Chapter

Genetic Determinant of Familial Dilated Cardiomyopathy and Genotype-Targeted Therapeutic Strategy

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Abstract

Dilated cardiomyopathy (DCM) is a myocardium disease characterized by left ventricular dilation and systolic dysfunction. Genetic susceptibility contributes significantly to the disease progression in familial DCM. Mutations in more than fifty different genes have been identified to cause DCM, accounting for up to 50% of familial DCM cases. Elucidation of genetic basis for the remaining familial DCM probands promises to substantially increase the efficiency of genetic testing for early disease diagnosis and intervention. Dissecting genetic pathways linked to DCM and related pathogenic mechanisms can provide valuable insights into the understanding of disease pathophysiology that can be leveraged for development of genotype-targeted therapeutic strategy. Here, we review genetic variants, with a focus on affected genes most commonly implicated in DCM, and highlight their underlying pathophysiological mechanisms of action. We discuss recent progress on gene-based therapeutic strategy which holds the opportunities to implement individualized medicine and ultimately to improve patient outcome in the future.

Keywords: dilated cardiomyopathy, genetic determinant, genetic testing, gene therapy, pathophysiology

1. Introduction

Heart failure afflicts about 26 million patients worldwide. The estimated economic burden related to heart failure is 120 billion US dollars [1]. Dilated cardiomyopathy (DCM), referred to a group of heart muscle disorders characterized by heart chamber dilation and systolic dysfunction, is a common cause of heart failure. DCM is the most common form of non-ischemic cardiomyopathy and the most common indication in patients who need heart transplantation [2, 3]. In the pediatric, nearly 50% of patients dying suddenly or undergoing cardiac transplantation are affected by cardiomyopathy, predominantly by DCM [4]. In the young, DCM is the most frequent cause of heart failure [5]. In the adult, it is the second most common cause of heart failure (after the coronary heart disease), underlying about one third of all heart failure cases [1]. While the true prevalence for DCM in general population is not fully defined yet due to lack of well-designed large-scale population based studies, its estimated prevalence ranges from 1 in 2500 to 1 in 250 people [6]. The annual incidence of DCM

is approximately 7 cases per 10,000 individuals, and males are about 3 times more frequently affected than females [2, 7, 8].

Classically, DCM is defined based on two major criteria: 1) left LV fractional shortening <25% (> 2 standard deviations [SD]) and/or LV ejection fraction <45% (>2 SD); and 2) LV end-diastolic diameter greater than 117% of the predicated value corrected for age and body surface area. DCM is diagnosed when any other known cause of myocardial diseases are excluded [9]. The updated definitions of DCM are left ventricular or biventricular systolic dysfunction and dilatation that are not explained by abnormal loading conditions or coronary artery disease [10].

DCM is caused by a variety of etiologies, including acquired, genetic and/or mixed origins. Acquired risk factors such as infection, myocarditis, drug toxin, autoimmune response, excess alcohol consumption and metabolic disorders are recognized to cause DCM. Primary DCM results when all these acquired factors are exclude, which can be either idiopathic or familial. Familial DCM (FDC) is classified when two or more family members are diagnosed in first-degree relatives. The prevalence of familial DCM differs in different patient cohorts, estimated to range from 20–50% of all DCM cases. The remaining DCM cases are thus classified as idiopathic [6, 11]. However, the frequency of familial DCM is believed to be underestimated, due to the limitation of large pedigree and family's availability for diagnostic screening. Genetic factors which predispose to DCM have been increasingly recognized. Since the discovery of the first disease causative gene to cardiomyopathy [12], to date, more than 50 genes have been identified to associate with DCM and the number is still increasing. Genetic mutations in all these genes combined can explain about 40–50% of familial DCM cases, underpinning the genetic determinant of this disease [6]. The familial DCM appears to be inherited as a monogenic trait and is mainly transmitted in an autosomal dominant inheritance pattern, manifesting incomplete, age-related penetrance and variable expression. Other patterns such as autosomal recessive, X-linked and mitochondrial inheritance also occurs in a small portion of the familial DCM cases [6, 13].

Current management of DCM is mainly focused on treating patients with symptoms following the standard guidelines, similar to treating other forms of heart failure with reduced ejection fraction (EF). The guideline driven therapies mostly adapt a "one-size-fits-all" approach that uses β-adrenergic blockers, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers to improve cardiac function and symptom by reducing congestion and management of arrhythmia [14, 15]. Recently, several new additions of armamentarium are also implemented in the treatment options [16–18]. Despite of advances in these treatment protocols, DCM remains one of the major reasons for patients needed for heart transplantation, and the morbidity and mortality rate of DCM still remains unacceptably high. Thus, the current management of DCM with the "one-size-fits-all" strategy is challenged. More novel and effective and individualized treatment options are desirable.

Considering the genetic determinant of DCM, and up to 50% of familial DCM patients have a genetic origin, genotype-targeted therapies, by directly targeting at the specific gene mutations, have emerged as promising strategies toward development of more effective and individualized treatment. In this chapter, we firstly review definitive genes linked to DCM and classify them based on their intracellular localization (**Figure 1**), with a major focus on genes most commonly implicated in DCM, and highlight their underlying pathophysiological mechanism of action if known (**Table 1**). Next, we discuss progress and challenges on the emerging genotype-based therapeutic strategies for effective and individualized medicine explored in the treatment of DCM (**Table 2**), which hold the opportunities to ultimately improve patient outcome in the future.

