cardiac metabolism *in vivo*. DCM is one of the main clinical areas in which this technique may provide insight into pathophysiological mechanisms [59]. MRS uses magnetic resonance signals from nuclei, such as phosphorus, hydrogen and sodium, to provide comprehensive metabolic and biochemical information about the cardiac muscle. This technique has been used in familial hypertrophic cardiomyopathy to identify phenotypes [60] and could be available for familial DCM in the future. Currently, MRS is only used in research settings; with further technical developments leading to improved temporal and spatial resolution. Cardiac MRS will dramatically advance our understanding of the pathophysiologic and metabolic nature of a number of cardiac conditions.

In fact, cardiac MR allows an integral evaluation of the heart including bi-ventricular geometry, volumes, mass and function, tissue characterization (myocardial fat and edema) and identification of focal or diffuse fibrosis. It helps to distinguish primary from secondary forms of DCM and, specifically, to ascertain whether an ischemic etiology is present. In addition, it permits cardiologists to obtain several clinically feasible and reproducible parameters, some of them constituting biomarkers with important prognostic implications, in patients with DCM.

2. GENETICS

Since 2009, the American Heart Failure guidelines include recommendations on genetic counseling and genetic testing in patients and families with certain cardiomyopathies. Almost 50% of familial DCM cases have been shown to be associated with a genetic alteration in at least one of the over 60 genes linked to this disorder **(Table 2).** As shown in **Figure 4,** most of these genes codified proteins related to cell structure, ion channels and

desmosomes. Most familial cases of DCM are transmitted in an autosomal dominant inheritance, although other inheritance patterns have been reported, such as autosomal recessive, X-linked and mitochondrial [6]. The *TTN* gene is responsible for nearly 30% of the DCM cases. The next most affected genes in familial DCM are *LMNA*, responsible for nearly 15% of cases, and a group of genes (*MYH7, TNNT2, RBM20, BAG3, TPM1, DSP, SCN5A, ACTC1 or MYBPC3*) involved in 5-10% of familial DCM cases. The other affected genes associated with this condition are responsible for 1-5% of cases [61].

Mutations in the *TTN* gene (ID: 7273, 2q31.2; size: 34350 amino acids; molecular mass: 3816030 Da) are frequently detected in DCM. This gene encodes the largest human protein, titin, which provides an external scaffold and interacts with both thin and thick filaments and plays an important role in sarcomere assembly and force generation. Titin provides a passive force and elasticity to preserve diastolic and systolic function, respectively [62]. To date, 37 pathogenic variants in the *TTN* gene have been associated with familial DCM, most of which are truncating variants (in 25% of cases). Despite the lack of phenotype-genotype segregation studies, some evidence suggests that men affected by DCM with a TTN mutation had adverse events at earlier ages [63].

The second most frequent mutation associated with DCM is found in the *LMNA* gene (ID: 4000, 1q22; size: 664 amino acids; molecular mass: 74139 Da). This gene encodes the lamin A/C protein located in the nuclear lamina (a fibrous structure underlying the inner nuclear membrane). The integrity and stability of the nuclear envelope is supported by the interaction between lamin and integral proteins of the inner membrane [64]. To date, 114 pathogenic variants in the gene have been associated with familial DCM. The phenotype of DCM patients carrying a pathogenic variant in this gene is extremely variable; however, conduction system disturbances are frequently presented (sinus bradycardia, atrioventricular conduction block and atrial tachyarrhythmia), as well as skeletal myopathies with or without

conduction-system disease. Eventually, these patients develop heart failure, and cardiac transplant is recommended. DCM subjects with *LMNA* mutations have the worst prognosis and serious cardiovascular problems, including sudden cardiac death and a higher rate of transplantations, in comparison with individuals with idiopathic DCM. Regarding copy number alterations, the deletion of several exons in *LMNA* leads to nuclear membrane abnormalities, which compromises the normal function of the lamina and reduces wild type protein levels [65].

Additional DCM-linked genes have been identified over the last several years using NGS platforms [56]. This technique has revolutionized clinical genetic screening because it allows for the analysis of a large number of genes in a cost-effective and time-efficient manner [66]. The large amount of data and genes identified using NGS has led to an overlapping of DCM and other cardiomyopathies such as hypertrophic cardiomyopathy and arrhythmogenic cardiomyopathy. Thus far, sarcomere or desmosome gene mutations have been primarily associated with hypertrophic cardiomyopathy and arrhythmogenic cardiomyopathy, respectively. The genetic complexity of DCM is currently being studied [67]. Another challenge for geneticists and clinicians is the interpretation of the large volume of data generated via high-throughput screening. Frequently, a large number of genetic variants are identified and their pathogenicity interpreted using computational biology tools. *In silico* tools are used for predicting pathogenicity in terms of pathogenic, likely pathogenic, unknown significance or benign. Co-segregation analysis of family members is essential to determine the relative importance of each gene [68]. Penetrance, defined as the percentage of mutation carriers who express the cardiomyopathy phenotype, is age- and gene-dependent but is so far poorly understood [69, 70].

Because of the high number of genes related to DCM, it is recommended that cardiologists perform a comprehensive genetic analysis using NGS technology in the best

cost-effective way that is currently available. The primary challenge currently for clinicians is the pathogenic interpretation of genetic variants identified as well as translation into clinical practice.

3. NOVEL BIOMARKERS: non-coding RNAs

Patients may benefit from an accurate, accessible and non-invasive test when being screened for familial DCM. Currently, there are no blood-based biomarkers available for monitoring cardiac alterations in patients with familial DCM. The development of a blood-based diagnostic and prognostic test to predict and/or monitor cardiac abnormalities during the subclinical stages of the disease could meet the clinical needs of the cardiologists.

MicroRNAs (miRNAs) are a family of small RNAs (19-25 nucleotides) that play a relevant role in post-transcriptional gene regulation by inducing the degradation or reducing the translation of the target mRNA. miRNAs participate in development, homeostasis and cellular response to stress [71]. Indeed, the dysregulation of intracellular miRNA expression is a hallmark of several cardiovascular disorders [72]. Focusing on DCM, several research groups have demonstrated the involvement of miRNAs in the pathophysiology of the disease [73]. Inhibition of miRNA biogenesis in cardiac myocytes causes cardiac dysfunction and eventually DCM [74]. Targeted deletion of specific miRNAs, such as miR-22, sensitized animal models to left ventricular dilation after stimulation by pressure overload [75]. Wijnen *et al.* suggested a role for miR-30c in the onset of the disease through the impairment of mitochondrial function [76]. Dysregulated miR-699a expression has been associated with severe progression of DCM in dystrophic mice [77]. The same authors proposed the use of miR-699a as a therapeutic strategy to slow down the progression of DCM [77]. In humans, the miR-199/214 cluster has been associated with cardiac mass loss during dilation [78]. A

close relationship between expression levels of miR-208 [79] and let-7i [80] in endomyocardial biopsies and adverse clinical events in patients with DCM has been described, suggesting a possible clinical application for intracellular miRNAs as biomarkers. Evidence on the role of miRNAs in familial DCM is very limited. Nevertheless, some indirect data point to the participation of miRNAs in familial DCM. Indeed, Sylvius *et al.* demonstrated that miRNA expression is affected in laminopathies. This group identified a signature of 16 miRNAs differently expressed in skeletal muscle from patients with *LMNA*-related muscular dystrophy compared to control subjects [81]

miRNAs have also been detected in body fluids, including blood [82]. Extracellular miRNAs have a key role in intercellular communication and regulate gene expression and the phenotype of the recipient cells [83]. Similar to their intracellular forms, the c-miRNAs are involved in the onset and development of cardiovascular disease [84]. Interestingly, their release into the extracellular milieu and bloodstream in response to cell stress or damage supports the study of miRNAs as potential biomarkers of pathophysiological conditions [85]. Extracellular miRNAs meet optimal biochemical and physiological properties to become excellent biomarkers [86]: i) specific profiles of miRNAs are released in response to different physiological and pathological stimuli; ii) miRNA secretion into microvesicles and miRNA-protein complexes gives them outstanding stability; iii) miRNAs have evolutionarily highly conserved sequences, which facilitate their analysis; iv) samples are stable and have a long half-life; and v) their determination is performed relatively inexpensively with high sensitivity and specificity through standard techniques already available in clinical laboratories (e.g., quantitative reverse transcription PCR).

