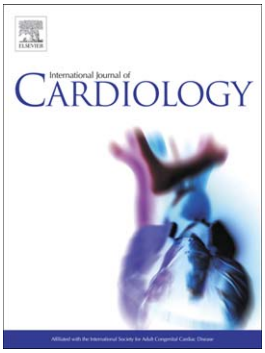

Genetic Basis of Dilated Cardiomyopathy

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PII: S0167-5273(16)32357-9
DOI: doi:10.1016/j.ijcard.2016.09.068
Reference: IJCA 23675

To appear in: *International Journal of Cardiology*

Received date: 13 May 2016
Revised date: 15 September 2016
Accepted date: 17 September 2016

Please cite this article as: Pérez-Serra Alexandra, Toro Rocio, Sarquella-Brugada Georgia, de Gonzalo-Calvo David, Cesar Sergi, Carro Esther, Llorente-Cortes Vicenta, Iglesias Anna, Brugada Josep, Brugada Ramon, Campuzano Oscar, Genetic Basis of Dilated Cardiomyopathy, *International Journal of Cardiology* (2016), doi:10.1016/j.ijcard.2016.09.068

Genetic Basis of Dilated Cardiomyopathy

Running title

Genetics and dilated cardiomyopathy

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CONFLICT OF INTEREST

All authors declare no conflict of interest

Number of words: 15.160

Number Figures/tables: 4

ABSTRACT

Dilated cardiomyopathy is a rare cardiac disease characterized by left ventricular dilatation and systolic dysfunction leading to heart failure and sudden cardiac death. Currently, despite several conditions have been reported as aetiologies of the disease, a large number of cases remain classified as idiopathic. Recent studies determine that nearly 60% of cases are inherited, therefore due to a genetic cause. Progressive technological advances in genetic analysis have identified over 60 genes associated with this entity, being *TTN* the main gene, so far. All these genes encode a wide variety of myocyte proteins, mainly sarcomeric and desmosomal, but physiopathologic pathways are not yet completely unraveled. We review the recent published data about genetics of familial dilated cardiomyopathy.

Keywords

Dilated Cardiomyopathy, Sudden Cardiac Death, Genetics

1. Introduction

Dilated cardiomyopathy (DCM) is a rare cardiac entity representing a vital health issue both in the adult and paediatric population [1]. It is characterized by dilatation of heart muscle, ventricular walls become thin and systolic impairment of left or both ventricles emerge (Figure 1). The prevalence of DCM in remains to clarify despite a recent study suggests a prevalence of 1 in 2,500 individuals. The incidence is 7 per 100,000, and males are more frequently affected than females (3:1). In the paediatric population, DCM is the predominant type of cardiomyopathy with an incidence of 0,57 per 100,000 cases [2, 3]. DCM has high rates of morbidity, mortality and is the most frequent cause of heart failure (HF) in the young. Classical criteria for the diagnosis of DCM are the presence of left ventricular fractional shortening <25% and/or left ventricular ejection fraction <45%, and left ventricular end-diastolic dimensions >117% of the predicted value by the Henry formula [4, 5]. The latest published definitions are left ventricular or biventricular systolic dysfunction and dilatation that are not explained by abnormal loading conditions or coronary artery disease, measured by echocardiography or CMR, considering left ventricular dilatation when left ventricular end-diastolic volumes or diameters >2 SD from normal according to normograms corrected by body surface area and age or body surface area and gender [6]. These alterations can lead to HF or premature death and patients could need a cardiac transplantation but is usually a last resort given the limited availability of donor organs, complicated clinical course management and associated morbidity/mortality [7]. Regarding HF guidelines for DCM therapy, angiotensin converting enzyme (ACE) inhibitors represent class 1 indication for patients with structural heart disease with/without symptoms (stages B and C of the heart failure stages) as they reduce morbidity and mortality in asymptomatic-to-moderate symptomatic HF. Similarly, beta-blockers have been demonstrated to reduce mortality and morbidity in HF and their use is a class 1 indication for patients with structural heart disease and past or present symptoms (stage C HF) [8]. Despite of that, no trial has demonstrated an outcome benefit of any pharmacotherapy in children with HF.

In last 20 years, continuous improvements in clinical and genetics basis of DCM has identified several factors as cause of DCM, such as infectious agents leading to myocarditis, some chemical agents like drugs or toxins, peripartum period, nutritional deficiencies, autoimmune disorders or a combination of some of these elements [6] . However, in a high proportion of cases, the etiology remains unsolved or also called idiopathic. Idiopathic DCM is defined as an enlarged LV with systolic function depressed in absence of pressure or volume overload, nor ischemic disease [9]. Within the group of idiopathic DCM, nearly 30-50% of diagnosed cases affects families, being thus inherited and classified of genetic origin. DCM is considered familial when two or more members meet the diagnostic criteria [10]. In this sense, although the diagnosis of familial DCM rarely begins with the identification of a genetic mutation, it is rational to incorporate genetic testing [11-13].

The heritability pattern could be autosomal dominant, recessive, or X-linked although chromosomal abnormalities may also be present and even mitochondrial inheritance cases have been reported [14]. Most part of these discoveries has been reported taking advantage of technological advances in the genetic field. To date, over 400 pathogenic variants has been identified in nearly 60 genes. These genes encode different proteins of nucleus, sarcomere, cytoskeletal or membrane. In all cases, genetic alteration indices structural and functional consequences that impair myocardial force generation, force transmission and cell viability. Thus, genetic diagnosis in the clinical routine can help to identify early diagnosis and adoption of preventive measures both in affected and asymptomatic patients at risk in order to prevent an episode of sudden cardiac death (Figure 2). In addition it allows us to identify non-carriers who do not need a clinical follow-up, avoiding both clinical cost and psychological stress in individual. In concordance, current clinical guidelines recommended genetic test as part of diagnosis in familial cases [15, 16].

Use of Next Generation Sequencing (NGS) platforms has increased the number of genes analyzed associated with DCM [17]. This technique has revolutionized the clinical genetic screening because it analyzes a large number of genes in a cost-effectives way and in a short time [18, 19]. Use of NGS technology in DCM has been suggested in recent reports [20]. However, the amount of data and genes identified using NGS technology has induced a genetic overlap between DCM and other cardiomyopathies such as hypertrophic cardiomyopathy (HCM), and arrhythmogenic cardiomyopathy (AC) [21]. So far, pathogenic variants identified in sarcomeric or desmosomal proteins have being predominating associated with HCM and AC, respectively [22]. However, to date we begin to discover the complexity of genetic basis of DCM. The other main challenge for geneticist and clinicians lies in the interpretation of the large amount of data generated by this high-throughput analysis. Frequently, high quantity of genetic variants of unknown significance is detected and the pathogenicity of them can be tested in computational biology tools. We can predict the pathogenicity *in silico* in terms of pathogenic, likely pathogenic, VUS and benign. Co-segregation in family members is essential to pick out the relative importance of each one.

2. Genetics

As mentioned before, nearly 60% of familial DCM cases show any genetic alteration in one of over 60 genes associated with DCM, and mainly following autosomal pattern of inheritance [23]. Recent studies classify pathogenic variants in the *TTN* gene as the main responsible for familial DCM [24, 25]. Thus, nearly 30%-35% of families diagnosed of DCM show any alteration in this gene. The second gene most prevalent in familial DCM is *LMNA*, responsible for nearly 10%-15%% of cases. Other several genes have been associated with this entity, being responsible all together nearly 5%-10% of all familial DCM cases (Table 1). Finally,

other genetic alterations such as Copy Number Variations (CNV) have been associated with DCM cases but in a low frequency.