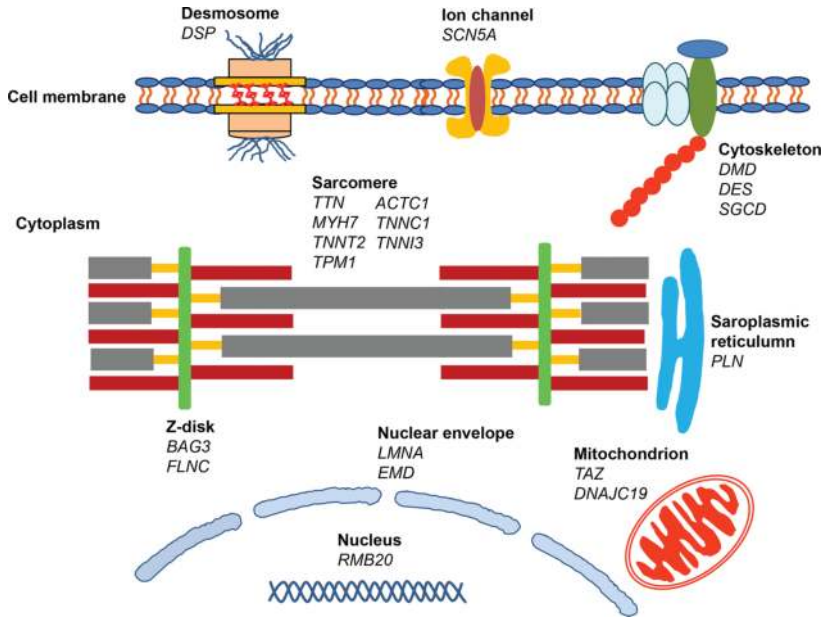

Figure 1.
Subcellular localization of the protein encoded by dilated cardiomyopathy disease genes. The graphic shows schematic representations and approximate intracellular localization of the encoded proteins by genes strongly implicated in dilated cardiomyopathy disease.

Gene	Protein	Function	Frequency and overlapping phenotype	Inheritance pattern
Sarcomere				
ACTC1	Alpha cardiac actin	Structural component of thin filament	<1% of DCM; HCM, LVNC	Autosomal dominant
MYH7	Beta-myosin heavy chain	Cardiac muscle contraction	5% of DCM; HCM, ACM, LVNV	Autosomal dominant
TNNC1	Cardiac troponin C	Cardiac muscle contraction	<1% of DCM; HCM, LVNC	Autosomal dominant
TNNI3	Cardiac troponin I	Cardiac muscle contraction	<1% of DCM; HCM	Autosomal recessive
TNNT2	Cardiac troponin T	Cardiac muscle contraction	3% of DCM; HCM, ACM, LVNC	Autosomal dominant
TPM1	Tropomyosin alpha-1 chain	Cardiac muscle contraction	1–2% of DCM; HCM, LVNC	Autosomal dominant
TTN	Titin	Sarcomere scaffold	15–25% of DCM; HCM	Autosomal dominant
Cytoskeleton				
DES	Desmin	Contractile force transduction	<1% of DCM; Desminopathies	Autosomal dominant
DMD	Dystrophin	Contractile force transduction	<1% of DCM; Duchenne/Becker muscular dystrophy	X-linked
SGCD	δ-sarcoglycan	Structural component of dystrophin-glycoprotein complex	<1% of DCM; HCM, LGMD2E	Autosomal recessive
Nuclear envelope				
EMD	Emerin	Nuclear membrane anchorage	<1% of DCM; EMDM	X-linked

Gene	Protein	Function	Frequency and overlapping phenotype	Inheritance pattern
LMNA	Lamin A/C	Nuclear envelope structure	6% of DCM; EMDM, LGMD1B	Autosomal dominant
Nucleus				
RBM20	RNA-binding protein 20	Regulator of cardiac gene splicing	2% of DCM	Autosomal dominant
Ion channel				
SCN5A	Sodium voltage-gated channel alpha subunit 5	Sodium channel protein	2–3% of DCM; LQTs Brugada syndrome	Autosomal dominant
Z-disk				
BAG3	BCL2-associated athanogene 3	Co-chaperone, inhibition of apoptosis	3% of DCM; Myofibrillar myopathy	Autosomal dominant
FLNC	Flamin-C	Actin filament crosslinking	<1% of DCM; HCM, RCM	Autosomal dominant
Desomosome				
DSP	Desmoplakin	Structural component of desomosome, cell–cell mechanotransmission	2% of DCM; ACM	Autosomal recessive
Mitochondrion				
DNAJC19	DnaJ heat shock protein family homolog, C19	Protein transporter	<1% of DCM; 3-Methylglutaconic aciduria, type V	Autosomal recessive
TAZ	Tafazzin	Phospholipid transacylase	<1% of DCM; HCM, Barth syndrome	X-linked
Sarcoplasmic reticulum				
PLN	Cardiac phospholamba	Regulator of calcium pump	<1% of DCM; HCM, ACM	Autosomal dominant

ACM, Arrhythmogenic cardiomyopathy; DCM, dilated cardiomyopathy; EDMD, Emery–Dreifuss muscular dystrophy; LVNC, left ventricular noncompaction; HCM, hypertrophic cardiomyopathy; LGMD, limb-girdle muscular dystrophy; LQTs, long QT syndrome; RCM, restrictive cardiomyopathy.

Table 1.
Genes linked to dilated cardiomyopathy with strong evidence.