Circulating miRNAs (c-miRNAs) are biological markers of a wide variety of cardiovascular conditions [87]. Indeed, c-miRNAs have been proposed as potent biomarkers of cardiac disease including heart failure and different cardiomyopathies [88], in some cases

with higher clinical value than the current gold standard [89]. Thus, the clinical application of c-miRNA as biomarkers of cardiovascular diseases has been proposed in the short- to medium-term [90]. During this decade, a number of studies focusing on circulating miRNAs as potential biomarkers of DCM have been published [91-96] **(Table 3)**. Nevertheless, whether circulating miRNAs can be useful diagnostic and prognostic tools in familial DCM still remains to be elucidated.

Long non-coding RNAs (lncRNAs) are transcripts of more than 200 nucleotides in length that function as epigenetic regulators [97]. LncRNAs are key mediators of cardiac development, function and disease [98]. Similar to miRNAs, lncRNAs have been proposed as potential clinical biomarkers [99]. Assays with clinical applications have been developed for urine lncRNA PCA3, which is a highly specific biomarker of prostate cancer [100]. Recent studies have identified circulating lncRNAs as useful diagnostic and prognostic tools with clinical applications for cardiovascular conditions [101-104]. However, no previous investigation has evaluated the circulating levels of lncRNAs as indicators of cardiac abnormalities in patients with DCM or familial DCM.

Studies based on the circulating miRNA and lncRNA signature in familial DCM are a promising strategy for the development of novel diagnostic, prognostic and therapeutic biomarkers for the management of this disease. Further investigations focusing on non-coding RNAs will elucidate the real clinical application of these novel biomarkers in this interesting field.

4. CONCLUSIONS

Here, we highlight the relevance of multidisciplinary teams for the management of familial DCM. Imaging biomarkers can detect the presence of the disease but are of little

value for characterizing the earlier stages of the disease when the disease is not yet clinically apparent. Genetic biomarkers provide insights into disease susceptibility. Nonetheless, genetic testing does not supply any information about whether subclinical disease has developed yet or not. Circulating biomarkers could provide relevant data about the disease process, with some biomarkers reflecting activity in biological pathways that precede the clinical presentation of the disease. However, the development of useful circulating indicators is in pre-clinical stages. An integrated assessment of familial DCM including imaging, genetic and blood-based biomarkers should lead to a novel understanding of familial DCM and, therefore, how to improve patient management.

5. PERSPECTIVES

- The combination of different methodologies is fundamental for a proper diagnosis of familial DCM. Management of familial DCM will require collaboration among multidisciplinary teams with representation from multiple different specialties.
- The definition of echocardiography patterns specific for each genetic alteration would be of diagnostic and prognostic utility for risk stratification of familial DCM. New imaging techniques could contribute to clinical management. Furthermore, the application of novel blood-based biomarkers should be considered.
- The integration of different types of biomarkers is fundamental for not only the diagnosis and prognosis but also for risk stratification of patients, as well as for decisions regarding changes in medical treatment, implantation of defibrillators and the need for cardiac transplantation.

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DISCLOSURES

None.

AUTHOR CONTRIBUTIONS

All of the authors have approved the final version of this article. All of the authors have made substantial contributions to the following: (1) the conception of the article and a critical review of the bibliography, (2) the drafting of the article or revising it critically for important intellectual content, and (3) the final approval of the version to be submitted.

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. 29 (2008) 270-276.

[2] Dec GW, The natural history of acute dilated cardiomyopathy, Trans Am Clin Climatol Assoc. 125 (2014) 76-86; discussion 86-77.

[3] Mestroni L, Brun F, Spezzacatene A et al., Genetic Causes of Dilated Cardiomyopathy, Prog Pediatr Cardiol. 37 (2014) 13-18.

[4] Towbin JA, Bowles NE, The failing heart, Nature. 415 (2002) 227-233.

[5] Hershberger RE, Morales A, Siegfried JD, Clinical and genetic issues in dilated cardiomyopathy: a review for genetics professionals, Genet Med. 12 (2010) 655-667.

[6] Hershberger RE, Siegfried JD, Update 2011: clinical and genetic issues in familial dilated cardiomyopathy, J Am Coll Cardiol. 57 (2011) 1641-1649.

[7] Hershberger RE, Lindenfeld J, Mestroni L et al., Genetic evaluation of cardiomyopathy--a Heart Failure Society of America practice guideline, J Card Fail. 15 (2009) 83-97.

[8] Michels VV, Moll PP, Miller FA et al., The frequency of familial dilated cardiomyopathy in a series of patients with idiopathic dilated cardiomyopathy, N Engl J Med. 326 (1992) 77-82.

[9] Juliani PS, Costa EF, Correia AT et al., Idiopathic dilated cardiomyopathy: computerized anatomic study of relashionship between septal and free left ventricle wall, Rev Bras Cir Cardiovasc. 29 (2014) 338-343.

[10] Henn MC, Lawrance CP, Kar J et al., Dilated Cardiomyopathy: Normalized Multiparametric Myocardial Strain Predicts Contractile Recovery, Ann Thorac Surg. 100 (2015) 1284-1291.

[11] Lang RM, Badano LP, Mor-Avi V et al., Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging, J Am Soc Echocardiogr. 28 (2015) 1-39 e14.

[12] Olszewski R, Timperley J, Szmigielski C et al., The clinical applications of contrast echocardiography, Eur J Echocardiogr. 8 (2007) S13-23.

[13] Fatkin D, Familial dilated cardiomyopathy: Current challenges and future directions, Glob Cardiol Sci Pract. 2012 (2012) 8.

[14] Flachskampf FA, Breithardt OA, Daniel WG, [Diagnostic value of tissue Doppler parameters in the early diagnosis of cardiomyopathies], Herz. 32 (2007) 89-96.

[15] Levine YC, Matos J, Rosenberg MA et al., Left ventricular sphericity independently predicts appropriate implantable cardioverter-defibrillator therapy, Heart Rhythm. 13 (2016) 490-497.

[16] Karaca O, Avci A, Guler GB et al., Tenting area reflects disease severity and prognosis in patients with non-ischaemic dilated cardiomyopathy and functional mitral regurgitation, Eur J Heart Fail. 13 (2011) 284-291.

[17] Tischler MD, Niggel J, Borowski DT et al., Relation between left ventricular shape and exercise capacity in patients with left ventricular dysfunction, J Am Coll Cardiol. 22 (1993) 751-757.

[18] Dorosz JL, Lezotte DC, Weitzenkamp DA et al., Performance of 3-dimensional echocardiography in measuring left ventricular volumes and ejection fraction: a systematic review and meta-analysis, J Am Coll Cardiol. 59 (2012) 1799-1808.

[19] Marwick TH, Leano RL, Brown J et al., Myocardial strain measurement with 2-dimensional speckle-tracking echocardiography: definition of normal range, JACC Cardiovasc Imaging. 2 (2009) 80-84.