2.1 Chromosomal abnormalities

Regarding gross abnormalities, few cases suffering of DCM and showing alteration in number of copies has been reported so far [26]. In this case, the deletion of several exons in the *LMNA* gene causes nuclear membrane abnormalities, compromising the normal function of the lamina and reducing wild type protein levels. This is critical consequence for nuclear envelope integrity (see below). In 2011, Norton et al reported a familial suffering of DCM and carrying a heterozygous large deletion in the *BAG3* gene [27]. This gene (ID: 9531, 10q25.2-q26.2) encodes BCL2-associated athanogene 3 protein. BAG proteins compete with Hip for binding to the Hsc70/Hsp70 ATPase domain and promote substrate release. The protein encoded by this gene contains a WW domain in the N-terminal region and a BAG domain in the C-terminal region. The BAG domains of BAG1, BAG2, and BAG3 interact specifically with the Hsc70 ATPase domain in vitro and in mammalian cells. All 3 proteins bind with high affinity to the ATPase domain of Hsc70 and inhibit its chaperone activity in a Hip-repressible manner. Also in 2011, Norton performed an analysis of CNV in a cohort of DCM cases but none was identified, concluding that point mutations are the major cause of DCM [28].

2.2 Autosomal dominant

Most of familial DCM cases are due to pathogenic variants following an autosomal pattern of inheritance. These pathogenic variants have been identified in several genes encoding proteins with different functions, such as ion channels, transcription factors, sarcomeric, desmosomal, and nuclear proteins, among others.

Ion channels

Ion channels are membrane protein complexes and their function is to facilitate the diffusion of ions across biological membranes, such as in myocytes. Pathogenic variants in cardiac ion channels lead to muscular contraction deterioration. Currently, three genes have been associated with DCM: *SCN5A, KCNQ1* and *ABCC9*.

The first gene is *SCN5A* (ID: 6331, 3p21), which encodes sodium channel, voltage gated, type V alpha subunit. In cardiomyocytes, sodium cannels are implicated in the fast depolarization of the myocardium and have an important role in the maintenance of impulse conduction [29]. The sodium channels are responsible for the rapid depolarization of the myocardium and are critical for the maintenance of the cardiac impulse

conduction [30]. Pathogenic variants in the *SCN5A* gene modify electrical excitability of sodium cannel that causes an imbalance in the currents of the different ions involved in the contraction process of the cardiomyocytes. This way, it induces ventricular remodeling and DCM. The first pathogenic variant in *SCN5A* associated with DCM was reported in 2004[31, 32]. To date, a total of 12 pathogenic variants have been associated with DCM (10 missense –CM087603, CM124743, CM087605, CM113111, CM055530-, CM113113, CM113112, CM137776, CM055526, CM088419 and CM087604-, and 2 small insertions -CI055774 and CI132939-) [33-41].

The *KCNQ1* gene has been also associated with DCM in patients carrying a genetic variant that provokes a loss of function of potassium cannel or auto-immune deficiency [42]. This gene (ID: 3784, 11p15.5) encodes the potassium channel, voltage gated KQT-like subfamily Q, member 1, required for repolarization phase of the cardiac action potential.

The third gene is *ABCC9* (ID: 10060, 12p12.1). The protein encoded by this gene (ATP-binding cassette, sub-family C (CFTR/MRP), member 9) is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. The first association between this gene and DCM was reported in 2004. Currently, 3 pathogenic variants have been associated with DCM (1 missense –CM1410876-, 1 nonsense –CM040975-, and 1 small indel –CX041212-) [43-46].

Transcription factors

In terms of regulation, cardiac pathogenic variants in transcription factors (TF) genes have been also described involved in cardiogenesis. Often alterations in these proteins cause heart malformations. This abnormal cardiac remodeling was associated with a decrease in transcriptional activation and it contributes to the development of DCM [47]. The *TBX5* (ID: 6910, 12q24.1), *TBX20* (ID: 57057, 7p14.3), and *NKX2-5* (ID: 1482, 5q34) genes are TF which activate the transcription of a set of gens expressed during cardiac development and structural remodeling. DCM patients with T-box 5 or T-box 20 altered show a decrease in transcriptional activity [48]. These loss-of-function alterations were identified in families suffering of DCM, inherited in an autosomal dominant pattern and showing a complete penetrance. Hence, 2 missense variants in *TBX5* have been associated with DCM (CM155953 and CM153188) [47]. In *TBX20*, only one variants has been reported in DCM (CM157143). Recently, Yuan et al reported a pathogenic variant in the *NK2-5* gene (NK2 homebox 5) associated with a significantly reduced transcriptional activity in familial DCM [49].

In addition, *GATA4* (ID: 2626, 8p23.1-p22, GATA binding protein 4), *GATA5* (ID: 140628, 20q13.33, GATA binding protein 5), and *GATA 6* (ID: 2627, 18q11.1-q11.2, GATA binding protein 6) regulate the expression of

cardiac structural and regulatory genes as expression for sarcomeric proteins and atrial natriuretic factors. Currently, only 2 missense pathogenic variants in *GATA4* have been reported in DCM (CM139943 and CM141469). Regarding *GATA6*, in 2014 was reported the only pathogenic variant associated with DCM [50] . Also, in *GATA5* another mutation was described [51].

Other TF associated with DCM, and sensorineural deafness, is the *EYA4* gene (ID: 2070, 6q23) which encodes a member of the eyes absent (EYA) family of proteins (EYA transcriptional coactivator and phosphatase 4). The encoded protein may act as a transcriptional activator through its protein phosphatase activity, and it may be important for eye development, and for continued function of the mature organ of Corti. To date, only one gross deletion in this gene have been associated with postlingual, progressive, autosomal dominant sensorineural hearing loss (SNHL) with DCM (CG052657) [52].

Finally, the *FOXD4* gene (ID: 2298, 9p24.3) encodes the protein forkhead box D4. This protein is a member of the forkhead/winged helix-box (FOX) family of transcription factors. FOX transcription factors play critical roles in the regulation of multiple processes including metabolism, cell proliferation and gene expression during ontogenesis. The forkhead/winged helix box (FOX) gene family comprises at least 43 different genes encoding transcriptional factors with a highly conserved DNA-binding domain. To date, only one missense pathogenic variant has been reported but associated with a complex phenotype consisting of familial DCM, obsessive-compulsive disorder, and suicidality (CM073074) [53].

Sarcomere

The sarcomere is the contraction unit for striated muscle limited by Z bands. Interaction between thin filaments and thick filaments in conjunction with titin, tropomyosin and other proteins induces muscle contraction. Pathogenic variants in sarcomeric genes are associated to decrease in contractile function [54]. To date, several sarcomeric genes have been associated with familial DCM following an autosomal pattern of inheritance: *ACTC1, MYBPC3, MYH6, MYH7, TNNC1, TNNI3, TNNT2, TPM1,* and *TTN*.

The *ACTC1* gene (ID: 70, 15q14) encodes Actin Alpha (Cardiac Muscle 1) protein. Actins are highly conserved proteins that are involved in various types of cell motility. Polymerization of globular actin (G-actin) leads to a structural filament (F-actin) in the form of a two-stranded helix. Each actin can bind to four others. The protein encoded by this gene belongs to the actin family which is comprised of three main groups of actin isoforms, alpha, beta, and gamma. The alpha actins are found in muscle tissues and are a major constituent of the contractile apparatus. To date, 7 pathogenic variants have been associated with DCM (6 missense -CM1010343, CM122826, CM122827, CM1410877, CM980030, and CM980031-, and 1 splicing -CS1410878-) [44, 55-60].