Gene	Variant type	Clinical phenotype	Molecular pathophysiology	Genotype-based therapy	Reference
DMD	Nonsense Deletion	DCM, DMD, Becker muscular dystrophy, premature death	Absence of functional dystrophin protein, replacement of muscle by fibrotic and adipose tissue, contraction weakness	1) Exon skipping; 2) Mini dystrophin gene replacement; 3) CRISPRA/Cas9 genome editing; 4) Stop codon readthrough	[60–62, 67, 71–73, 80]
LMNA	Nonsense Deletion	DCM with high penetrance, high risk of arrhythmia, early lethality	Lamins A and C proteins haploinsufficiency, nuclear malformations, biomechanical defects, activation of p38 kinase pathway	1) Preventive therapy, lower the threshold for cardiac defibrillator implantation; 2) p38 inhibition; 3) Trans-splicing; 4) Stop codon readthrough	[29, 30, 58, 75, 79]

Gene	Variant type	Clinical phenotype	Molecular pathophysiology	Genotype-based therapy	Reference
TTN	Truncating	DCM, HCM, ventricular arrhythmias	Titin protein haploinsufficiency, sarcomere insufficiency, metabolic and energetic adaption, increased sensitivity to mechanical stress	Exon-skipping	[82]
PLN	Missense Deletion	Ventricular dilation, contractile dysfunction and ventricular arrhythmias, heart failure by middle age	Abnormal PLN protein subcellular localization, calcium handling defects, electrical instability	TALEN genome editing	[22, 84]
SGCD	Deletion	DCM, HCM	δ-sarcoglycan protein deficiency, sarcolomma instability, increased myocyte apoptosis, reduced expression of miRNA-669a	1) Gene replacement; 2) microRNA overexpression	[83, 88, 89]

Table 2.
Examples of gene-targeted therapeutic strategies.

2. Genetic causes of familial DCM

Extensive studies on genetic basis of DCM underscored profound heterogeneous nature of DCM disease. DCM variants mutate genes encoding a broad spectrum of proteins with distinct functions and intracellular localization have been identified (**Figure 1** and **Table 1**). For example, mutations in genes encoding sarcomere protein involving in mechanosensing and force transmission, encoding nuclear envelope proteins required for the protection of biochemical forces, encoding desmosomal proteins required for maintaining the structural integrity of desomosome, encoding ion channels and molecules involving in calcium handling, encoding chaperone proteins, transcription factors and RNA-binding proteins have all been identified to cause DCM. Specific variants in these genes may alter various signaling pathways and cellular structures in cardiomyocyte that can disrupt the mechanism of cardiac muscle contraction and function, leading to common phenotypes of DCM.

2.1 Sarcomere genes

Sarcomere is the basic contractile unit mainly composed of thin and thick filaments. Mutations in sarcomere genes are one of the most important causes of DCM, which have been identified in about one third of DCM [19–21]. In addition to cause DCM, mutations in sarcomere genes often lead to overlapping phenotypes of hypertrophic cardiomyopathy (HCM), another major genetic type of cardiomyopathy characterized by ventricular wall thickness and diastolic dysfunction. Genes with definitive evidence, supported by studies from multiple centers/groups, with both

clinical linkage data and confirmative data from animal models, include *ACTC1, MYH7, TNNT2, TNNC1, TRM1,TTN*. Among which, *TTN, MYH7* and *TTNT2* are most commonly implicated in DCM, accounting for about 25%, 5% and 3% of familial DCM, respectively.

TTN gene encodes the titin, the largest known protein in human, consisting of 364 exons and about 35,000 amino acids that spans about a half of the sarcomere. It has long been recognized as a sarcomere scaffolding protein that serves as a blue print for sarcomerogenesis and myofibrillar assembly. In addition, titin also serves as a molecular spring that provides passive tension to regulate sarcomere contraction. With the advent of next-generation sequencing, human genetic analysis had revealed that *TTN*-truncating variants (*TTNtvs*) represent the most prevalent gene mutations in DCM patients, accounting for up to 25% of familial DCM cases [19]. Notebly, *TTNtvs* are also found in about 1% of general population [22, 23]. While not all of these healthy individuals with the *TTNtvs* are expected to develop DCM, they showed significantly increased left ventricular volumes and mild reduction of contractility [23]. Initially, it was hypothesized that *TTNtvs* caused DCM through a dominant negative mechanism [24]. This hypothesis is compromised by that truncated titin peptides were not detected from DCM hearts that harbored *TTNtvs*. Instead, there is evidence to support that premature truncations in the *TTN* transcripts trigger nonsense-mediated decay in rat models with *TTNtvs* [23]. Together with recently cumulative data derived from studies in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) and human cardiac tissues, the hypothesis of haploinsufficiency mechanisms is now more widely recognized [25].

MYH7 encodes the beta isoform of myosin heavy chain (MHC-β) that is predominantly expressed in the heart. MHC-β is a major component of the thick filament responsible for hydrolyzing ATP to produce force for cardiac muscle contraction. The Arg403Gln missense mutation of *MYH7* was first identified to cause HCM back to 1990 [12]. Since then, many *MYH7* variants were identified to associate with both HCM and DCM, accounting for approximately 40% of HCM and 5% of DCM cases, respectively. The definitive mechanisms underlying how *MYH7* mutations cause different types of cardiomyopathies remain to be elusive. Clinically, HCM is often characterized by hypercontractility. In contrast, DCM is characterized by hypocontractility. Mutations in myosin head domain consume more energy, leading to hypercontractility. One observation is that the *MYH7* variants associated with HCM are located more in the region encoding the myosin head domain, while *MYH7* mutations causal to DCM appear to disperse across the entire gene. The Arg403Gln variant causal to HCM, for example, increased energy usage due to impaired catalytic cycle of ATP hydrolysis, resulting in increased contractility [26]. In contrast, mutations associated with DCM showed an increased tension cost, with more energy consumption, have reduced force-generating capacity, thus causes a hypocontractility, leading to DCM [27, 28]. For example, the ASN1918LYS variant, causal to DCM, is located to the coiled-coil rod region, which is hypothesized to impair the incorporation of myosin into the myofilaments.