[20] Okada M, Tanaka H, Matsumoto K et al., Subclinical myocardial dysfunction in patients with reverse-remodeled dilated cardiomyopathy, J Am Soc Echocardiogr. 25 (2012) 726-732.

[21] Cordero-Reyes AM, Youker K, Estep JD et al., Molecular and cellular correlates of cardiac function in end-stage DCM: a study using speckle tracking echocardiography, JACC Cardiovasc Imaging. 7 (2014) 441-452.

[22] Fujita N, Duerinekx AJ, Higgins CB, Variation in left ventricular regional wall stress with cine magnetic resonance imaging: normal subjects versus dilated cardiomyopathy, Am Heart J. 125 (1993) 1337-1345.

[23] Popescu BA, Beladan CC, Calin A et al., Left ventricular remodelling and torsional dynamics in dilated cardiomyopathy: reversed apical rotation as a marker of disease severity, Eur J Heart Fail. 11 (2009) 945-951.

[24] Kobayashi M, Izawa H, Cheng XW et al., Dobutamine stress testing as a diagnostic tool for evaluation of myocardial contractile reserve in asymptomatic or mildly symptomatic patients with dilated cardiomyopathy, JACC Cardiovasc Imaging. 1 (2008) 718-726.

[25] La Vecchia L, Paccanaro M, Bonanno C et al., Left ventricular versus biventricular dysfunction in idiopathic dilated cardiomyopathy, Am J Cardiol. 83 (1999) 120-122, A129.

[26] Gulati A, Ismail TF, Jabbour A et al., The prevalence and prognostic significance of right ventricular systolic dysfunction in nonischemic dilated cardiomyopathy, Circulation. 128 (2013) 1623-1633.

[27] Ghio S, Recusani F, Klersy C et al., Prognostic usefulness of the tricuspid annular plane systolic excursion in patients with congestive heart failure secondary to idiopathic or ischemic dilated cardiomyopathy, Am J Cardiol. 85 (2000) 837-842.

[28] La Vecchia L, Zanolla L, Varotto L et al., Reduced right ventricular ejection fraction as a marker for idiopathic dilated cardiomyopathy compared with ischemic left ventricular dysfunction, Am Heart J. 142 (2001) 181-189.

[29] Janda S, Shahidi N, Gin K et al., Diagnostic accuracy of echocardiography for pulmonary hypertension: a systematic review and meta-analysis, Heart. 97 (2011) 612-622.

[30] Agricola E, Stella S, Figini F et al., Non-ischemic dilated cardiopathy: prognostic value of functional mitral regurgitation, Int J Cardiol. 146 (2011) 426-428.

[31] Hirashiki A, Kondo T, Adachi S et al., Prognostic value of pulmonary hypertension in ambulatory patients with non-ischemic dilated cardiomyopathy, Circ J. 78 (2014) 1245-1253.

[32] Chang PP, Longenecker JC, Wang NY et al., Mild vs severe pulmonary hypertension before heart transplantation: different effects on posttransplantation pulmonary hypertension and mortality, J Heart Lung Transplant. 24 (2005) 998-1007.

[33] McCarthy KP, Ring L, Rana BS, Anatomy of the mitral valve: understanding the mitral valve complex in mitral regurgitation, Eur J Echocardiogr. 11 (2010) i3-9.

[34] Lancellotti P, Moura L, Pierard LA et al., European Association of Echocardiography recommendations for the assessment of valvular regurgitation. Part 2: mitral and tricuspid regurgitation (native valve disease), Eur J Echocardiogr. 11 (2010) 307-332.

[35] Zile MR, Brutsaert DL, New concepts in diastolic dysfunction and diastolic heart failure: Part II: causal mechanisms and treatment, Circulation. 105 (2002) 1503-1508.

[36] Vanoverschelde JL, Raphael DA, Robert AR et al., Left ventricular filling in dilated cardiomyopathy: relation to functional class and hemodynamics, J Am Coll Cardiol. 15 (1990) 1288-1295.

[37] Thomas DE, Wheeler R, Yousef ZR et al., The role of echocardiography in guiding management in dilated cardiomyopathy, Eur J Echocardiogr. 10 (2009) iii15-21.

[38] Kim RJ, Wu E, Rafael A et al., The use of contrast-enhanced magnetic resonance imaging to identify reversible myocardial dysfunction, N Engl J Med. 343 (2000) 1445-1453.

[39] Whalley GA, Doughty RN, Gamble GD et al., Pseudonormal mitral filling pattern predicts hospital re-admission in patients with congestive heart failure, J Am Coll Cardiol. 39 (2002) 1787-1795.

[40] Moreo A, Ambrosio G, De Chiara B et al., Influence of myocardial fibrosis on left ventricular diastolic function: noninvasive assessment by cardiac magnetic resonance and echo, Circ Cardiovasc Imaging. 2 (2009) 437-443.

[41] Akinrinade O, Ollila L, Vattulainen S et al., Genetics and genotype-phenotype correlations in Finnish patients with dilated cardiomyopathy, Eur Heart J. 36 (2015) 2327-2337.

[42] Yoskovitz G, Peled Y, Gramlich M et al., A novel titin mutation in adult-onset familial dilated cardiomyopathy, Am J Cardiol. 109 (2012) 1644-1650.

[43] Perez-Serra A, Toro R, Campuzano O et al., A novel mutation in lamin a/c causing familial dilated cardiomyopathy associated with sudden cardiac death, J Card Fail. 21 (2015) 217-225.

[44] Volpi L, Ricci G, Passino C et al., Prevalent cardiac phenotype resulting in heart transplantation in a novel LMNA gene duplication, Neuromuscul Disord. 20 (2010) 512-516.

[45] Sparrow PJ, Merchant N, Provost YL et al., CT and MR imaging findings in patients with acquired heart disease at risk for sudden cardiac death, Radiographics. 29 (2009) 805-823.

[46] Francone M, Role of cardiac magnetic resonance in the evaluation of dilated cardiomyopathy: diagnostic contribution and prognostic significance, ISRN Radiol. 2014 (2014) 365404.

[47] Shehata ML, Turkbey EB, Vogel-Claussen J et al., Role of cardiac magnetic resonance imaging in assessment of nonischemic cardiomyopathies, Top Magn Reson Imaging. 19 (2008) 43-57.

[48] Assomull RG, Prasad SK, Lyne J et al., Cardiovascular magnetic resonance, fibrosis, and prognosis in dilated cardiomyopathy, J Am Coll Cardiol. 48 (2006) 1977-1985.

[49] Nishii T, Kono AK, Shigeru M et al., Cardiovascular magnetic resonance T2 mapping can detect myocardial edema in idiopathic dilated cardiomyopathy, Int J Cardiovasc Imaging. 30 Suppl 1 (2014) 65-72.

[50] Barison A, Grigoratos C, Todiere G et al., Myocardial interstitial remodelling in non-ischaemic dilated cardiomyopathy: insights from cardiovascular magnetic resonance, Heart Fail Rev. 20 (2015) 731-749.

[51] Kono AK, Croisille P, Nishii T et al., Cardiovascular magnetic resonance tagging imaging correlates with myocardial dysfunction and T2 mapping in idiopathic dilated cardiomyopathy, Int J Cardiovasc Imaging. 30 Suppl 2 (2014) 145-152.

[52] Mahrholdt H, Wagner A, Judd RM et al., Delayed enhancement cardiovascular magnetic resonance assessment of non-ischaemic cardiomyopathies, Eur Heart J. 26 (2005) 1461-1474.

