Chapter

Left Ventricular Noncompaction Cardiomyopathy: From Clinical Features to Animal Modeling

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Abstract

Cardiomyopathy or disease of the heart muscle involves abnormal enlargement and a thickened, stiff, or spongy-like appearance of the myocardium. As a result, the function of the myocardium is weakened and does not sufficiently pump blood throughout the body nor maintain a normal pumping rhythm, leading to heart failure. The main types of cardiomyopathies include dilated hypertrophic, restrictive, arrhythmogenic, and noncompaction cardiomyopathy. Abnormal trabeculations of the myocardium in the left ventricle are classified as left ventricular noncompaction cardiomyopathy (LVNC). Myocardial noncompaction most frequently is observed at the apex of the left ventricle and can be associated with chamber dilation or muscle hypertrophy, systolic or diastolic dysfunction, or both, or various forms of congenital heart disease. Animal models are incredibly important for uncovering the etiology and pathogenesis involved in this disease. This chapter will describe the clinical and pathological features of LVNC in humans and present the animal models that have been used for the study of the genetic basis and pathogenesis of this disease.

Keywords: animal models, noncompaction, cardiomyopathy, mutation, cardiac development

1. Introduction

Left ventricular noncompaction (LVNC) is a unique form of cardiomyopathy that is distinguished by a distinctive ("spongy") morphological appearance of the left ventricle (LV) myocardium [1]. This "spongy" appearance encompasses hypertrabeculation, deep intertrabecular recesses or sinusoids, and a bilayered ventricular myocardium with a noncompacted endocardium and compacted epicardium [2]. Although LVNC is rare, the prevalence of LVNC is reported to be <0.3% in adults and < 0.0001% in children. It is the third most common form of inherited cardiomyopathies and accounts for 9% of all pediatric cardiomyopathy cases [3]. Prevalence was reported as 0.014–1.3% in adult patients who underwent echocardiography. In heart failure patients, the prevalence of LVNC is estimated at 3–4% [4]. LVNC is characterized as genetic, primary cardiomyopathy by the 2006 American Heart Association classification model, whereas the European Society of Cardiology has not classified LVNC as distinct cardiomyopathy due to its phenotypic heterogeneity [5, 6].

Clinical presentation of LVNC is variable, and Towbin et al. have described nine distinct subtypes: 1) benign, 2) arrhythmogenic, 3) dilated, 4) hypertrophic, 5) mixed hypertrophic and dilated, 6) restrictive, 7) right ventricular with the normal left ventricle, 8) biventricular, and 9) associated with congenital heart disease [7]. Some of the LVNC phenotypes are shown in **Figure 1**. Complications of LVNC include chronic heart failure, arrhythmias, cardioembolism, chest pain, dyspnea, syncope, myocardial infarction, and sudden cardiac death (SCD) [8]. Patients with LVNC associated with neuromuscular disease may present with exercise intolerance, fatigue, muscle pain, muscle stiffness, and muscle weakness. Heart failure associated with LVNC is often due to either systolic or diastolic ventricular dysfunction. Electrocardiogram (ECG) abnormalities are very common (88–94% and 88%, respectively) in both adult and pediatric cases. Common arrhythmias are atrial fibrillation, atrial flutter, paroxysmal supraventricular tachycardia, and atrioventricular block. Heart failure and arrhythmias are the greatest cause of concern for mortality in LVNC patients [9].