TNNT2 encodes the cardiac muscle isoform of troponin T (cTnT). cTnT is one of the major tropomyosin-binding subunits of troponin on the thin filament that regulates cardiac muscle contraction. *TNNT2* variants are thought to account for approximately 5% of HCM, up to 3% of DCM, and a small fraction of arrhythmogenic cardiomyopathy (ACM). Many mutations in *TNNT2* causing DCM are located in both the middle and C-terminal regions of cTnT. These mutations mostly impair the cTnT's interaction with the thin filament regulatory system, myofilament calcium sensitivity, and/or the myosin ATPase activity, thus cause DCM.

TPM1 encodes the alpha tropomyosin chain protein that belongs to the tropomyosin family of highly conserved thin filament proteins. In association with the

troponin complex, tropomyosin mostly participates in the calcium regulation of cardiac muscle contraction and interaction of actin and myosin. Mutations in *TPM1* cause both HCM and DCM phenotypes [29, 30], accounting for about 5% of HCM and 1% of DCM cases, respectively. Functional characterization of *TPM1* mutations associated with both cardiomyopathies has led to a better understanding of the primary effects and consequence triggered by mutations in the long-range communication of the thin filament and specific phenotypes [31].

2.2 Nucleus and nuclear envelope genes

Mutations in gene encoding nucleus or nuclear envelope localized proteins that definitively link to DCM such as *LMNA, EMD* and *RBM20* were reported. *LMNA* is the second most common gene implicated in DCM, and *LMNA* variants account for about 6–10% of genetic DCM [32, 33]. *LMNA* encodes the nuclear envelope localized lamins A and C resulted from differential splicing at the 3′ end. Both lamins A and C are intermediate filament structural proteins, playing major roles in supporting cells with stability and strength. *LMNA* mutations linked to DCM can be both missense and frameshift across the coding region. Both dominant negative and haploinsufficiency mechanisms were proposed for *LMNA* mutations caused DCM. Beyond their roles as structural proteins, both lamins A and C involve in many different cellular process including regulation of gene expression, mechanosensing and nuclear to cytoplasmic transport. Functional study in animal models revealed that ERK1/2, JNK and p38 kinase pathways were drastically activated in *LMNA*-associated DCM. By targeting to the p38 kinase pathway through using a specific p38 kinase inhibitor ARRY-371797, Muchir and colleagues showed that LV dilation and deterioration of EF were effectively blocked, and *LMNA*-related severe biomechanical defects were significantly rescued in neonatal rat ventricular myocytes [34, 35]. Based on their encouraging and other related data, a clinical trial to study the protective effect of ARRY-371797 on patients with symptomatic DCM due to *LMNA* gene mutations was initiated (NCT03439514). This represents one of the first clinical trials involving genotype-specific therapy particularly for DCM.

The RNA binding motif protein 20 (*RBM20)* gene encodes a nucleus localized RNA-binding protein. RBM20 protein mostly functions as a regulator of post-transcriptional splicing of a subset of genes involved in cardiomyopathy, ion-homeostasis, and sarcomere biology [36]. *RBM20* is predominantly expressed in skeletal and cardiac muscles. Loss of function mutations in the *RBM20* were firstly linked to familial DCM and account for 2–5% of DCM cases [37, 38]. Of the many targets regulated by *RBM20*, aberrant splicing of *TTN* is believed to be the main determinant of *RBM20* mutations caused DCM. Calcium/calmodulin-dependent kinase II delta (*CAMK2D*) is another pivotal cardiac gene transcriptionally regulated by *RBM20*. A recent study showed that *RBM20* mutations carriers also had increased risk of malignant ventricular arrhythmias and sudden cardiac death (SCD), likely resultant from disturbed Ca^{2+} handling and arrhythmic Ca^{2+} cycling [39].

2.3 Cytoskeletal protein coding genes

Other genes such as *DES, DMD* and *FLNC* that encode components of cytoskeleton localized proteins are also identified to link to DCM pathogenesis. Pathogenic mutations in these genes causing DCM often accompany with additional phenotypes, most notably skeletal myopathy. *DES* encodes desmin, a muscle-specific component of the intermediate filament presented at the Z-disk and intercalated discs that integrates sarcolemma, Z-disk and nuclear membrane to maintain the structural and functional integrity of sarcomeric contractile apparatus [40].

Mutations in *DES* have been associated with a spectrum of cardiomyopathies, mostly notably DCM, in about 1–2% of cases [41]. Overlapping phenotypes of *DES* mutations including arrhythmia, cardiac conduction diseases, and skeletal myopathy and smooth muscle defects are frequently observed.