[53] Satoh H, Sano M, Suwa K et al., Distribution of late gadolinium enhancement in various types of cardiomyopathies: Significance in differential diagnosis, clinical features and prognosis, World J Cardiol. 6 (2014) 585-601.

[54] Kehr E, Sono M, Chugh SS et al., Gadolinium-enhanced magnetic resonance imaging for detection and quantification of fibrosis in human myocardium in vitro, Int J Cardiovasc Imaging. 24 (2008) 61-68.

[55] Puntmann VO, Voigt T, Chen Z et al., Native T1 mapping in differentiation of normal myocardium from diffuse disease in hypertrophic and dilated cardiomyopathy, JACC Cardiovasc Imaging. 6 (2013) 475-484.

[56] Hong YJ, Park CH, Kim YJ et al., Extracellular volume fraction in dilated cardiomyopathy patients without obvious late gadolinium enhancement: comparison with healthy control subjects, Int J Cardiovasc Imaging. 31 Suppl 1 (2015) 115-122.

[57] aus dem Siepen F, Buss SJ, Messroghli D et al., T1 mapping in dilated cardiomyopathy with cardiac magnetic resonance: quantification of diffuse myocardial fibrosis and comparison with endomyocardial biopsy, Eur Heart J Cardiovasc Imaging. 16 (2015) 210-216.

[58] Barison A, Del Torto A, Chiappino S et al., Prognostic significance of myocardial extracellular volume fraction in nonischaemic dilated cardiomyopathy, J Cardiovasc Med (Hagerstown). 16 (2015) 681-687.

[59] Hudsmith LE, Neubauer S, Magnetic resonance spectroscopy in myocardial disease, JACC Cardiovasc Imaging. 2 (2009) 87-96.

[60] Ashrafian H, Redwood C, Blair E et al., Hypertrophic cardiomyopathy:a paradigm for myocardial energy depletion, Trends Genet. 19 (2003) 263-268.

[61] Garcia-Pavia P, Cobo-Marcos M, Guzzo-Merello G et al., Genetics in dilated cardiomyopathy, Biomark Med. 7 (2013) 517-533.

[62] LeWinter MM, Granzier HL, Cardiac titin and heart disease, J Cardiovasc Pharmacol. 63 (2014) 207-212.

[63] Haas J, Frese KS, Peil B et al., Atlas of the clinical genetics of human dilated cardiomyopathy, Eur Heart J. 36 (2015) 1123-1135a.

[64] Vaikhanskaya T, Sivitskaya L, Danilenko N et al., LMNA-related dilated cardiomyopathy, Oxf Med Case Reports. 2014 (2014) 102-104.

[65] Tesson F, Saj M, Uvaize MM et al., Lamin A/C mutations in dilated cardiomyopathy, Cardiol J. 21 (2014) 331-342.

[66] Kummeling GJ, Baas AF, Harakalova M et al., Cardiovascular genetics: technological advancements and applicability for dilated cardiomyopathy, Neth Heart J. 23 (2015) 356-362.

[67] Morini E, Sangiuolo F, Caporossi D et al., Application of Next Generation Sequencing for personalized medicine for sudden cardiac death, Front Genet. 6 (2015) 55.

[68] McNally EM, Puckelwartz MJ, Genetic Variation in Cardiomyopathy and Cardiovascular Disorders, Circ J. 79 (2015) 1409-1415.

[69] Herman DS, Lam L, Taylor MR et al., Truncations of titin causing dilated cardiomyopathy, N Engl J Med. 366 (2012) 619-628.

[70] van Rijsingen IA, Nannenberg EA, Arbustini E et al., Gender-specific differences in major cardiac events and mortality in lamin A/C mutation carriers, Eur J Heart Fail. 15 (2013) 376-384.

[71] Mendell JT, Olson EN, MicroRNAs in stress signaling and human disease, Cell. 148 (2012) 1172-1187.

[72] Small EM, Olson EN, Pervasive roles of microRNAs in cardiovascular biology, Nature. 469 (2011) 336-342.

[73] Naga Prasad SV, Karnik SS, MicroRNAs--regulators of signaling networks in dilated cardiomyopathy, J Cardiovasc Transl Res. 3 (2010) 225-234.

[74] Rao PK, Toyama Y, Chiang HR et al., Loss of cardiac microRNA-mediated regulation leads to dilated cardiomyopathy and heart failure, Circ Res. 105 (2009) 585-594.

[75] Gurha P, Abreu-Goodger C, Wang T et al., Targeted deletion of microRNA-22 promotes stress-induced cardiac dilation and contractile dysfunction, Circulation. 125 (2012) 2751-2761.

[76] Wijnen WJ, van der Made I, van den Oever S et al., Cardiomyocyte-specific miRNA-30c over-expression causes dilated cardiomyopathy, PLoS One. 9 (2014) e96290.

[77] Quattrocelli M, Crippa S, Montecchiani C et al., Long-term miR-669a therapy alleviates chronic dilated cardiomyopathy in dystrophic mice, J Am Heart Assoc. 2 (2013) e000284.

[78] Baumgarten A, Bang C, Tschirner A et al., TWIST1 regulates the activity of ubiquitin proteasome system via the miR-199/214 cluster in human end-stage dilated cardiomyopathy, Int J Cardiol. 168 (2013) 1447-1452.

[79] Satoh M, Minami Y, Takahashi Y et al., Expression of microRNA-208 is associated with adverse clinical outcomes in human dilated cardiomyopathy, J Card Fail. 16 (2010) 404-410.

[80] Satoh M, Minami Y, Takahashi Y et al., A cellular microRNA, let-7i, is a novel biomarker for clinical outcome in patients with dilated cardiomyopathy, J Card Fail. 17 (2011) 923-929.

[81] Sylvius N, Bonne G, Straatman K et al., MicroRNA expression profiling in patients with lamin A/C-associated muscular dystrophy, Faseb J. 25 (2011) 3966-3978.

[82] Mitchell PS, Parkin RK, Kroh EM et al., Circulating microRNAs as stable blood-based markers for cancer detection, Proc Natl Acad Sci U S A. 105 (2008) 10513-10518.

[83] Hergenreider E, Heydt S, Treguer K et al., Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs, Nat Cell Biol. 14 (2012) 249-256.

[84] Bang C, Batkai S, Dangwal S et al., Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy, J Clin Invest. 124 (2014) 2136-2146.

[85] de Gonzalo-Calvo D, Cenarro A, Civeira F et al., microRNA expression profile in human coronary smooth muscle cell-derived microparticles is a source of biomarkers, Clin Investig Arterioscler. (2016)

[86] Gupta SK, Bang C, Thum T, Circulating microRNAs as biomarkers and potential paracrine mediators of cardiovascular disease, Circ Cardiovasc Genet. 3 (2010) 484-488.

[87] Romaine SP, Tomaszewski M, Condorelli G et al., MicroRNAs in cardiovascular disease: an introduction for clinicians, Heart. 101 (2015) 921-928.

[88] Roncarati R, Viviani Anselmi C, Losi MA et al., Circulating miR-29a, among other up-regulated microRNAs, is the only biomarker for both hypertrophy and fibrosis in patients with hypertrophic cardiomyopathy, J Am Coll Cardiol. 63 (2014) 920-927.

[89] Wang GK, Zhu JQ, Zhang JT et al., Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans, Eur Heart J. 31 (2010) 659-666.

[90] Rayner K, Dimmeler S, Calin GA et al., Novel biomarkers for acute myocardial infarction: is microRNA the new kid on the block?, Clin Chem. 60 (2014) 812-817.

[91] Enes Coskun M, Kervancioglu M, Oztuzcu S et al., Plasma microRNA profiling of children with idiopathic dilated cardiomyopathy, Biomarkers. 21 (2016) 56-61.