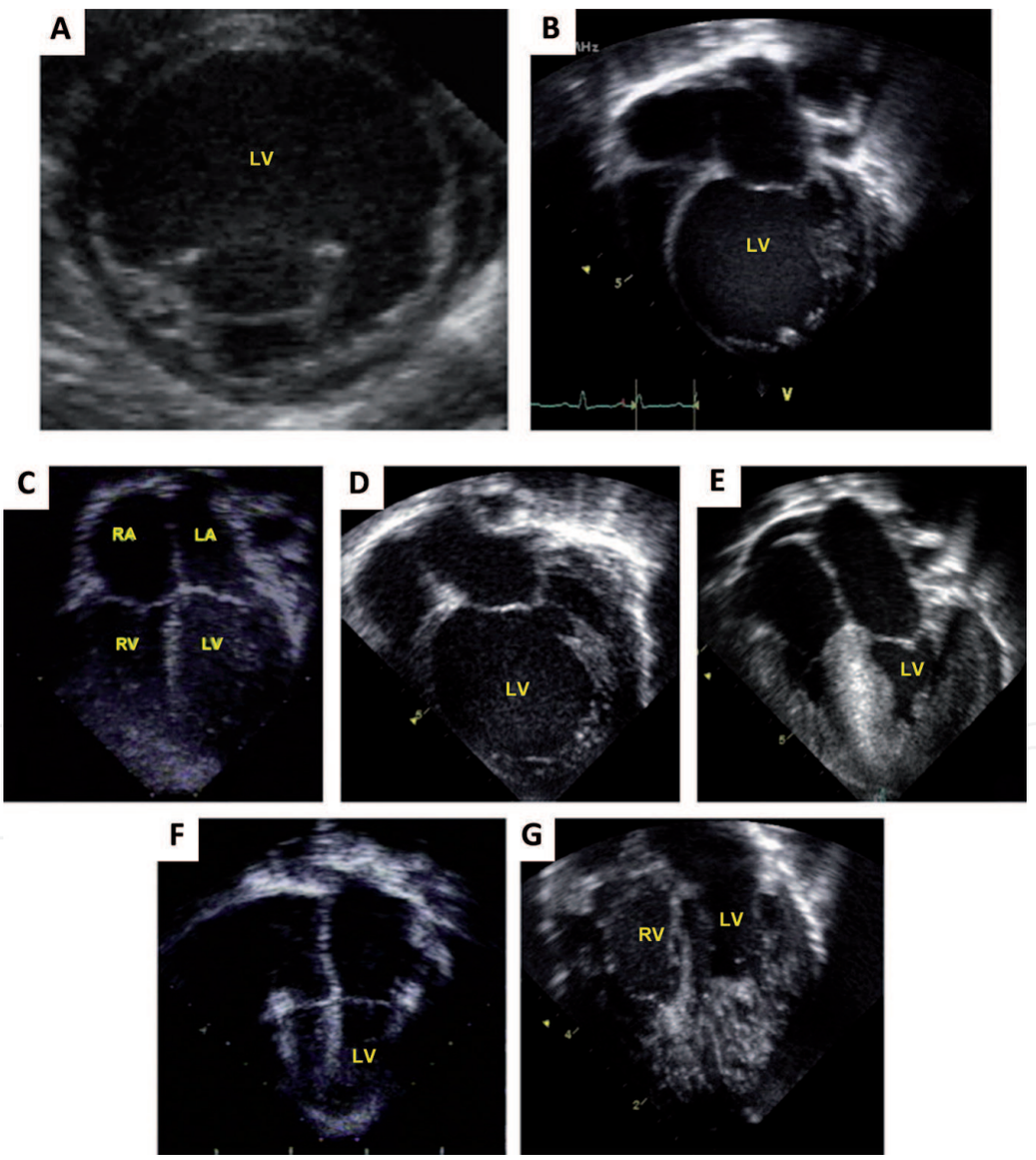

Figure 1.
Echocardiographic images of heterogeneous forms of left ventricular noncompaction phenotype. A. Parasternal short-axis view of a dilated form of LVNC with trabeculations noted at the apex. B. An apical 4-chamber view with trabeculations noted at the right side of the image. C-G, Heterogeneous phenotypes associated with LVNC. LVNC with normal LV size, thickness, and function (C), dilated form of LVNC (D), hypertrophic form of LVNC (E), restrictive form of LVNC (F), biventricular LVNC (G). LV, left ventricle; RV, right ventricle; LA, left atrium, RA, right atrium.