Mutations in the gene encoding dystrophin (*DMD*) cause severe muscle weakness and DCM in Duchenne muscular dystrophy (DMD). Because the *DMD* gene is located in the short arm of X chromosome, pathogenic mutations causing DMD mostly affect boys. The frequency of *DMD* caused muscular dystrophy and DCM is rare, with an estimated incidence 1 in 3500 male births worldwide [42]. Dystrophin protein is a key component of dystrophin-glycoprotein complex and plays a critical role in maintaining the structural integrity of sarcolemma during repeated cycles of muscle contraction and relaxation [43]. Mutations in *DMD* result in loss of the dystrophin protein expression that causes primary muscular dystrophy in males presenting with progressive muscle wasting at early childhood. Subsequently, cardiac dysfunction is involved and more than 90% of affected individuals manifest DCM and patient often died of cardiac and respiratory muscle failure [44].

2.4 Z-disk gene

The Z-disk is an anchoring plane for the actin (thin) filaments to attach and stabilize in the sarcomere. Mutations in many Z-disk-associated proteins coding genes result in cardiac disorders. *BAG3* encodes a highly conserved, Z-disk localized co-chaperone protein that is predominantly expressed in heart and skeletal muscle. BAG3 binds to the ATPase domain of the heat shock protein (Hsp) 70 and exerts multiple functions in regulating apoptosis, preserving the integrity of sarcomere, mediating unfolded protein response and autophagy. *BAG3* variants linked to DCM were firstly reported by two independent genome-wide association studies (GWASs). Later on, *BAG3* mutations were identified in 2–7% of DCM cases [45–47]. Genotype–phenotype correlation study revealed that DCM attributed by *BAG3* mutations is characterized by high penetrance in carriers more than 40 years of age. Patients with *BAG3* mutations are at a higher risk of developing a more severe and progressive heart failure compared with patients without *BAG3* mutations [46]. The level of BAG3 protein was reduced by about a half in both animal models of heart failure and DCM patients as well. Based on the evidence that truncation or deletion mutations in *BAG3* are associated with BAG3 haploinsufficiency which co-segregates with affected DCM family members, it was proposed that the decreased levels of BAG3 protein is the cause of DCM. BAG3 is also an independent heart failure risk factors associated with subclinical LV dysfunction. Thus, cumulative data support that *BAG3* as a bona-fide disease susceptibility gene for DCM [48].

2.5 Ion channel gene

Mutations in the ion channel coding gene *SCN5A* are identified to cause DCM with strong supporting evidence. *SCN5A* encodes the sodium channel Nav1.5 that is mainly expressed in the cardiac muscle [49]. Mutations in *SCN5A* are associated primarily with conduction disorder, arrhythmia and DCM. Incidence of pathogenic *SCN5A* variants is estimated to be 2–4% in all DCM cases [50]. Missense mutations such as R222Q variant located in a voltage-sensing domain exert activating effects on sodium channel function and were thought to cause DCM. While guideline-based heart failure therapies have moderate effect, drugs that have sodium channel-blocking properties such as amiodarone or flecainide could substantially reduce DCM phenotype in patients with R222Q carriers [51]. Moreover, a recent report showed that quinidine treatment of a DCM patient with R222Q mutation achieved

a rapid and significant reduction of ventricular tachyarrhythmia and an improvement in the myocardial function [52]. These interesting genotype–phenotype association studies thus provide another successful example of elucidation of the genetic basis of familial DCM which can lead to effective genotype-tailored therapeutic strategy.

2.6 Other DCM genes

PLN encodes phosphlamban, a transmembrane protein localized to the sarcoplasmic reticulum. Mutations in *PLN* cause variable DCM phenotype, with underlying mechanisms proposed through inhibiting the sarcoplasmic reticulum Ca2 + -ATPase (SERCA2a) [53]. While founder mutation R14del mutation in *PLN* is associated with severe phenotype with high risk for lethal ventricular arrhythmias and end-stage heart failure in the European [54], a milder phenotype had been reported from others [55], suggesting that genetic background might have a big impact on modifying the disease progression associated with *PLN* mutations caused DCM.

Mutations in genes other encoding the sarcoglycans (α, β, γ, δ) were also identified to cause DCM. The sarcoglycans are transmembrane proteins mainly expressed in heart and skeletal muscle that interact with dystrophin. α-, β-, γ-, and δ-sarcoglycans form the sarcoglycan complex that is key components of the dystrophin-associated glycoprotein complex, conferring structural integrity and stability to the sarcolemma through connecting the muscle fiber cytoskeleton to the extracellular matrix, and protecting muscle fibers from mechanical stress during muscle contraction. Mutations in sarcoglycans coding genes cause primary limb-girdle muscular dystrophy presented with early onset muscle weakness and associate with significant DCM [56]. Notably, mutations in the δ- sarcoglycan coding gene lead to DCM without involvement of obvious muscular dystrophy phenotypes [57].

Mutations in nuclear encoded mitochondrial genes such as *TAZ* and *DNAJC19* were also identified to cause DCM. *TAZ* encodes a mitochondrial localized Tafazzin protein that is predominantly expressed in cardiac and skeletal muscle. Tafazzin functions as a phospholipid transacylase that catalyzes the remodeling of cardiolipin that is required for oxidative phosphorylation. Mutations in the TAZ gene cause X-linked Barth syndrome and DCM, leading to premature death [58]. Mechanistically, mutations in *TAZ* result in Taffazin deficiency and cause mitochondrial dysfunction and impaired mitophagy and increased oxidative stress, leading to DCM [59].