[92] Fan KL, Zhang HF, Shen J et al., Circulating microRNAs levels in Chinese heart failure patients caused by dilated cardiomyopathy, Indian Heart J. 65 (2013) 12-16.

[93] Gupta MK, Halley C, Duan ZH et al., miRNA-548c: a specific signature in circulating PBMCs from dilated cardiomyopathy patients, J Mol Cell Cardiol. 62 (2013) 131-141.

[94] Miyamoto SD, Karimpour-Fard A, Peterson V et al., Circulating microRNA as a biomarker for recovery in pediatric dilated cardiomyopathy, J Heart Lung Transplant. 34 (2015) 724-733.

[95] Nair N, Kumar S, Gongora E et al., Circulating miRNA as novel markers for diastolic dysfunction, Mol Cell Biochem. 376 (2013) 33-40.

[96] Voellenkle C, van Rooij J, Cappuzzello C et al., MicroRNA signatures in peripheral blood mononuclear cells of chronic heart failure patients, Physiol Genomics. 42 (2010) 420-426.

[97] Devaux Y, Zangrando J, Schroen B et al., Long noncoding RNAs in cardiac development and ageing, Nat Rev Cardiol. 12 (2015) 415-425.

[98] Thum T, Condorelli G, Long Noncoding RNAs and MicroRNAs in Cardiovascular Pathophysiology, Circ Res. 116 (2015) 751-762.

[99] Xu L, Fengji L, Changning L et al., Comparison of the Prognostic Utility of the Diverse Molecular Data among lncRNA, DNA Methylation, microRNA, and mRNA across Five Human Cancers, PLoS One. 10 (2015) e0142433.

[100] de Kok JB, Verhaegh GW, Roelofs RW et al., DD3(PCA3), a very sensitive and specific marker to detect prostate tumors, Cancer Res. 62 (2002) 2695-2698.

[101] Cai Y, Yang Y, Chen X et al., Circulating 'lncRNA OTTHUMT00000387022' from monocytes as a novel biomarker for coronary artery disease, Cardiovasc Res. (2016)

[102] Kitow J, Derda AA, Beermann J et al., Mitochondrial long noncoding RNAs as blood based biomarkers for cardiac remodeling in patients with hypertrophic cardiomyopathy, Am J Physiol Heart Circ Physiol. (2016) ajpheart 00194 02016.

[103] Kumarswamy R, Bauters C, Volkmann I et al., Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure, Circ Res. 114 (2014) 1569-1575.

[104] Vausort M, Wagner DR, Devaux Y, Long noncoding RNAs in patients with acute myocardial infarction, Circ Res. 115 (2014) 668-677.

[105] Pugh TJ, Kelly MA, Gowrisankar S et al., The landscape of genetic variation in dilated cardiomyopathy as surveyed by clinical DNA sequencing, Genet Med. 16 (2014) 601-608.

[106] Li D, Tapscoft T, Gonzalez O et al., Desmin mutation responsible for idiopathic dilated cardiomyopathy, Circulation. 100 (1999) 461-464.

[107] Lakdawala NK, Funke BH, Baxter S et al., Genetic testing for dilated cardiomyopathy in clinical practice, J Card Fail. 18 (2012) 296-303.

[108] Ackerman MJ, Splawski I, Makielski JC et al., Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing, Heart Rhythm. 1 (2004) 600-607.

[109] Ng D, Johnston JJ, Teer JK et al., Interpreting secondary cardiac disease variants in an exome cohort, Circ Cardiovasc Genet. 6 (2013) 337-346.

[110] Kamisago M, Sharma SD, DePalma SR et al., Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy, N Engl J Med. 343 (2000) 1688-1696.

[111] Norton N, Li D, Rieder MJ et al., Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy, Am J Hum Genet. 88 (2011) 273-282.

[112] Brauch KM, Karst ML, Herron KJ et al., Mutations in ribonucleic acid binding protein gene cause familial dilated cardiomyopathy, J Am Coll Cardiol. 54 (2009) 930-941.

[113] Vatta M, Mohapatra B, Jimenez S et al., Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction, J Am Coll Cardiol. 42 (2003) 2014-2027.

[114] Hirtle-Lewis M, Desbiens K, Ruel I et al., The genetics of dilated cardiomyopathy: a prioritized candidate gene study of LMNA, TNNT2, TCAP, and PLN, Clin Cardiol. 36 (2013) 628-633.

[115] Andreasen C, Nielsen JB, Refsgaard L et al., New population-based exome data are questioning the pathogenicity of previously cardiomyopathy-associated genetic variants, Eur J Hum Genet. 21 (2013) 918-928.

[116] Zimmerman RS, Cox S, Lakdawala NK et al., A novel custom resequencing array for dilated cardiomyopathy, Genet Med. 12 (2010) 268-278.

[117] Vasile VC, Will ML, Ommen SR et al., Identification of a metavinculin missense mutation, R975W, associated with both hypertrophic and dilated cardiomyopathy, Mol Genet Metab. 87 (2006) 169-174.

[118] Norgett EE, Hatsell SJ, Carvajal-Huerta L et al., Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma, Hum Mol Genet. 9 (2000) 2761-2766.

[119] Schonberger J, Wang L, Shin JT et al., Mutation in the transcriptional coactivator EYA4 causes dilated cardiomyopathy and sensorineural hearing loss, Nat Genet. 37 (2005) 418-422.

[120] Murphy RT, Mogensen J, Shaw A et al., Novel mutation in cardiac troponin I in recessive idiopathic dilated cardiomyopathy, Lancet. 363 (2004) 371-372.

[121] Muntoni F, Cau M, Ganau A et al., Brief report: deletion of the dystrophin muscle-promoter region associated with X-linked dilated cardiomyopathy, N Engl J Med. 329 (1993) 921-925.

[122] Mook OR, Haagmans MA, Soucy JF et al., Targeted sequence capture and GS-FLX Titanium sequencing of 23 hypertrophic and dilated cardiomyopathy genes: implementation into diagnostics, J Med Genet. 50 (2013) 614-626.

[123] Man E, Lafferty KA, Funke BH et al., NGS identifies TAZ mutation in a family with X-linked dilated cardiomyopathy, BMJ Case Rep. 2013 (2013)

[124] Coral-Vazquez R, Cohn RD, Moore SA et al., Disruption of the sarcoglycan-sarcospan complex in vascular smooth muscle: a novel mechanism for cardiomyopathy and muscular dystrophy, Cell. 98 (1999) 465-474.

[125] Duboscq-Bidot L, Xu P, Charron P et al., Mutations in the Z-band protein myopalladin gene and idiopathic dilated cardiomyopathy, Cardiovasc Res. 77 (2008) 118-125.

[126] Zhao Y, Feng Y, Zhang YM et al., Targeted next-generation sequencing of candidate genes reveals novel mutations in patients with dilated cardiomyopathy, Int J Mol Med. 36 (2015) 1479-1486.

[127] Garcia-Pavia P, Syrris P, Salas C et al., Desmosomal protein gene mutations in patients with idiopathic dilated cardiomyopathy undergoing cardiac transplantation: a clinicopathological study, Heart. 97 (2011) 1744-1752.

[128] Elliott P, O'Mahony C, Syrris P et al., Prevalence of desmosomal protein gene mutations in patients with dilated cardiomyopathy, Circ Cardiovasc Genet. 3 (2010) 314-322.

[129] Parry TL, Melehani JH, Ranek MJ et al., Functional Amyloid Signaling via the Inflammasome, Necrosome, and Signalosome: New Therapeutic Targets in Heart Failure, Front Cardiovasc Med. 2 (2015) 25.