The *MYBPC3* gene (ID: 4607, 11p11.2) encodes the cardiac isoform of myosin-binding protein C. Myosin-binding protein C is a myosin-associated protein found in the cross-bridge-bearing zone (C region) of A bands in striated muscle. MYBPC3, the cardiac isoform, is expressed exclusively in heart muscle. Regulatory phosphorylation of the cardiac isoform in vivo by cAMP-dependent protein kinase (PKA) upon adrenergic stimulation may be linked to modulation of cardiac contraction. To date, a total of 56 pathogenic variants have been associated with DCM: 31 missense, 2 nonsense –CM115896, CM0911463, CM-, 4 splicing (CS115916, CS103025, CS063341 and CS1010363), 12 small deletions, 4 small insertions (CI1010244, CI1010242, CI1010243 and CI1010245), 2 small indels (CX122832 and CX1010246), and 1 gross deletion (CG1010362) [44, 57, 60-64].

The *MYH6* gene (ID: 4624, 14q12) encodes the Myosin Heavy Chain 6 (Cardiac Muscle, Alpha) protein. Cardiac muscle myosin is a hexamer consisting of two heavy chain subunits, two light chain subunits, and two regulatory subunits. Changes in the relative abundance of this protein and the alpha (or fast) heavy subunit of cardiac myosin correlate with the contractile velocity of cardiac muscle. To date, a total of 11 variants have been associated with DCM (10 missense –CM103026, CM103027, CM052256, CM052257, CM1510986, CM103028, CM1510987, CM103030, CM052259, CM103029-, and 1 small indel -CX103031-)[46, 65-68].

The *MYH7* gene (ID: 4625, 14q11.2) encodes Myosin Heavy Chain 7 (Cardiac Muscle, Beta) protein. It is expressed predominantly in normal human ventricle. To date, a total of 99 pathogenic variants have been associated with DCM (94 missense/nonsense, 1 splicing -CS1010356-, 3 small deletions -CD1010247, CD1010368 and CD1010248-, and 1 small insertion –CI1010367-) [57, 60, 69, 70].

Other gene is *TNNI3* (ID: 7137, 19q13.4). This gene encodes the TnI-cardiac protein and is exclusively expressed in cardiac muscle tissues. Troponin I (TnI), along with troponin T (TnT) and troponin C (TnC), is one of 3 subunits that form the troponin complex of the thin filaments of striated muscle. TnI is the inhibitory subunit; blocking actin-myosin interactions and thereby mediating striated muscle relaxation. The TnI subfamily contains three genes: TnI-skeletal-fast-twitch, TnI-skeletal-slow-twitch, and TnI-cardiac. Pathogenic variants have been reported following an autosomic dominant but also a recessive pattern of inheritance (see data below). Regarding dominant pattern, a total of 10 pathogenic variants have been reported, 10 missense (CM109301, CM094825, CM0910150, CM0910151, CM117176, CM103040, CM122824, CM122825, and CM094826), and 1 small deletion (CD1010249) [57, 60, 71-76].

The *TNNT2* gene (ID: 7139; 1q32) encodes the Troponin T Type 2 (Cardiac) protein which is located on the thin filament of striated muscles and regulates muscle contraction in response to alterations in intracellular calcium ion concentration. To date, a total of 38 pathogenic variants have been associated with DCM. Of

them 29 are missense/nonsense, 4 splicing (CS146730, CS1010370, CS146731 and CS146727), 4 small deletions (CD132937, CD138796, CD117175 and CD003106), and 1 small insertion (CI1010374) [54, 60, 69, 75].

The *TNNC1* gene (ID: 7134, 3p21.1) encodes the Troponin C Type (Slow) protein -TnC-. The binding of calcium to TnC abolishes the inhibitory action of TnI, thus allowing the interaction of actin with myosin, the hydrolysis of ATP, and the generation of tension. To date, a total of 8 missense pathogenic variants have been associated with DCM (CM1510989, CM103038, CM109623, CM088074, CM085751, CM103037, CM103039 and CM043593) [46, 68, 76-78].

The *TPM1* gene (ID: 7168, 15q22.1) encodes the Tropomyosin 1 (Alpha) protein, the predominant tropomyosin of striated muscle, where it also functions in association with the troponin complex to regulate the calcium-dependent interaction of actin and myosin during muscle contraction. This gene has been associated with HCM and DCM. To date, a total of 20 pathogenic variants have been associated with DCM. Of them, 19 were missense and 1 splicing (CS1010373) [60].

Alterations in the *TTN* gene (ID: 7273, 2q31) are the main cause of DCM. It is a key sarcomeric, which encodes a huge protein, titin. This one is an important giant protein that provides an external scaffold and interacts with both thin and thick filaments, playing an important role in sarcomere assembly and in force generation. *TTN* provides a passive force and elasticity to preserve diastolic and systolic function respectively. TTN truncating mutations are a common cause of DCM, occurring in approximately 25% of familial cases of idiopathic dilated cardiomyopathy and in 18% of sporadic case. Clinical manifestation in titin mutation are similar related to symptoms, morbidity and mortality, but the disease is more aggressive with adverse events in men at earlier ages [79]. To date, a total of 37 pathogenic variants have been associated with DCM (16 missense/nonsense, 2 splicing –CS1410930and CS133421-, 14 small deletions -CD1410925, CD1410926, CD1410927, CD1410928, CD1410929, CD1410933, CD1410934, CD1410937, CD133391, CD072504 and CD072503-, 5 small insertions –CI1410939, CI020367, CI1410940, CI122912 and CI133390-) [44, 65, 80-83]. In addition, induced Pluripotent Stem Cell–Derived Cardiomyocytes studies have shown the pathogenicity of *TTN* truncating missense variants which lead to cardiomyocytes contraction deficiency. These mutations produce stable proteins but they can not interact properly with the sarcomere protein network resulting in a clinically detectable dysfunction [25].

Guo et al proposed a model where the less efficiency of protein encode by the *RBM20* gene results in altered protein expression from sarcomere, among them titin, modifying structure and cardiac function [84]. The *RBM20* gene has been linked to DCM previously [85]. It is a cardiac splicing regulator and selects alternative

splice sites in pre-mRNA. Its regulatory activity join in a pathway that regulates cardiac morphology carry weight in DCM.

Desmosome

Desmosome is a myocyte structure responsible of mechanical intercellular junctions. In combination with the adherents and gap junctions, connect myocardial cells which maintain both the mechanical and electrical integrity of the heart. Desmosomal genes are mainly associated with AC. However, several pathogenic variants in familial DCM cases have been identified in 4 desmosomal genes: *DSP, DSG2, DSC2* and *PKP2*. In most part of these families, autosomal dominant pattern of inheritance was observed. Only in *DSP* has been reported a pathogenic variant following an autosomal recessive pattern of inheritance in DCM. It is important to remark that some of these pathogenic variants have been described in cases showing a mixed phenotype of isolated DCM and AC but without fulfilling the diagnostic criteria for AC.