3. Genetic testing

The advent of next-generation sequencing enables cost-effective genetic testing in familial DCM which can define the precise genetic cause of disease. Genetic testing can also help optimize risk stratification and assess prognostics of patients and their relatives. With the identification of a pathogenic mutation and early diagnostic certainty, clinical management of affect individuals could be tailored and patients' survival can be improved. One best example is related to clinical practice of early intervention of DCM patients with L*MNA* mutations. *LMNA*-related DCM usually accompanied by significant conduction system disease, atrial fibrillation, ventricular tachycardia, and sudden cardiac death (SCD). Thus, *LMNA* mutations are often associated with a higher disease penetrance and more severe morbidity and high mortality [60]. Studies in several different cohorts of DCM patients with *LMNA* mutations identified non-missense mutations, LVEF<45% and higher

AV blocker as significant risk factors for disease malignancy [61, 62]. Because of this well-determined genotype–phenotype knowledge, an actionable prognostic genotype–phenotype association, implementation of a lower threshold and earlier implantable cardioverter defibrillator (ICD) therapy than current guidelines recommend in patients with LMNA mutations, was demonstrated to significantly improve patient outcome and survival [63].

Based on the Genetic Testing Registry (https://www.ncbi.nlm.nih.gov/gtr/), there are from 40 to 80 genes included in the testing panels for most commercially available genetic testing for DCM. In familial DCM, the yield of genetic testing, resulting in identification of pathogenic mutations, can thus far reach up to 40%, in a comparable level to that of other inherited cardiac disorders such as HCM and long QT syndrome. The sensitivity of genetic testing is compromised partially due to that not all genes implicated in DCM are included in the gene panels for testing. This is especially the case for those identified from sporadic DCM cases or single family. Furthermore, limited by human genetics approaches that heavily rely on pedigree availability and candidate gene approaches, variants for more than half of familial DCM cases have not been identified yet. As technique advances in genetics of cardiomyopathy, identification of the remaining genetic causes in inherited DCM cases and elucidation of the underlying pathogenic mechanisms leading to the phenotype are evolving rapidly. Targeted gene panels for genetic testing are increasing in an unprecedented scale. With further characterization and functional validation, the ever expanding gene panels of genetic testing promise to increase the rate of positive identification and provide individuals and families with a more comprehensive and conclusive genetic testing.

4. Genotype-targeted therapies

By directly targeting at specific pathogenic mutations causing DCM, genotype-targeted genetic approaches have emerged as promising strategies for effective and individualized therapies to ameliorate disease phenotypes. To carry out genotype-based therapy, many customized genetic strategies were explored. For examples: for loss-of-function mutations that cause reduced or insufficient protein levels, the straightforward gene replacement strategy can be employed. In this scenario, full-length or partial functional cDNA for corresponding mutated gene can be transferred to cardiac tissue to supplement the reduced gene dosage using appropriate gene delivery approach. For mutations that cause dominant-negative effects on a particular gene, exon-skipping or trans-splicing approaches can be considered to remove or modify the mutant transcripts. For pathogenic mutations that cause other protein dysfunction, the highly efficient CRISPRA/Cas9 (Clustered regularly interspaced short palindromic repeats/CRISPR-associated endonuclease) system can be explored to directly correct the mutant variants. In addition, other genotype-based therapeutic strategies such as manipulation of the downstream pathways evoked by specific DCM mutations were also explored. Below, we summarize several examples of gene-targeted therapeutic strategies that produced encouraging results in the treatment of DCM (**Table 2**), which hold the opportunities to ultimately improve patient outcome in the future.

4.1 Dystrophin mutations-targeted gene therapies

Mutations in the dystrophin (*DMD*) gene cause muscular dystrophy patients and X-linked familial DCM in early childhood and patients often die of cardiac and respiratory failure [44]. Currently, there are no effective cures for DMD yet and management

of DMD mostly focuses on preserving the limited muscle strength and ameliorating disease symptoms [64]. Mechanistically, dystrophin protein deficiency due to *DMD* gene mutations is the primary cause of DMD and subsequent DCM and heart failure. And the disease severity is mostly correlated with the dystrophin protein level. Thus, a plausible treatment strategy would be to restore the expression level of dystrophin protein [44]. To cure this devastating disease, dystrophin gene-targeted therapeutic strategies such as gene replacement, exon skipping and CRISPRA/Cas9 genome editing techniques are mostly employed, which have shown encouraging results in restoration of dystrophin protein expression and recovery of dystrophin protein function in both animal models and clinical trials.

Gene replacement strategy involves delivering a functioning gene to replace or supplement the mutant gene to the target organ and cells to ease the disease phenotype caused by genetic mutation. This approach often utilizes the adeno-associated virus (AAV) as a vector to mediate gene transfection into the skeletal and cardiac muscle cells. However, as the dystrophin gene has 79 exons and its transcript is about 14 Kb, its size is too large to fit in currently available gene construction vector for gene transfection. Alternatively, a mini- or micro- dystrophin gene coding for a functionally similar to dystrophin but smaller in size was thus used. The authors Wang and colleagues firstly defined the minimal functional region of the dystrophin protein, later referred as mini-dystrophin. The authors then packed this min-dystrophin gene into an AAV vector to mediate muscle transfection and demonstrated the effectiveness of gene delivery and restoration of dystrophin gene function [65]. After this study, several other independent groups latter confirmed the effectiveness of a shortened albeit functional dystrophin gene replacement strategy mediated by the AAV gene delivery system in preservation of cardiac and skeletal muscle function and extending the lifespan in the dystrophic mice [66, 67]. Notably, further studies in animal models of muscular dystrophy and human patients detected certain immunologic responses to the shortened dystrophin peptide, which need to be carefully considered in future clinical application [68–70].