[130] Karkkainen S, Miettinen R, Tuomainen P et al., A novel mutation, Arg71Thr, in the delta-sarcoglycan gene is associated with dilated cardiomyopathy, J Mol Med (Berl). 81 (2003) 795-800.

[131] Gianni D, Li A, Tesco G et al., Protein aggregates and novel presenilin gene variants in idiopathic dilated cardiomyopathy, Circulation. 121 (2010) 1216-1226.

FIGURES LEGENDS

Figure 1. Current methodologies and novel technologies proposed for the clinical management of familial dilated cardiomyopathy. NGS: Next-generation sequencing; SSFP: Steady state free precession; ST: speckle tracking; T2 – STIR: T2 short tau inversion recovery.

Figure 2. Balance Steady State Free Precession image (bSSFP) oriented in a four-chamber view revealing a severe dilatation of the left ventricle in a patient with idiopathic dilated cardiomyopathy. Note the thinner walls of the heart, which are less than 6 mm in the lateral wall (blue arrow). There is also increased trabeculation in the apical lateral segment without fulfilling the Petersen criteria for MR-based diagnosis of non–compaction cardiomyopathy (yellow arrow). Secondary mitral insufficiency with a regurgitant jet present at the A2P2 segment of the mitral valve (green arrow) is also noted. This is in keeping with dilated cardiomyopathy.

Figure 3. Late gadolinium enhancement (LGE) sequence oriented in a short axis view revealing a linear midwall enhancement at the midventricular septal segments of the left ventricle, which is suggestive of fibrosis (white arrows). This pattern of distribution is indicative of a non-ischemic origin of the DCM. Those patients have a higher risk of developing intraventricular systolic dyssynchrony and arrhythmias.

Figure 4. Schematic representation of the primary genes associated with dilated cardiomyopathy.

Table 1. Cardiac magnetic resonance parameters.

Sequence	Aim	Parameter	Clinical importance and prognosis
Cine SSFP	Ventricular geometry, size, contractility and function	RVEDVI, LVEF, LVCI	- LVEF: Strong predictor of mortality. - LVCI: Prognostic of cardiac death and SCD. - RVEDVI: Increased risk of cardiac death and SCD secondary to arrhythmias and decompensated heart failure.
T2 mapping and T2 – STIR	Water content in the myocardium	T2 ratio index T2 values	- Increment of water content with disease progression.
Late Gadolinium enhancement	Assessment of focal or replacement fibrosis	Visual assessment	- Increased risk of mortality, heart failure, tachycardia and SCD.
T1 mapping	Assessment of diffuse interstitial fibrosis	Native T1, post – contrast T1, Gdλ and ECV	- Association with left ventricular systolic function and impaired ventricular function. - Differentiation of "early" DCM, "overt" DCM and normal controls. - Prognostic predictor of death, heart failure and malignant arrhythmias.
Cardiac MR spectroscopy	Evaluation of tissue metabolism	Visual assessment	- Associated with impaired ventricular filling, systolic function and life-threatening arrhythmias. - Higher incidence of SCD and tachycardia. - Increased risk of death and hospitalization. - Risk stratification for device implantation.

ECV: extracellular volume; Gdλ: partition coefficient; LVEF: left ventricle ejection fraction; LVCI: left ventricle cardiac index; RVEDVI: right ventricle end diastolic volume index; SCD: sudden cardiac death; SSFP: steady state free precession; T2 – STIR: T2 short tau inversion recovery.

Table 2. The genes associated with familial dilated cardiomyopathy classified as pathogenic or as likely pathogenic according to previously published studies, although the classification is not selective.

Clinical significance: pathogenic

Symbol	Name	% of FDC caused by the pathogenic mutation	Protein Affected/Function	Reference
TTN	titin	20-30%	*Sarcomere*	[105]
LMNA	lamin A/C	6%	Nuclear	[105]
MYBPC3	myosin binding protein C, cardiac	4%	Sarcomere	[105]
DES	desmin	4%	Desmosome	[106]
MYH7	myosin, heavy chain 7, cardiac muscle, beta	4%	Sarcomere	[107]
SCN5A	sodium voltage-gated channel alpha subunit 5	4%	Ion Channel	[108]
DES	desmin	4%	Desmosome	[106]
MYH6	myosin, heavy chain 6, cardiac muscle, alpha	3-4%	Sarcomere	[109]
TNNT2	troponin T2, cardiac type	3%	Sarcomere	[110]
BAG3	BCL2 associated athanogene 3	2.5%	Z-disc	[111]
RBM20	RNA binding motif protein 20	2%	Sarcomere	[112]
LDB3	LIM domain binding 3	1%	Sarcomere	[113]
TCAP	titin-cap	1%	Z-disc	[114]
TMPO	thymopoietin	1%	Nuclear	[115]
TNNC1	troponin C1, slow skeletal and cardiac type	1%	Sarcomere	[105]
TPM1	tropomyosin 1 (alpha)	1%	Sarcomere	[116]
VCL	vinculin	1%	Cytoskeleton	[117]
ACTN2	actinin alpha 2	<1%	Z-disc	[116]
DSP	desmoplakin	<1%	Desmosome	[118]

PLN	phospholamban	<1%	Sarcoplasmic Reticulum	[105]
EYA4	EYA transcriptional coactivator and phosphatase 4	<1%	Transcription Factor	[119]
CSRP3	cysteine and glycine rich protein 3	<1%	Z-disc	[116]
TNNI3	troponin I3, cardiac type	<1%	Sarcomere	[120]
DMD	dystrophin	<1%	Cytoskeleton	[121]
LAMP2	lysosomal associated membrane protein 2	<1%	Cytoskeleton	[122]
TAZ	tafazzin	<1%	Cytoskeleton	[123]
NEXN	nexilin	<1%	Cytoskeleton	[115]
SGCD	sarcoglycan delta	<1%	Sarcomere	[124]
MYPN	myopalladin	<1%	Z-disc	[125]
ANKRD1	ankyrin repeat domain 1	<1%	Sarcomere	[109]
ACTC1	actin, alpha, cardiac muscle 1	<1%	Sarcomere	[105]
EMD	emerin	<1%	Nuclear	[126]
JUP	junction plakoglobin	<1%	Desmosome	[127]
DSC2	desmocollin 2	<1%	Desmosome	[128]
TTR	transthyretin	<1%	Sarcomere	[129]
SGCD	sarcoglycan delta	<1%	Sarcoglycan Complex	[130]
PSEN1	presenilin-1	<1%	Gamma secretase activity	[131]
PSEN2	presenilin-2	<1%	Gamma secretase activity	[131]

Table 3. A summary of the studies measuring circulating miRNAs in dilated cardiomyopathy.

Reference	Study Design	miRNA source	miRNA Biomarker
[96]	7 patients with non-ischemic DCM; 8 patients with ischemic cardiomyopathy; 9 control subjects. Validation in 19 patients with non-ischemic DCM; 15 patients with ischemic cardiomyopathy; 19 control subjects	PBMC	miR-29b, miR-107, miR-139, miR-142-3p, miR-142-5p
[92]	45 DCM patients; 39 age- and sex-matched controls	Plasma	miR-423-5p
[93]	48 non-failing controls; 44 patients with relatively stable CHF associated with DCM. Validation in 41 non-failing; 37 failing patient samples	PBMC	miRNA-548 family
[95]	8 patients with diastolic function and preserved systolic function; 10 patients with stable compensated DCM; 13 patients with decompensated congestive heart failure; 9 normal healthy individuals	Buffy coat	miR-142-3p, miR-124-5p
[94]	55 children <18 years old	Serum	miR-155, miR-636, miR-646, miR-639
[91]	23 children with idiopathic DCM; 26 healthy controls	Plasma	miR-147, miR-194, miR-205, miR-302a, miR-454, miR-518f, miR-618, miR-518f, miR-875-3p

Figure 1.