The *DSP* gene (ID: 1832, 6p24) encodes desmoplakin protein. The N-terminus of desmoplakin is required for localization to the desmosome and interacts with the N-terminal region of plakophilin 1 and plakoglobin. The C-terminus of desmoplakin binds with intermediate filaments. In the mid-region of desmoplakin, a coiled-coiled rod domain is responsible for homodimerization. Although pathogenic variants in desmosomes are mainly associated with AC, pathogenic variants on desmosome proteins associated with DCM have also been described, following both a recessive (see above, autosomal recessive), and dominant pattern of inheritance. It has been reported only a pathogenic variant resulting a truncated desmoplakin protein missing the C-domain of the tail region in patients with epidermal disease and DCM [86]. To date, 12 variants have been associated with DCM: 8 missense (CM1413539, CM101082, CM117223, CM1510354, CM117226, CM117224, C1010383 and CM117225), 1 splicing (CS105371), 1 small deletion (CD105375), and 2 small insertions (CI1410882 and CI117550) [44, 65, 87-90].

The *DSG2* gene (ID: 1829, 18q12.1) encodes desmoglein 2, a calcium-binding transmembrane glycoprotein component of desmosomes. Currently, only 2 pathogenic variants have been associated with DCM (CM1010385 and CM117227) [46, 88, 91].

The *DSC2* gene (ID: 1824, 18q12.1) encodes desmocolin 2. Desmocollins, along with desmogleins, are cadherin-like transmembrane glycoproteins that are major components of the desmosome. Desmosomes are cell-cell junctions that help resist shearing forces and are found in high concentrations in cells subject to mechanical stress. Regarding DCM cases, only 2 missense variants have been reported so far (CM117222 and CM1010386) [65, 88, 91].

Finally, the *PKP2* gene (ID: 5318, 12p11) encodes plakophilin 2, a member of the arm-repeat (armadillo) and plakophilin gene families. Plakophilin proteins contain numerous armadillo repeats, localize to cell desmosomes and nuclei, and participate in linking cadherins to intermediate filaments in the cytoskeleton. This gene product may regulate the signaling activity of beta-catenin. To date, only 1 genetic variant has been associated with DCM (CM1010384) [91].

Nuclear Proteins

The cardiomyocyte nucleus is a cellular region where occurs an interaction of several proteins which regulate the nucleon-cytoskeleton interplay. It plays a key role both in chromatin organization and transcriptional activity. Currently, 3 gens of the nucleus have been associated with familial DCM: *LMNA, EMD,* and *TMPO*. However, only *LMNA,* and *TMPO* follow an autosomal dominant pattern of inheritance. The *EMD* has X-linked inheritance (see X-linked section).

The *TMPO* gene (ID: 7112, 12q22) encodes thymopoietin protein, which play a role in the assembly of the nuclear lamina, and thus help maintain the structural organization of the nuclear envelope. So far, only one pathogenic variant has been reported associated with familial DCM (CM055559) [65, 92].

Regarding the *LMNA* gene (ID: 4000, 1q22), it encodes lamin A/C protein, located in nuclear lamina. The nuclear lamina is a fibrous structure underlying the inner nuclear membrane. Lamina filaments are composed by three polypeptides type called Lamin A, lamina B and lamin C. The *LMNA* gene gives rise to both protein types by alternative splicing [93]. Lamina B is encoded by the *LMNB* gene. The nuclear envelope is supported by the interaction between Lamin and integral proteins of the inner membrane who provide integrity and stability at the nuclear membranes nuclear pore complexes. *LMNA* is the second gene responsible of DCM with mostly an autosomal dominant inheritance pattern. Pathogenic variants in *LMNA* explain 6% of all cases of DCM [13], and 7,5% are familial forms and up to 11% are sporadic forms [94]. To date, 114 pathogenic variants located in the *LMNA* gene have been associated with DCM: 73 missense/nonsense, 13 splicing (CS129892, CS083946, CS1410869, CS129893, CS055592, CS129894, CS050003, CS132933, CS088079, CS1210610, CS1210609, CS129895, CS132934), 13 small deletions (CD035667, CD119158, CD131622, CD035724, CD000266, CD122839, CD065750, CD122840, CD084158, CD003350, CD065751, CD1413532), 9 small insertions (CI032151, CI117821, CI129891, CI117174, CI084290, CI081289, CI050001, CI1010359, CI022596), 1 small indel (CX096065), 3 gross deletions (CG135991, CG077066 and CG100913), and 2 gross insertions (CN066506 and CN094948) [44, 60, 75, 95-101].

The phenotype of DCM patients carrying a pathogenic variant in this gene is extremely variable but frequently exist an alteration of conduction system (sinus bradycardia, atrioventricular conduction block,

atrial tachyarrhythmias), and skeletal movement involvement with or without conduction-system disease, eventually they develop heart failure and cardiac transplant is recommended [102]. Individuals affected show mostly conduction disturbances such as atrioventricular conduction disturbances or left bundle branch block, atrial and ventricular arrthymias, and a sequential findings in electrical and mechanical dysfunction; in addition, the penetrance is very high, with almost all the carriers manifesting some aspect of the DCM in early ages [103]. Usually, lamin A/C DCM has a worst prognosis, serious cardiovascular problems, including SCD, and most frequent transplantations than idiopathic DCM.

Z-disc

The Z-disc or Z-band is a dark thin protein band to which actin filaments are attached in a striated muscle fiber, marking the boundaries between adjacent sarcomeres. It contains a multitude of regulatory proteins which transmit the generated power of muscle contraction to adjacent sarcomeres. To date, it has been reported pathogenic alterations in multiple Z-band proteins encoded by *ACTN2, ANKRD1*, *BAG3, CSRP3, LDB3*, *MYPN*, *NEBL, NEXN*, *PDLIM3*, and *TCAP*.

The *ACTN2* gene (ID: 88, 1q42-q43) encodes a muscle-specific, alpha actinin isoform that is expressed in both skeletal and cardiac muscles, the actinin alpha 2. Alpha actinins belong to the spectrin gene superfamily which represents a diverse group of cytoskeletal proteins, including the alpha and beta spectrins and dystrophins. Alpha actinin is an actin-binding protein with multiple roles in different cell types. In non-muscle cells, the cytoskeletal isoform is found along microfilament bundles and adherents-type junctions, where it is involved in binding actin to the membrane. In contrast, skeletal, cardiac, and smooth muscle isoforms are localized to the Z-disc and analogous dense bodies, where they help anchor the myofibrillar actin filaments. Currently, 6 variants have been associated with DCM (5 missense -CM034714, CM103193, CM1310241, CM1410879, CM103194-, and 1 small deletion -CD103195-) [44, 46, 60, 65].

The *ANKRD1* gene (ID: 27063, 10q23.31) encodes ankyrin repeat domain 1 protein, also named CARP. This protein is localized to the nucleus of endothelial cells and is induced by IL-1 and TNF-alpha stimulation, and interacts with myopalladin or titin. A total of 7 missense variants have been associated with DCM (CM095439, CM095440, CM094349, CM094350, CM094351, CM095441 and CM095438) [46, 104, 105].

Other gene is *BAG3* (see above). Regarding association between DCM and BAG3, a total of 25 genetic variants have been reported so far. Of them, 15 were missense/nonsense (CM111936, CM111933, CM1111346, CM111937, CM1111347, CM111934, CM1110061, CM111938, CM1111349, CM1111350, CM147667, CM1111351, CM11110062, CM1111352, CM111939). In addition, 7 were small deletions (CD111935, CD1111348, CD147668,

CD147669, CD1111354, CD1111353 and CD143737), and 2 gross deletions (CG147670 and CG111940) [27, 106-108].