The etiology, pathogenesis, and natural history of LVNC are not clearly understood. The genetic causes of LVNC are heterogeneous [10, 11], involving final common pathways initiated by primary (the sarcomere) and developmental (NOTCH pathway) genetic abnormalities, often *via* a disturbance of protein–protein binding caused by the primary genetic mutation [12]. Doppler echocardiography, cardiac magnetic resonance imaging, or LV angiography are used for the diagnosis. Due to heritable nature, patients with LVNC and at-risk first-degree relatives are recommended to undergo genetic screening and counseling [13, 14]. Clinical symptoms associated with myocardial dysfunction, significant arrhythmias, congenital heart disease, or neuromuscular disease combined with the results of genetic testing dictate the outcome and therapeutic management of LVNC [15]. Family studies and animal models are incredibly important for uncovering the genetic basis and pathways involved in this disease. In this chapter, we describe trabeculation and compaction events during cardiogenesis, morphopathological features of LVNC, and possible genetic mechanisms of LVNC. We will also describe the animal models that have been used for the study of LVNC.

2. Embryonic development of compacted myocardium

The underlying mechanisms for LVNC remain largely unknown, but many studies associate it with the failure of compaction of trabecular myocardium during embryogenesis [16]. The development of the functionally competent, compacted, and multilayered myocardial wall is a two-part process consisting of trabeculation followed by a compaction process set at the midgestational period of cardiogenesis [17]. When the myocardial spiral system enfolds, myocyte recruitment and proliferation lead to myocardial maturation with the development of protrusions into the lumen. Endocardial cells invaginate, and cardiomyocytes in specific regions along the inner wall of the heart form sheet-like protrusions into the lumen to give rise to the trabecular myocardium [16]. The intertrabecular recesses communicate with the blood-filled cavity of the heart tube to increase the surface area for gas exchange and blood. This mechanism favors the concomitant increase in myocardial mass despite the absence of a distinct epicardial coronary circulation [18].

The trabecular myocardium starts undergoing compaction between weeks 5 and 8 of human embryonic development and coincides with the invasion of the developing coronary vasculature from the epicardium. Compaction is gradual, from the epicardial to the endocardial surface and from the basal segments of the ventricle moving toward the apex [19]. Vascular endothelial growth factor (VEGF) and angiopoietin-1 may be involved with triggering compaction [2]. As a result of the compaction process, the intertrabecular recesses disappear almost entirely leaving a smooth endocardial ventricular surface. Compaction is more pronounced in the LV than in the right ventricle (RV), therefore the RV endomyocardial surface is more heavily trabeculated (**Figure 2**) [19]. On the other hand, noncompaction indicates failure of compact myocardium formation, leaving spongy myocardium and deep intertrabecular recesses [20].

2.1 Etiopathophysiology of LVNC and genotype: phenotype correlation

While the etiology of LVNC is not clearly understood, it is largely considered that hypertrabeculation or noncompaction in LVNC has a genetic origin with typically autosomal dominant inheritance if the implicated genes encode components of the sarcomere, Z-disc, or cytoskeleton [21]. Autosomal recessive, X-linked, and mitochondrial inheritance patterns have also been found [3, 22]. One large retrospective multicenter study showed that nearly one-third of the LVNC patients had genetic variants in at least one cardiomyopathy-causative gene [14]. LVNC