Exon skipping is an RNA-based splice-switching approach that causes cells to "skip" the exon containing the pathogenic variant, resultant in-frame transcripts that produce a shorter peptide, albeit still at least partially functional protein to ameliorate disease phenotypes. This approach was initially developed to mask the pathogenic mutations containing exon 51 using an antisense oligonucleotide (AON), resulting in genetic correction of the open reading frame of the *DMD* gene and partial restoration of dystrophin protein expression and improvement of muscle pathology and function [71–73]. These encouraging studies lead to at least two ongoing clinical trials to evaluate the efficacy and safety of this exon skipping approach targeting to the pathogenic exon 51 or 53 (NCT02255552, NCT02310906).

With the revolutionary development of the CRISPRA/Cas9 genome editing technologies that enable precise and efficient genetic modifications from single-nucleotide alternation, insertion of gene of interest to deletion of chromosomal regions, numerous studies have demonstrated the feasibility and high efficiency of this technique in restoration dystrophin expression and function in skeletal and cardiac muscle, both in vitro and in vivo [74, 75]. For example, a recent study carried out by El Refaey and colleagues packaged SaCas9 (Cas9 from *Staphylococcus aureus*)/gRNA constructs into an AAV serotype rh74 and delivered it to the mdx/Utr$^{+/-}$ dystrophic mice through a retro-orbital approach [76]. The authors showed this CRIPRA-mediated genome editing strategy could efficiently excise the mutant exon 23 of dystrophin in the mice model, resulting in the restoration of the dystrophin protein expression and cardiac myofiber architecture, and significantly improved the cardiac contractility in vivo. A follow up study using this AAV-mediated CRISPR genome editing approach found that restored dystrophin expression and improved cardiac function were consistently

detected at up to 19 months [77]. Similarly, Hakim and colleagues modified the dose of AAV.SaCas9 and AAV-9.gRNA vector that directs Cas9 to introns of 22 and 23 and performed the AAV-9 CRISPRA gene therapy in the mdx mice that carry a nonsense mutation in exon 23 of the dystrophin gene [78, 79]. The authors found significantly increased dystrophin restoration and reduced fibrosis in both skeletal and cardiac muscle and improved muscle function and cardiac hemodynamics at up to 18 months.

4.2 *LMNA* mutations-tailored therapeutic strategy

A genetic approach referred as trans-splicing approach was initially carried out to correct *LMNA* mutations in the *Lmna* DelK32 knock-in mouse harboring a *LMNA*-related congenital muscular dystrophy mutation in the exon 1 [80, 81]. The *Lmna* DelK32/DelK32 mice exhibited severe muscular and cardiac defects and early premature death. The trans-splicing approach allows converting the targeted endogenous mutated pre-mRNA to a therapeutic pre–trans-spliced molecule containing the wildtype coding sequence, resulting in a processed mRNA transcript devoid of the exon harboring the pathogenic variant [82, 83]. To perform the trans-splicing rescue in the *Lmna* DelK32 knock-in mouse model, 5'-RNA pre-trans-splicing molecules containing the first five exons of *Lmna* and targeting intron 5 of *Lmna* pre-mRNA were firstly developed. After confirmed the induced trans-splicing events on endogenous *Lmna* mRNA in vitro, AAV mediated delivery system was then evaluated in vivo in the newborn mice. Similarly, trans-splicing events were successfully detected in both skeletal and heart muscle of mice up to 50 days after AAV delivery. However, the tans-splicing occurring efficiency needs to be further improved to exert significant rescue effects on cardiac function and premature death.

To target at the *LMNA* pathogenic variants resulting in deficiency of the protein level, another strategy referred as stop codon readthrough was explored through inactivating the molecules participating in nonsense-mediated decay at the RNA level [84]. This approach applies to the scenario when pathogenic variants result in frameshifts and premature stops, subsequently leading to protein deficiency because of increased nonsense-mediated decay of mutant RNAs. PTC124, also known as ataluren, is a chemical compound that can selectively induce ribosomal readthrough of premature but not normal termination codons [85]. PTC124 was tested in iPSC-CMs derived from patients carrying different nonsense and frameshift mutations in the *LMNA* gene [85]. In one of the three frameshift mutants tested, administration of PTC124 significantly increased the translation of full-length LMNA protein and partially restored the protein function, as shown by reduced cardiomyocyte apoptosis and improved excitation-contraction coupling [84].

4.3 *TTN* truncating variants-targeted gene therapy

While the pathogenicity of *TTN* missense mutations to DCM remains largely undetermined, *TTN* truncating variants (*TTNtvs*) are the most common cause to DCM. To date, experimental evidence generated largely from rodent animal models, human induced pluripotent stem cell-derived cardiomyocyte, and patient tissues mostly support the mechanism of titin protein haploinsufficiency. Correction of the titin protein deficiency by using traditional viral-mediated gene replacement strategy to increase the expression of titin protein, just as that has been successfully employed to restore the dystrophin deficiency, however, is challenging. Because the *TTN* gene spans 294 Kb of genomic region and its spliced transcript is more than 100 Kb, a size that is way bigger than any currently available cargo capacity for AAV

mediated gene transfection. Alternatively, exon skipping, a genetic approach that had been initially employed to treat the DMD, through using an AON to mask the pathogenic mutations containing exon 51 or 53 and restoration of DMD phenotype [72], as aforementioned, was explored to treat *TTNtvs* related DCM. Notably, this strategy had been also successfully utilized for treating Mybpc3 mutations caused HCM in a knock-in mouse model [86].