Next gene is *CSRP3* (ID: 8048, 11p15.1). This gene encodes the cysteine and glycine-rich protein 3 (cardiac LIM protein). It is a member of the CSRP family of LIM domain proteins, which may be involved in regulatory processes important for development and cellular differentiation. The LIM/double zinc-finger motif found in this protein is found in a group of proteins with critical functions in gene regulation, cell growth, and somatic differentiation. To date, 4 missense variants have been associated with DCM (CM023060, CM103199, CM034732 and CM087598) [60, 65, 69, 109-111].

The *LDB3* gene (ID: 11155, 10q22.3-q23.3) encodes LIM domain binding 3, a PDZ domain-containing protein (also named Cypher/ZASP). PDZ motifs are modular protein-protein interaction domains consisting of 80-120 amino acid residues. PDZ domain-containing proteins interact with each other in cytoskeletal assembly or with other proteins involved in targeting and clustering of membrane proteins. The protein encoded by this gene interacts with alpha-actinin-2 through its N-terminal PDZ domain and with protein kinase C via its C-terminal LIM domains. This protein also interacts with all three members of the myozenin family [69, 112]. To date, only 2 missense variants have been reported associated with DCM (CM033941 and CM033942) [65, 113].

The *MYPN* gene (ID: 84665, 10q21.3) encodes myopalladin, a protein which interacts with nebulin in skeletal muscle or nebulette in cardiac muscle and alpha-actinin. In addition, this gene product can interact with a protein with the I-band indicating it has a regulatory as well as structural function. To date, a total of 13 pathogenic variants have been reported associated with DCM (12 missense –CM122989, CM122982, CM1413540, CM124343, CM1510983, CM122980, CM122979, CM139271, CM139270, CM086686, CM086688 and CM086689-, and 1 small deletion –CD086687-)[114].

The *NEBL* gene (ID: 10529, 10p12) encodes nebulette, a sarcomeric Z-disk protein abundantly expressed in cardiac muscle and involved in mechanosensing and force generation via its interaction with actin and tropomyosin-troponin complex. To date it has been reported 3 pathogenic variants associated with DCM (CM106905, CM106907 and CM106908) [65, 115].

The *NEXN* gene (ID: 91624, 1p31.1) encodes nexilin (F actin binding protein), a filamentous actin-binding protein that may function in cell adhesion and migration. To date, 5 variants have been associated with DCM (2 missense –CM097739 and CM097741-, 1 splicing –CS1410902-, an 2 small deletions –CD1410903 and CD097742-) [44, 65, 116].

The *PDLIM3* gene (ID: 27295, 4q35) encodes the Z line proteins PDZ and LIM domain protein 3, indicating that it may be involved in cytoskeletal assembly. To date, only one pathogenic small insertion has been reported associated with DCM (CI072597) [117].

Finally, the gene *TCAP* (ID: 8557, 17q12), also named *LGMD2G*, encodes a titin-cap protein (also called Telethonin) which is found in striated and cardiac muscle that binds to the titin Z1-Z2 domains and is a substrate of titin kinase, interactions thought to be critical to sarcomere assembly. This gene has been associated with limb-girdle muscular dystrophy type 2G, HCM and DCM. Concretely, it has been reported 5 missense variants associated with DCM (CM087600, CM087601, CM025914, CM043590 and CM138797) [65, 69, 109, 118, 119].

Gamma secretase activity

Currently, one gene involved in gamma-secretase has been associated with familial DCM: PSEN1 (ID:5663, 14q24.3) which encodes presenilin 1. The presenilins, in concert with other proteins, form the γ-secretase complex that acts on numerous protein substrates but also in the cleavage of the Notch receptor, such that they either directly regulate gamma-secretase activity or themselves are protease enzymes. In this gene, only 2 variants have been reported so far (1 regulatory -CR101830-, and 1 small deletion -CD101829-) [115]. In Alzheimer's patients, DCM has been also reported associated with pathogenic variants in *PSEN1* and *PSEN2* [120]. Likewise, pathogenic variants in the *SLC22A5* gene has been associated with primary carnitine deficiency [121].

Redox activity

Thioredoxin reductase is a group of enzymes that catalyze the reduction of thioredoxin (redox protein). Three enzyms of this category have been described in mammalian. One of them, encoded by the *TXNRD2* gene, has been associated with DCM [122]. Sibbing et al. showed the relevant role of *TXNRD2* in cardiomyocytes and normal heart function, such that a deficiency regulation of this gene causes disturbed mitochondrial redox homeostasis [123].

Sarcoplasmic Reticulum

The Sarcoplasmic Reticulum or endoplasmic reticulum is a network of tubules and sacs in skeletal muscle fibers that plays an important role in muscle contraction and relaxation by releasing and storing calcium ions. To date, only pathogenic variants in one gene have been associated with familial DCM.

The *PLN* gene (ID: 5350, 6q22.1) encodes phospholamban protein. This protein is found as a pentamer and is a major substrate for the cAMP-dependent protein kinase in cardiac muscle. The encoded protein is an inhibitor of cardiac muscle sarcoplasmic reticulum Ca (2+)-ATPase in the unphosphorylated state, but inhibition is relieved upon phosphorylation of the protein. The subsequent activation of the Ca (2+) pump leads to enhanced muscle relaxation rates, thereby contributing to the inotropic response elicited in heart by beta-agonists. The encoded protein is a key regulator of cardiac diastolic function [124, 125]. To date, 8 pathogenic variants have been associated with DCM (5 missense -CM030490, CM1110161, CM1110160, CM1410904, and CM1010357-, 1 nonsense -CM030697-, 1 small insertion -CI1410905-, and 1 gross insertion -CN1414680-) [44, 46, 60, 126-128]

Other genes

To date, several other minoritary genes have been associated with DCM following an autosomal dominant pattern of inheritance: *ADRB1, BRAF, CRYAB, CTF1, DES, DNM1L, FHL2, FKRP, LAMA4, MURC, PLEC, SGCD, SYNE1,* and *VCL.*

The *ADRB1* gene (ID: 153, 10q25.3) encodes adrenoreceptor beta 1. The adrenergic receptors (subtypes alpha 1, alpha 2, beta 1, and beta 2) are a prototypic family of guanine nucleotide binding regulatory protein-coupled receptors that mediate the physiological effects of the hormone epinephrine and the neurotransmitter norepinephrine. To date, only one repor has been published suggesting the association between this gene and DCM [129].

The *BRAF* gene (ID: 673, 7q34) encodes B-Raf proto-oncogene, a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion. To date, only one report has been published focused on *RAF1* and DCM [130].

The *CRYAB* gene (ID: 1410, 11q22.3-q23.1) encodes crystalline alpha B. Alpha crystallins are composed of two gene products: alpha-A and alpha-B, for acidic and basic, respectively. Alpha crystallins can be induced by heat shock and are members of the small heat shock protein (HSP20) family. They act as molecular chaperones although they do not renature proteins and release them in the fashion of a true chaperone; instead they hold them in large soluble aggregates. Two additional functions of alpha crystallins are an autokinase activity and participation in the intracellular architecture. To date, 3 pathogeni variants have been associated with DCM (2 missense -CM062568, CM060948-, and 1 nonsense -CM142212-) [65, 131, 132].

The *CTF1* gene (ID: 1489, 16p11.2) encodes cardiotrophin 1. This protein is a secreted cytokine that induces cardiac myocyte hypertrophy in vitro. It has been shown to bind and activate the ILST/gp130 receptor. Currently, only 1 missense pathogenic variant has been associated with DCM (CM003924) [133].