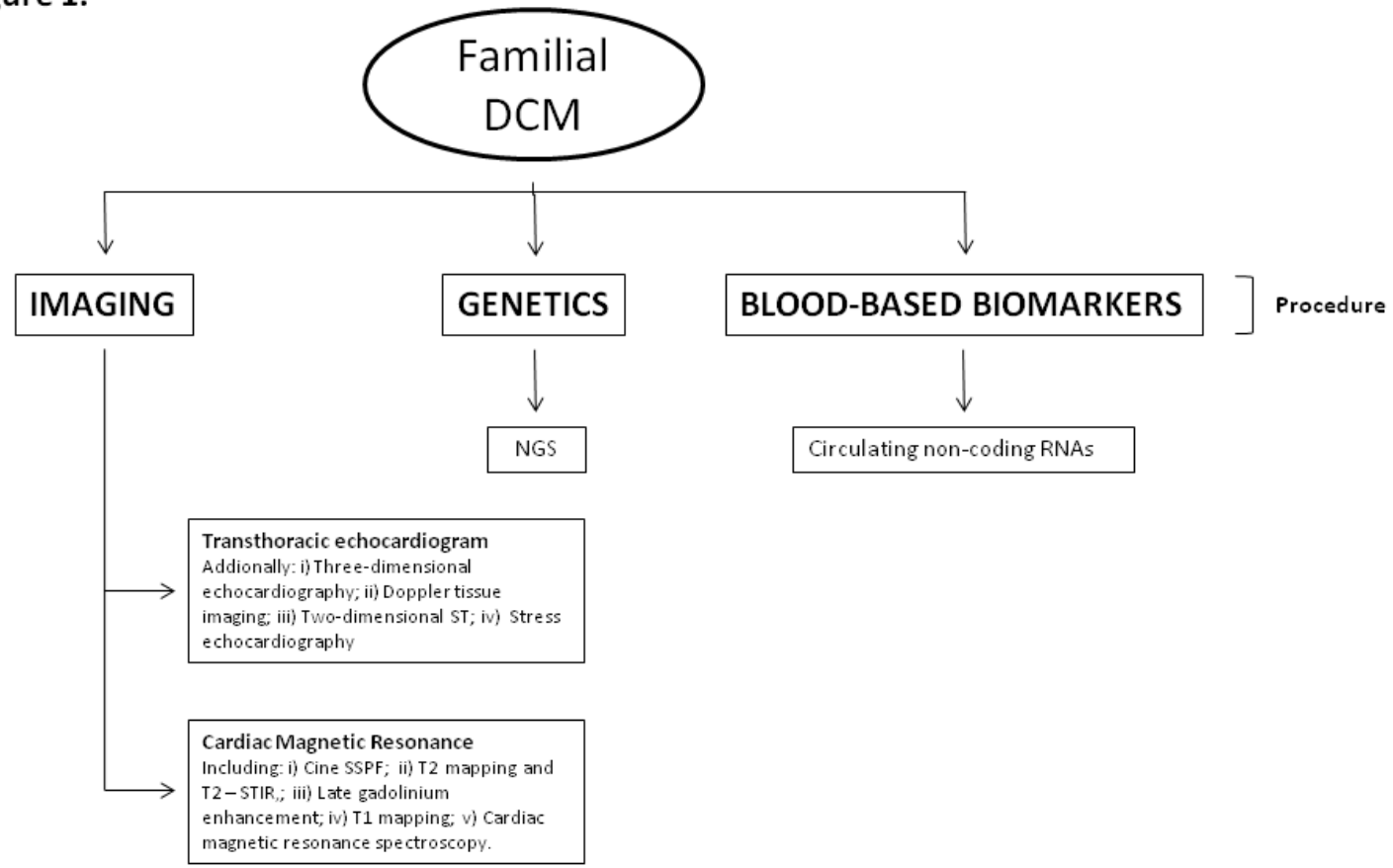


Figure 2.

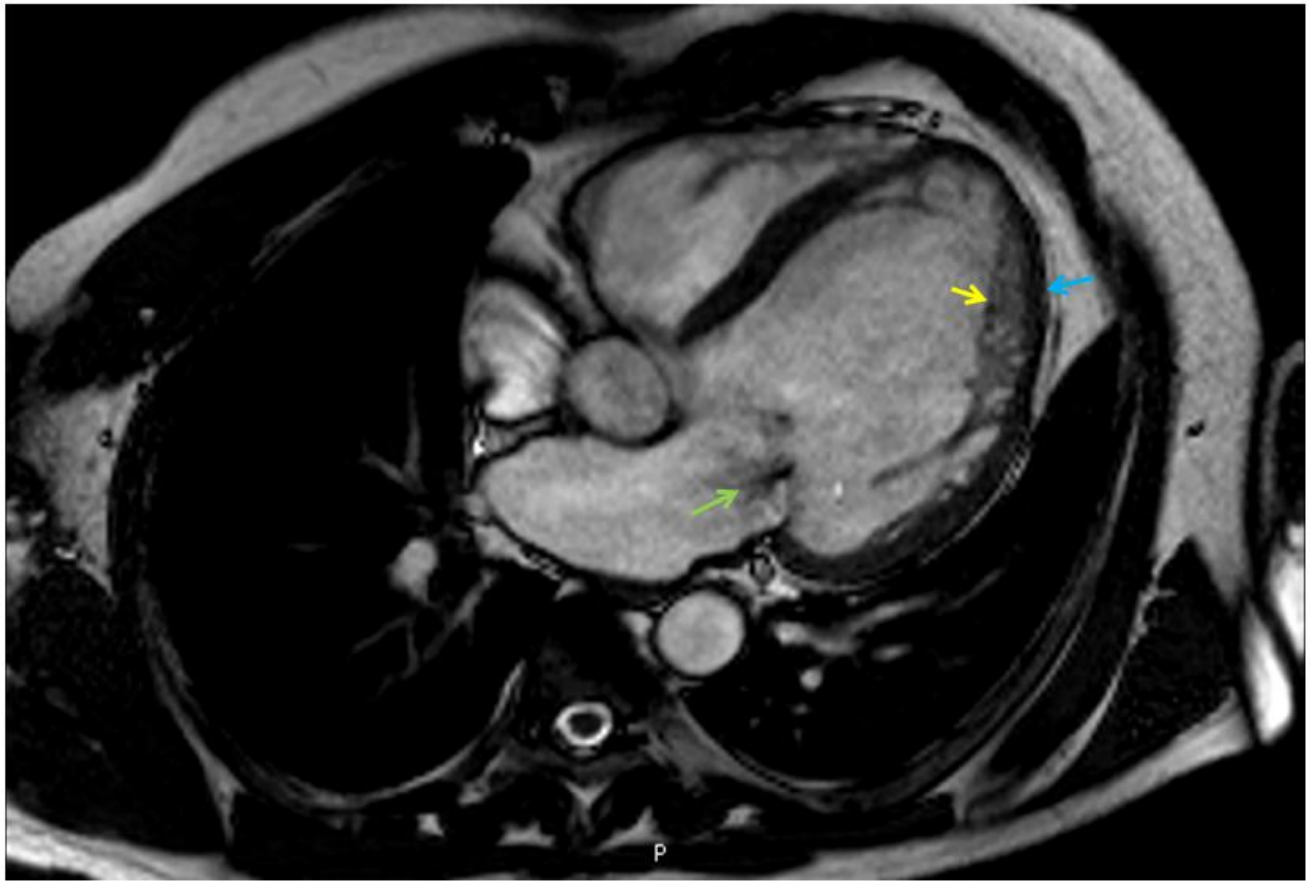


Figure 3.