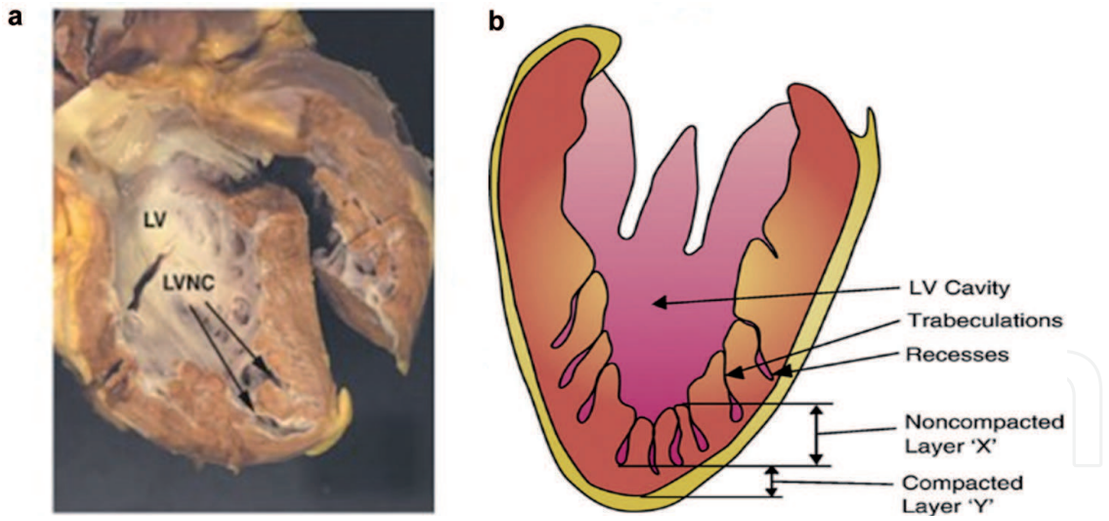

Figure 2.
Gross morphopathologic appearance of LVNC. a. Heart from a individual with left ventricular noncompaction. Note the spongy appearance of the ventricular wall, caused by 'holes' in the myocardium, which represent deep trabeculations. The heart is thick and has a dilated chamber (that is, hypertrophic and dilated). In life this ventricle functioned poorly. LV, left ventricle; LVNC, trabeculations of left ventricular noncompaction. b. Numerous excessive prominent trabeculations and deep intertrabecular recesses is noted by arrows. The trabecular zone (noncompacted layer, X) in the LV is at least twice thick as the compact layer (Y) of the ventricular wall. (Adopted from Towbin and Bowles, Nature 415, 227–233. 2002).

has also been reported in many complex syndromes [23, 24] and neuromuscular disorders [25–27]. LVNC can also be considered congenital or acquired, and several hypotheses have been proposed for the development of LVNC [28]. The primary hypothesis for congenital LVNC is the embryonic hypothesis, which attributes the hypertrabeculation of LVNC to the arrest in normal ventricular compaction during myocardial embryogenesis [29]. The etiology of LVNC can be described as having two components, congenital and modifier factors.

Genetically, LVNC is heterogeneous and has been associated with chromosomal defects and genetic mutations in myosin heavy chain 7 (*MYH7*) [21, 30], LIM domain-binding protein 3 (*ZASP*), α-dystrobrevin (*DTNA*), tafazzin (*TAZ/G4.5*), ion channels, and proteins found in the sarcomere, cytoskeleton, and mitochondria. Alterations in the NOTCH signaling pathway, associated with morphological development, and WNT pathway signaling, embryonically involved in body axis patterning and cell polarity, are also linked to LVNC [20, 31]. In some categories of LVNC, the genotype–phenotype correlation is identifiable. Tafazzin mutations, one of the first mutations linked to LVNC, are characteristic of Barth syndrome, an X-linked genetic disorder that commonly presents with LVNC. Tafazzin, an inner mitochondrial membrane protein, catalyzes phospholipid cardiolipin synthesis, which is essential for mitochondrial integrity and energy production in cardiomyocytes [29, 32]. Family studies have identified mutations in hyperpolarization-activated cyclic nucleotide-gated channel 4 (*HCN4*), sodium voltage-gated channel alpha subunit 5 (*SCN5A*), and ankyrin 2 (*ANK2*) as genetic abnormalities underlying sinus bradycardia-associated LVNC [33]. Lamin A/C (*LMNA*) mutations, which are also found in dilated cardiomyopathies, are associated with the early onset of advanced atrioventricular block [34]. A 6.8-megabase locus on chromosome 11p15, containing muscle LIM protein (*MLP/CSRP3*) and SOX6, was implicated in an autosomal dominant pedigree of LVNC [35]. The V470I variant in bone morphogenetic protein 10 (*BMP10*) and W143X variant in neuregulin (*NRG1*) were identified in two unrelated LVNC probands and their affected family members [36]. Impaired BMP receptor binding ability, perturbed proliferation and differentiation processes, and intolerance to stretch in mutant cardiomyoblasts may underlie myocardial noncompaction in these families.