Other gen is *DES* (ID: 1674, 2q35), mainly associated with AC. It encodes a muscle-specific class III intermediate filament. In mature striated muscle, desmin filaments connect the sarcomere to the sarcolemmal, extracellular matrix and the nuclear lamina, maintain structural interactions at the Z-bands and intercalated discs, and interact with the mitochondria, ensuring their proximity to the A- and I-bands in the sarcomere. To date 12 variants have been reported in *DES* associated with DCM: 10 missense (CM070888, CM159728, CM1514290, CM1310426, CM070891, CM070887, CM070890, CM1510984, CM000369, and CM070889), 1 splicing (CS068099), and 1 small deletion (CD103220) [60, 65, 68, 134-137].

Other gene is *DNM1L* (ID: 10059, 12p11.21) which encodes dynamin 1-like protein, a member of the dynamin superfamily of GTPases. This protein mediates mitochondrial and peroxisomal division, and is involved in developmentally regulated apoptosis and programmed necrosis. To date only one report has been published in DCM.

The *FHL2* gene (ID: 2274, 2q12.2) encodes four and a half LIM domains 2 protein. Family members contain two highly conserved, tandemly arranged, zinc finger domains with four highly conserved cysteines binding a zinc atom in each zinc finger. This protein is thought to have a role in the assembly of extracellular membranes. Also, this gene is down-regulated during transformation of normal myoblasts to rhabdomyosarcoma cells and the encoded protein may function as a link between presenilin-2 and an intracellular signaling pathway. Arimura et al suggested that because FHL2 protein is known to tether metabolic enzymes to titin/connectin, pathogenic variants in this gene may be involved in the pathogenesis of DCM via impaired recruitment of metabolic enzymes to the sarcomere [138].

The *FKRP* gene (ID: 79147, 19q13.32) encodes the fukutin related protein. This protein is targeted to the medial Golgi apparatus and is necessary for posttranslational modification of dystroglycan. Usually, pathogenic variants in this gene have been associated with Limb-girdle muscular dystrophy (LGMD2I). However, Müller et al reported a family suffering of DCM and without muscular alterations [139]. Specifically, Murakami et al described some families with DCM and mild or no limb-girdle muscle involvement, where mutations in the *FKTN* gene keep autosomal recessive inheritance pattern [140]. Moreover, Hobbiebrunken et al. have recently published an exon deletion which explains a DCM in a patient with Mild Limb-Girdle Muscular Dystrophy [141].

The *LAMA4* gene (ID: 3910, 6q21) encodes the laminin, alpha 4 protein. Laminins, a family of extracellular matrix glycoproteins, are the major noncollagenous constituent of basement membranes. They have been

implicated in a wide variety of biological processes including cell adhesion, differentiation, migration, signaling, neurite outgrowth and metastasis. Laminins are composed of 3 non identical chains: laminin alpha, beta and gamma (formerly A, B1, and B2, respectively) and they form a cruciform structure consisting of 3 short arms, each formed by a different chain, and a long arm composed of all 3 chains. Each laminin chain is a multidomain protein encoded by a distinct gene. To date, only 3 pathogenic variants have been suggested as cause of DCM (2 missense -CM073173 and CM1510356-, and 1 nonsense -CM073174-) [65, 89, 142].

The *MURC* gene (ID: 347273, 9q31.1) encodes the muscle-related coiled-coil protein. The encoded protein promotes Rho/ROCK (Rho-kinase) signaling in cardiac muscles cells, and may facilitate myofibrillar organization. Currently, 6 pathogenic variants have been associated as DCM (CM116735, CM116738, CM116739, CM116736, CM116737 and CM116740) [65, 143].

The *PLEC* gene (ID: 5339, 8q24) encodes plectin protein. Plectin is a prominent member of an important family of structurally and in part functionally related proteins, termed plakins or cytolinkers, that are capable of interlinking different elements of the cytoskeleton. Plakins, with their multi-domain structure and enormous size, not only play crucial roles in maintaining cell and tissue integrity and orchestrating dynamic changes in cytoarchitecture and cell shape, but also serve as scaffolding platforms for the assembly, positioning, and regulation of signaling complexes. To date, only one study published a pathogenic variant in this gene associated with a case of adult-onset biventricular DCM but also showing epidermolysis bullosa simplex with muscular dystrophy (EBSMD) [144].

The *SGCD* gene (ID: 6444, 5q33-q34) encodes sarcoglycan, delta (35kDa dystrophin-associated glycoprotein). This protein is one of the four known components of the sarcoglycan complex, which is a subcomplex of the dystrophin-glycoprotein complex (DGC). DGC forms a link between the F-actin cytoskeleton and the extracellular matrix. This protein is expressed most abundantly in skeletal and cardiac muscle. To date, only 3 pathogenic variants have been associated with DCM (2 missense –CM033460 and CM004337-, and 1 small deletion –CD1410908-) [44, 145, 146].

The *SYNE1* gene (ID: 23345, 6q25) encodes spectrin repeat containing, nuclear envelope 1 which is expressed in skeletal and smooth muscle, and peripheral blood lymphocytes, that localizes to the nuclear membrane. Nesprins are spectrin repeat-containing proteins that interact with lamin A/C and are components of the linker-of-nucleoskeleton-and-cytoskeleton (LINC) complex that connects the nuclear envelope to the actin cytoskeleton. Currently, only one pathogenic missense variant has been associated with DCM -CM101580- [147].

The *VCL* gene (ID: 7414, 10q22.2) encodes vinculin protein. Vinculin is a cytoskeletal protein associated with cell-cell and cell-matrix junctions, where it is thought to function as one of several interacting proteins involved in anchoring F-actin to the membrane. Vinculin and its splice variant, metavinculin (MV), are key elements of multiple protein assemblies linking the extracellular matrix to the actin cytoskeleton. Vinculin is expressed ubiquitously, whereas MV is mainly expressed in smooth and cardiac muscle tissue. The only difference in amino acid sequence between the isoforms is a 68-residue insert in the C-terminal tail domain of MV. Defects in cardio-specific exons of metavinculin (*VCL* gene) are the cause of DCM. Currently, a total of 12 pathogenic variants have been associated with DCM (6 missense -CM103197, CM1510985, CM1111746, CM026020, CM020193, and CM103198-, 3 nonsense - CM1410941, CM1410942, and CM1410943-, 1 splicing -CS026021-, 1 small deletion -CD020250-, and 1 small insertion -CI1410944-).

2.3. Autosomal recessive

Currently, few DCM cases following an autosomal recessive pattern of inheritance have been reported. The main feature in most cases is the early manifestation of the disease, at neonatal and adolescent ages [15, 148-150]. Currently, several variants have been identified in several genes: *TNNI3, DES, DOLK*, *DSP*, *GATAD1, SDHA*, and *TAX1BP3*.

The first gene, *TNNI3* (ID: 7137, 19q13.4), encodes the human cardiac troponin I-type 3- (see data mentioned before). C-terminal pathogenic variants in the *TNNI3* gene was described by Murphy et al. 2004 as an autosomal recessive inheritance pattern and the pathogenic variant caused an alteration in troponins interaction [148].

Other gene associated with DCM following an autosomal recessive pattern of inheritance is desmin (*DES*, ID: 1674, 2q35) (see data mentioned before) [151].

The *DOLK* gene (ID: 22845, 9q34.11) encodes dolichol kinase protein which catalyzes the CTP-mediated phosphorylation of dolichol. It is also involved in the synthesis of Dol-P-Man, which is an essential glycosyl carrier lipid for C- and O-mannosylation, N- and O-linked glycosylation of proteins, and for the biosynthesis of glycosyl phosphatidylinositol anchors in endoplasmic reticulum. To date, only 3 variants have been associated with DCM (CM1110851, CM111850 and CM1110849) [152].