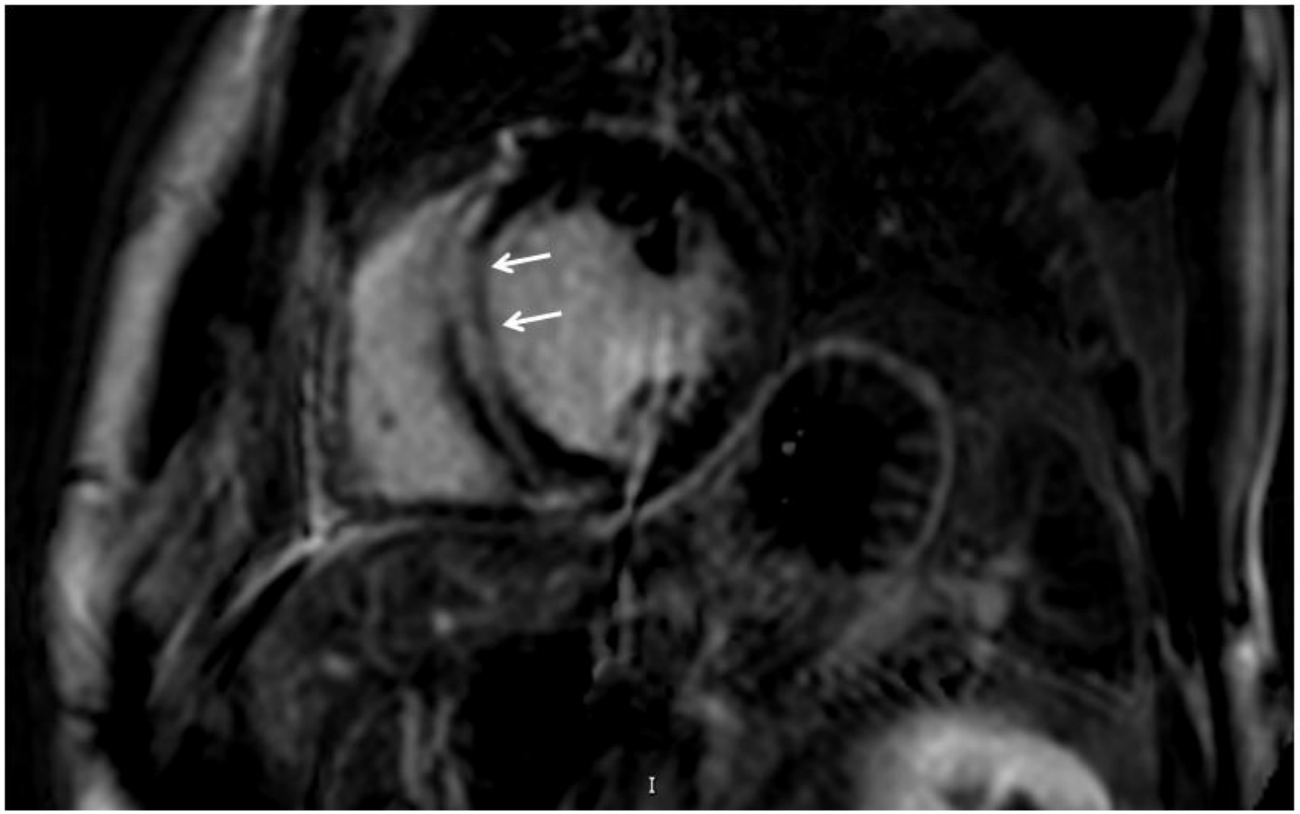
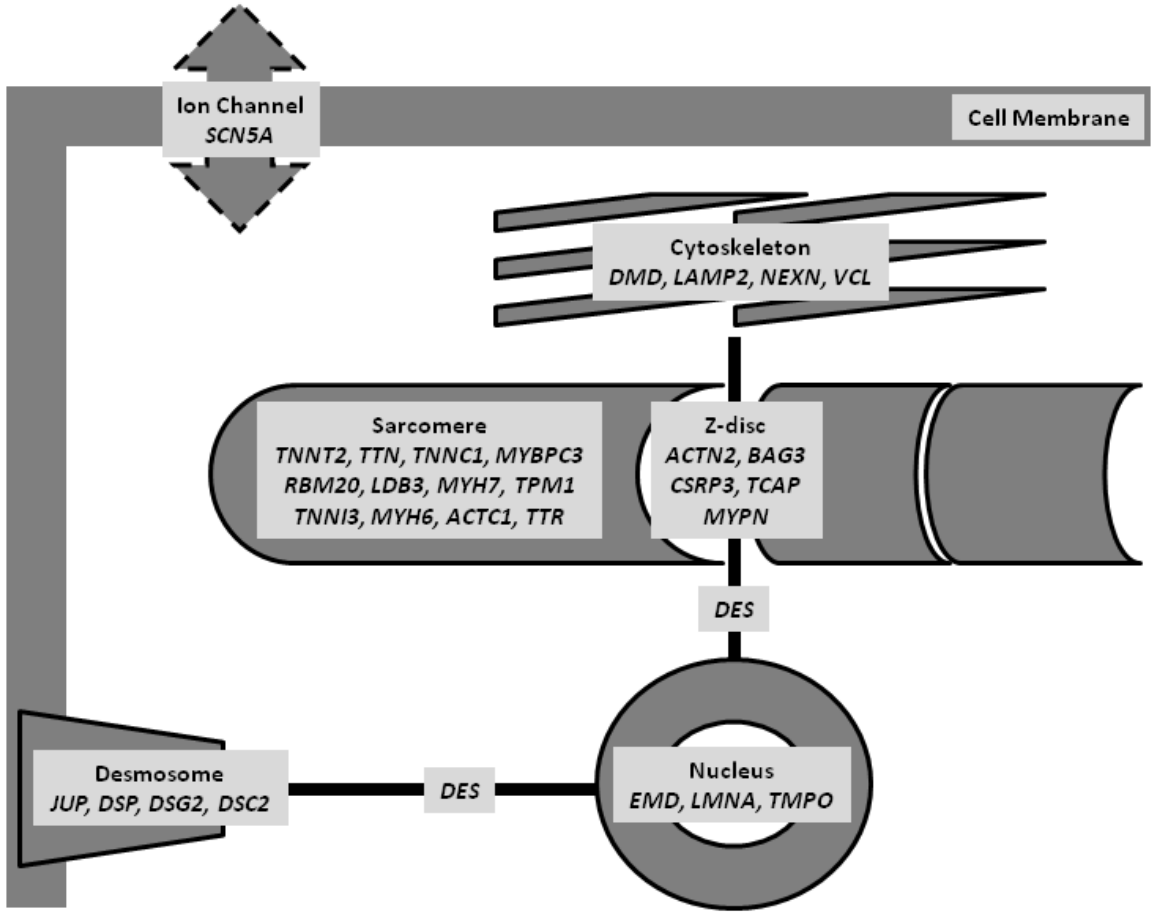


Figure 4.



HIGHLIGHTS

1. Familial dilated cardiomyopathies should be considering a multidisciplinary entity.

2. Imaging, genetic and serological techniques should be considered together in the evaluation and testing of familial dilated cardiomyopathy.

3. A multifocal focus could establish different genetic and cardiovascular imaging patterns in familial dilated cardiomyopathies useful for diagnosis and prognosis status.