The causal nature of genetic defects is further complicated by the overlap of genetic mutations in distinct cardiomyopathies. LVNC can be categorized based on its association with dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and other forms of heart muscle disease. Using next-generation sequencing, several groups revealed a wide range of pathogenic variants in LVNC patients and an association between pathogenic variants and poor prognoses, especially in those patients harboring multiple pathogenic variants [10, 11, 37, 38]. Variants in *MYH7* were associated with HCM, DCM, and restrictive cardiomyopathy (RCM). Patients with sarcomeric genes variants had more frequent findings of trabeculations and likelihood fibrosis in the interventricular septum of the myocardium [11]. Variants in mindbomb homolog 1 (*MIB1*), *LMNA,* and *MLP* were linked to LVNC associated with DCM. Myosin-binding protein C (*MYBPC3*) mutations are associated with LVNC-hypertrophic cardiomyopathy, while *SCN5A* and *DSP* variants are reported causative for arrhythmogenic cardiomyopathy (ACM), DCM, and cardiac conduction system dysfunction disorders including Brugada syndrome and long QT syndrome. Interestingly, truncating variants in the *LAMP2* gene that is causative for Danon disease were identified in LVNC patients by Li et.al [37]. In addition, mitochondrial genome mutations [39], chromosomal abnormalities such as 1p36 deletion, 7p14·3p14·1 deletion, 18p subtelomeric deletion, 22q11·2 deletion, distal 22q11·2, 8p23·1 deletion trisomies 18 and 13, and tetrasomy 5q35·2–5q35 have been associated with syndromic LVNC [40–44]. Patients with Coffin–Lowry syndrome (*RPS6KA3* mutation), Sotos syndrome (*NSD1* mutation), and Charcot–Marie–Tooth disease type 1A (*PMP22* duplication) have also been reported to manifest clinical signs of LVNC [23, 45–47]. Titin encoded by the *TTN* gene with 364 exons is the largest protein, expressed in striated muscles [48]. A missense variant *TTN* A178D identified by high throughput next-generation whole-genome sequencing techniques that have been implicated in clinical genetics practice over the last decade has recently been associated with autosomal dominant LVNC and DCM [49]. Nonetheless, a genotype–phenotype correlation may not be identifiable for all mutations and variants. Genetic defects may have incomplete penetrance and variable expressivity or have no causal relationship between genotype and phenotype [2].

The embryonic hypothesis does not explain acquired LVNC that presents after birth, some forms of which are potentially reversible. Acquired LVNC, has been identified in athletes, pregnant women, and patients with sickle cell anemia, myopathies, and chronic renal failure [50]. The etiology of acquired LVNC is merely speculative. One such hypothesis argues that mild LVNC can remain undetected until transient LV dilation allows LVNC to become visible under precise and accurate imaging [51]. It is also speculated that acquired LVNC may be due to cardiac remodeling from increased preload and altered hemodynamics [29]. Ventricular trabeculation in athletes, particularly in the LV apex, allows for increased compliance which reduces wall stress and strain [52]. Given the high risk of possible cardiac embolic events from thrombus formation in the intertrabecular recesses, clinical trials for thromboembolic events in isolated LVNC have been suggested [53–55].

2.2 Diagnosis and therapeutic strategy

The clinical manifestations of LVNC vary widely, including no symptoms, thromboembolic events (ventricular or systemic arterial), LV dilation, impaired contractility with heart failure leading to pulmonary edema, arrhythmia including ventricular tachycardia and atrial fibrillation, and sudden cardiac death. Patients with neuromuscular disorder and LVNC may present with elevation in muscle form of creatine kinase, CK-MM (creatine kinase, muscle isoform) consistent with skeletal myopathy [7].