The next gene is *DSP* (ID: 1832, 6p24) which encodes desmoplakin, a key component of desmosomes that anchors intermediate filaments to desmosomal plaques in the myocyte. (see data mentioned before). Regarding recessive inheritance, only one variant (7901delG) has been associated with a generalized striate keratoderma particularly affecting the palmoplantar epidermis, woolly hair and a dilated left ventricular cardiomyopathy [86].

In 2011, Theis et al reported a familial DCM caused by a pathogenic variant in homozygosis in the *GATAD1* gene, following an autosomal recessive pattern of inheritance (CM1110608) [153]. This gene (ID: 57798, 7q21-q22) encodes the GATA zinc finger domain containing 1 protein, and is thought to bind to a histone modification site that regulates gene expression.

In addition, the *SDHA* gene (ID: 6389, 5p15) has been also associated with familial neonatal DCM [154]. This gene encodes succinate dehydrogenase complex, subunit A flavoprotein (Fp), a major catalytic subunit of succinate-ubiquinone oxidoreductase, a complex of the mitochondrial respiratory chain. The complex is composed of four nuclear-encoded subunits and is localized in the mitochondrial inner membrane.

Finally, the *TAX1BP3* gene (ID: 30851, 17p13) encodes the Tax1 (human T-cell leukemia virus type I) binding protein 3. Recently, Reinstein et al reported a homozygous missense variant (CM152607) in the *TAX1BP3* gene [155], which encodes a small PDZ domain containing protein implicated in regulation of the Wnt/β-catenin signaling pathway, as a novel autosomal recessive syndrome characterized by dilated cardiomyopathy and septo-optic dysplasia.

2.4. X-linked

X-linked pathogenic variants involving progressive degeneration of the cardiac muscle have been described. These alterations produce a dilatation with consequent functional heart complications in affected patients. Often these pathogenic variants are lethal at early age in males and the transplantation is the best alternative. Currently, pathogenic variants have been identified in some genes: *DMD, TAZ, EMD, CLIC2,* and *LAMP2*.

Dystrophin protein, encoded by the *DMD* gene (ID: 1756, Xp21.2), plays a cohesive role between actin filaments and sarcolemmal protein complex. This gene was the first described associated with DCM [156]. Curiously, pathogenic variants in this gen are frequently located in regulatory regions or splice sites [156-158]. To date, 4 missense variants (CM024515, CM970419, CM023911 and CM024513), 5 splicing (CS961548, CS077351, CS024007, CS024533, CS117195), 1 small deletion (CD031831), 14 gross deletions (CG984289, CG087410, CG087409, CG0910134, CG004986, CG117199, CG117198, CG973442, CG0910138, CG0910135, CG0910136, CG117197, CG931275, and CG052656), 2 gross insertions (CN984493 and CN973565) and 2 complex rearrangements (CP005268 and CP005269) have been associated with DCM [65, 157, 159-169].

The *TAZ* gene (ID: 6901, Xq28) encodes tafazzin, an enzyme that catalyzes a phospholipid synthesis in the inner mitochondrial membrane that contributes to structure maintenance, cell energy metabolism and apoptotic pathway [170]. Pathogenic variants in this gene trigger alterations in phospholipids metabolic route contributing to a change in the structure of the mitochondrial membrane lipids and also it alters

function [171]. This gene is usually associated with Barth Syndrome. This syndrome affects males and it is characterized by cardiomyopathy, neutropenia, skeletal myopathy, prepubertal growth delay, and distinctive facial gestalt (most evident in infancy) despite not all features may be present together in a patient. Cardiomyopathy is the most prevalent clinical feature and it usually presents early in life (less than 5 years of age). It can present as DCM with or without endocardial fibroelastosis, left ventricular noncompaction or, less frequently, HCM [172]. To date 5 variants have been associated with DCM: 2 missense (CM1010358 and CM130622), 2 nonsense (CM1410909), 1 splicing (CS1111745) and 1 gross deletion (CG126031) [44, 60, 173].

The third gene is *EMD* (ID: 2010, Xq28), which encodes emerin protein, a serine-rich nuclear membrane protein and a member of the nuclear lamina-associated protein family. It mediates membrane anchorage to the cytoskeleton. It has been recently reported as a causal gene in DCM [68]. A deletion in exon one results in a complete loss of emerin protein in affected subjects whose pedigree was consistent with X-linked pattern of inheritance (CD147693). In 2013 other variant was reported (CM137775) associated with DCM despite its pathogenic role remains to clarify [174].

Other gene is *CLIC2* (ID: 1193, Xq28) which encodes a chloride intracellular channel protein. Chloride channels are a diverse group of proteins that regulate fundamental cellular processes including stabilization of cell membrane potential, transepithelial transport, maintenance of intracellular pH, and regulation of cell volume. This protein may play a role in inhibiting the function of ryanodine receptor 2 [175].

Finally, the gene *LAMP2* (ID: 3920, Xq24) is also associated with DCM. The protein encoded by this gene (lysosomal-associated membrane protein 2) is a member of a family of membrane glycoproteins. This glycoprotein provides selectins with carbohydrate ligands. LAMP2 fills an important function in the protection, maintenance, and adhesion of the lysosome. This gene is the first candidate in Danon disease. This pathology is characterized by heart and skeletal muscle failing. In woman patient with both DCM and HCM, they are the most common symptoms and this is also true in the familial phenotype [166]. In 2013, Mook et al. reported a variant (CM137777) associated with DCM [164]. LAMP2 has been also published related to tumor cell metastasis.

2.5. DCM and other pathologies

Notably, a large group of genes are linked to different pathologies associated with typical symptoms of DCM. In these cases, it is unclear whether the mutation is strictly liable for the DCM, or the heart disease is a consequence or collateral damage of the main disease. Here, we mention only some examples. Mutations in the *ALMS1* gene are linked to Alström syndrome and DCM, which is described as a complication [176]. ALMS1 protein seems to be involved in the organization of cellular microtubules, the transport of materials,

and the function of cilia. Other example is DCM related to ataxia syndrome where have been described mutations in the *DNAJC19* gene [177]. The lack of functional DNAJC19 protein alters the transport of other proteins into and out of the mitochondria and consequently energy production and mitochondrial survival can be reduced. The *HFE* gene is associated with porphyria cutanea tarda and haemochromatosis. The protein encoded by it interacts with others membrane's proteins to detect iron in the body. Iron overload cardiomyopathy (like DCM) has been linked to mutations in this gene. Finally, pathogenic variants in the *RyR2* gene have been also associated with DCM despite concomitant with other phenotypes such as CPVT [178] and LVNC [179]. However, the definite association between *RyR2* and DCM remain as a current matter of argue.

2.6. SNP pattern

A new category of studies begins to highlight. The mutated genes that account for a higher percentage of cases of DCM seem clear, as mentioned above. It seems easier to find isolated cases of specific patients or families with a particular mutation. SNPs pattern studies associated with DCM can become, in short, a very useful genetic support for the diagnostic purpose. In this sense, Xiaoping Li et al. published a polymorphism in the *ZBTB17* gene which seems to induce susceptibility to DCM in Chinese population. Other example is the association between intronic polymorphism and DCM in the *CTLA4* gene [180] where the variant confers susceptibility for the cardiomyopathy but it does not seem to interfere with the course of the disease.