To employ exon skipping strategy for treating *TTNtvs* caused DCM, Gramlich and colleagues targeted to the Ser14450fsX4 variant located in the exon 326 identified in a DCM patient that caused autosomal-dominant truncating frameshift mutation [87]. They used an AON-mediated exon skipping approach to remove the exon 326 in both patient cardiomyocytes in vitro and mouse heart in vivo. In vitro, skipping of the exon 326 containing the Ser14450fsX4 pathogenic variant restored the impaired myofibril assembly and stabilized its structural integrity and normalized expression of sarcomeric protein in the patient-specific cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs) model. Furthermore, in the corresponding Ttn knock-in mice model, skipping of the exon 326 significantly improved sarcomere formation and contractile function and prevented subsequent development of DCM phenotype. These results provide strong evidence to support that the RNA-based exon skipping strategy could be a potential treatment option for DCM caused by gene variants that are otherwise technically difficult to be delivered due to size limitation for gene replacement therapy.

4.4 Genotype-targeted therapies for other DCM genes

Mutations in the δ- sarcoglycan coding gene (*SGCD*) can lead to DCM without obvious involvement of skeletal muscle disorders [57]. The gene replacement strategy was firstly applied in the treatment of DCM caused by a deletion mutation in the *Sgcd* gene that disrupted the dystrophin-associated glycoprotein complex in a hamster model [88]. The authors Kawada and colleague constructed a recombinant AAV vector containing the full-length cDNA of *Sgcd* gene driven under the cardiac specific expression promoter of CMV and intramurally injected it to the *Sgcd* mutation induced DCM model in the hamster. *They detected* robust expression of the *Sgcd* gene and other types of sarcoglycans as well in the transfected myocardium. The restored expression of *Sgcd* and other sarcoglycans normalized the diameter of transduced cardiomyocytes, improved the contractile function and ultimately prolonged the life span of the animals harboring the *Sgcd* gene mutation. This study provides one of the earliest evidence to employ gene replacement strategy to treat DCM caused by a specific gene mutation with efficient and sustained transfection capability.

An earlier version of genome editing technique referred as transcription activator-affected nuclease (TALEN) was explored to directly correct the *PLN* R14del mutation that is associated with high risk for malignant ventricular arrhythmias and end-stage heart failure in DCM patient carriers [89]. The authors Karakikes and colleague firstly derived iPSC-CMs from a DCM patient harboring the *PLN* R14del mutation, followed by detailed phenotypic characterization of this iPSC-CMs model. Next, the authors made the effort to correct the *PLN* R14del mutation in the iPSC-CMs model using TALEN gene editing strategy. This approach successfully corrected the R14del mutation and attenuated the R14del-associated disease phenotypes in the iPSC-CMs model. Furthermore, the authors engineered an AAV6 vector that enabled knock-down the endogenous *PLN*, while simultaneously overexpress a codon-optimized *PLN*, to effectively reverse the disease phenotype in the iPSC-CMs model as well. Thus, this study provides another successful example to using genome editing approach to directly target at the pathogenic mutations associated with DCM

to achieve therapeutic benefit. Notably, while it provides evidence to support the effectiveness of genome editing technique to correct pathogenic variants causing DCM in vitro, germline correction of embryos harboring DCM variants had not been reported yet.

5. Conclusions

The ultimate goal of elucidating genetic basis for DCM is for early disease diagnosis, early disease intervention and treatment. To date, pathogenic mutations in more than 50 genes are associated with DCM. However, these mutations can only explain about 40–50% of familial DCM cases, and the genetic architecture of DCM still remains incompletely understood. Future research directions rely on technique advances for identification of the remaining up to 60% of genetic causes in familial DCM cases. After identifying the genetic causes, functional characterization and validation studies are needed to confirm the pathogenic variants. Extensive studies on delineation of genotype–phenotype relationships are necessary to address currently unmet issues for translational research. Further dissecting genetic pathways linked to DCM and elucidating the pathogenic mechanisms leading to the phenotype can provide valuable insights into the understanding of disease pathophysiology, laying solid foundation for future development of groundbreaking therapeutics.

Gene-based therapies, such as gene replacement, exon skipping and recent evolutionarily developed CRISPRA/Cas9 based genome-editing techniques that directly target at the underlying genetic mutations responsible for the DCM disease progression, have led to several clinical trials which have produced promising results. Thus, the last decade has witnessed encouraging advances in the development of genotype-targeted, personalized therapies. While the progress is promising, several technical issues need to be thoroughly assessed before implementation in clinical practice. For example, current gene delivery system mostly adapt the AAV system because of its many advantages such as long term transgene expression and choice of appropriate serotype for heart enriched expression. One primary limitation for this system, however, is that there exists certain level of anti-AAV antibodies in general human population causing the immunogenicity issue which can potentially lead to development of myocarditis [70, 90–92]. While the efficiency for exogenous delivery of preprocessed RNAs or oligonucleotides for trans-splicing or exon skipping needs to be further improved, a major concern of off-target issue needs to be seriously addressed for the highly efficient AAV9 mediated CRISPR/Cas9 genome editing system. Novel approaches are desirable to comprehensively evaluate any potential off-target mutagenesis in the heart and other tissues for future clinical application.

Conflict of interest

The authors declare no conflict of interest.

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