3.- NGS screening

Increasingly, clinicians are more and more familiar with genetic studies. Hopefully, the cost-efficiency of these studies is becoming more affordable. In this respect some evidence has been objectively reported [181]. Economic saving is derived from the calculation of the difference between the genetic testing cost and the cost of unnecessary follow-up involving both clinical and complementary tests such as ECG, ECO and so on, apart from absences from work. Some platforms already commercially offer their cardiac NGS kits and numerous public or private centers have also created their own custom panels. The massive candidate gene screening can identify not only punctual mutations but also insertions/deletions that can be unidentifiable with Sanger technology. Obviously, today, this type of analysis is indicated for cases in which the clinician has a clear suspected heart disease and therefore the risk of relatives is a serious concern. More importantly, we must emphasize the role of a specialist in clinical practice to explain to the mutation carriers the implications of the genetic abnormality as potentially responsible for a heart disease.

4. Conclusions

Familial DCM is a rare genetic entity with a heterogeneous clinical progression and outcome. Use of NGS technology has identified several new genes in recent years, helping to understand the pathophysiological pathways. However, DCM remains classified as a complex disease due to elevate number of genes associated with the pathology and different inheritance patterns. Further genotype-phenotype studies should be performed in large cohorts of families suffering of DCM in order to unravel new genetic causes of the disease. These advances will contribute to the translation of genetic data into clinical practice, helping clinicians to improve current diagnostic tools and management.

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Figures

Figure 1. Main clinical characteristics of DCM**. A**. ECG from a patient with idiopathic dilated cardiomyopathy with a *BAG3* deletion confirmed. Note the resting sinus tachycardia, left atrial enlargement and left ventricle hypertrophy in the anterior precordial leads. **B**. Paraesternal long-axis view from a 2-D echocardiogram from the same patient above. It shows a markedly dilated and hypokinetic left ventricle. The left atrium is also enlarged and there is reduced excursion of the mitral and aortic valves suggesting a reduced stroke volume. **C.** Balance *Steady State Free Precession* image (bSSFP) oriented in short axis view revealing a severe dilatation of the left ventricle. Note the thinner walls of it, especially in the lateral wall but also, the increased trabeculation in the antero-lateral segment (yellow arrow). **D**. Late gadolinium enhancement sequence oriented in a four chambers view revealing a linear mid wall enhancement at the lateral free wall of the left ventricle suggestive of fibrosis. This pattern of distribution is orientated of a non ischemic origin of the dilated cardiomyopathy.

Figure 2. Diagnostic steps forward in a family with a history of DCM.

Tables

Gene	Localization	Protein	Frequency, %
TTN	Sarcomere	Titin	25 - 30
LMNA	Nucleus	Lamin A/C	10 - 15
MYH7	Sarcomere	β-myosin heavy chain	5 – 10
MYH6	Sarcomere	α-myosin heavy chain	5 – 10
TNNT2	Sarcomere	Cardiac troponin T	5 – 10
ACTC1	Sarcomere	Cardiac actin	5 – 10
BAG3	co-chaperones	athanogene 3	1 – 5
DSP	Desmosome	Desmoplakin	1 – 5
MYBPC3	Sarcomere	Myosin-binding protein C	1 – 5
RBM20	regulator of mRNA splicing	RNA-binding protein 20	1 – 5
SCN5A	Ion channel	Sodium channel	1 – 5
TPM1	Sarcomere	α-tropomyosin	1 – 5

Table 1. Frequency of main genes associated with DCM. All other genes have a frequency minor than 1%.

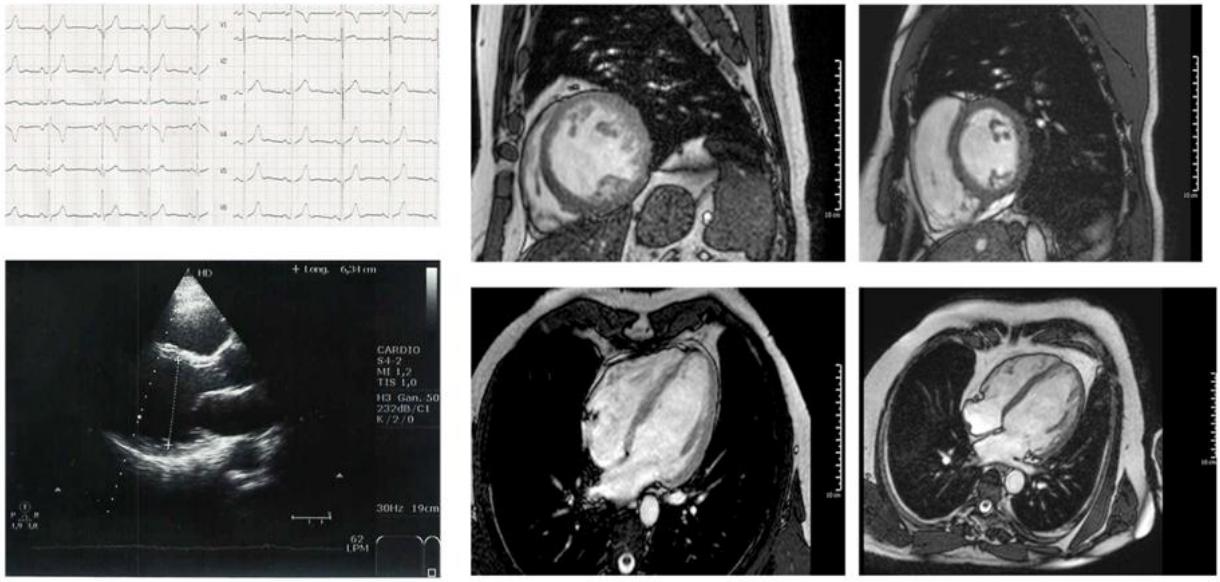

Fig. 1

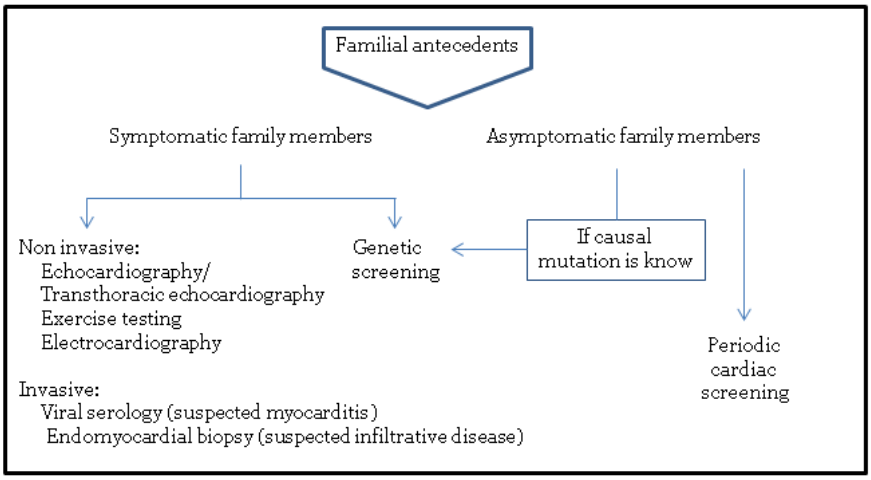

Fig. 2

Highlights

Familial DCM is a rare genetic entity with a heterogeneous clinical progression and outcome.

NGS technology helps to unravel genetic cause of disease in a cost-effective way.

Genotype-phenotype studies are crucial in order to clarify the pathogenic role of new genetic alterations identified.

Genetic advances help clinicians to improve current diagnostic tools and management.