Echocardiography is the first-line diagnostic routine and an accessible technique to detect abnormal trabeculations or a "spongy" appearance of the myocardium. Several diagnostic criteria have been developed to define LVNC through echocardiographic analysis. A ratio of >2:1 in thickness of noncompacted to compacted layers during diastole is deemed diagnostic for LVNC [56, 57]. Compared with echocardiography, cardiac magnetic resonance (CMR) imaging offers more in-depth anatomic and functional features of the noncompacted myocardium. Late gadolinium enhancement specifically provides detection of cardiac fibrosis. CMR criteria developed by Petersen et al. to accurately diagnose pathologic noncompaction is based on a noncompaction to compaction ratio at end-diastole of >2.3 [58]. Quantitative CMR criteria by Jacquier *et al*. define LV noncompacted mass > 20% of the total mass for accurate LVNC diagnosis [59], while Grothoff et al. propose LV mass > 25% of the total mass as well as a noncompacted mass > 15 g/m^2 [60]. Despite all these proposed criteria, there are wide inconsistencies and poor specificity, and it remains difficult to accurately differentiate normal variants in trabeculations from pathological LVNC [61]. Therefore, data matrices 0f echocardiography and CMR imaging measurements, electrocardiogram features, and clinical genetics of the patient and relatives are helpful for confirming the clinical diagnosis [7].

Genetic testing in patients with LVNC and family members has been important in identifying genetic causes of cardiac dysfunction. Testing can be done on genes known to be associated with LVNC and other forms of cardiomyopathy as well as genes involved in syndromic diseases such as metabolic abnormalities, mitochondrial dysfunction, Barth syndrome, and storage diseases. Identification of pathogenic variants in probands and family members can be followed by segregation studies in the family [15].

Treatment strategy in LVNC depends on clinical presentations and complications, and clinical needs are managed according to corresponding guidelines [62]. The key targets of clinical management are the treatment of heart failure (including beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin-II receptor blockers, aldosterone antagonists, diuretics, and heart transplantation), arrhythmias (including ablation and implantation of an implantable cardioverter-defibrillator in patients with life-threatening events), and oral anticoagulation. In patients with congenital heart disease and LVNC, surgery for the congenital abnormalities takes precedence when feasible. In many cases, palliative surgery ultimately fails because of the myocardial abnormality, and cardiac transplantation is required [63].

3. Animal models of LVNC

LVNC is an overlap disorder and it appears that any of these "final common pathways" can be involved depending on the specific form of LVNC [64]. Combining information about disease-causing genes with murine models is crucial in identifying pathways involved in ventricular noncompaction. For instance, Barth syndrome is caused by tafazzin mutations, and tafazzin knockdown mice were engineered using a short-hairpin RNA-inducible transgenic approach. These mice demonstrated hypertrabeculation and noncompaction, and the knockdown mice died prenatally at E12.5–14.5 [65]. New powerful cutting-edge gene-editing technologies using transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9 (CRISPR-Cas9) have been used for modeling LVNC [66–69]. Patient-specific induced pluripotent stem cells (iPSCs) derived cardiomyocytes have emerged as a useful tool for investigating pathological mechanisms of many cardiovascular diseases including LVNC [70, 71].

Gene	Animal model	Signaling	Ref #
FKBP12	KO mouse	Decrease in Notch1 activity, increase in BMP10	[79]
BMP10	KO E9.0–13.5 mouse	TGF-b	[80]
BMP10	Overexpression adult mouse	TGF-b	[81]
NUMB/NUMBL	Double KO mouse	Inhibition of Notch1, Smad6 and Smad7, WNT	[82]
SMAD7	Mutant mouse	BMP10, TGF-b	[86]
TBX20	Overexpression mouse	T-box family, TBX1	[87]
MIB1	Mutant zebrafish	Reduces Notch1	[89]
MIB1	Decifient zebrafish	Reduces Notch1	[89]
VEGF	Overexpression mouse	Notch1, Flk-1	[90]
Daam1	Decifient mouse	PCP and WNT	[92]
NKX2–5	KO mouse	MEF2, HAND1, HAND2, GATA, BMP10	[96]
NFATC1	Mutant mouse	NFAT	[99]
TAZ	Inducible knockdown	Cardiolipin remodeling	[102]
TAZ	KO mouse	Cardiolipin remodeling	[65]
TTN	Mutant mouse	Telethonin loss	[104]

Table 1.
Animal models of LVNC.

Several signaling pathways such as the Dll4-NOTCH [72], MIB1 [73], BMP [74], and TGF-β [71] have been demonstrated to regulate myocardial trabecular compaction as well as to be involved in the development of LVNC. While zebrafish and *Drosophila* have been used to study LVNC in addition to patient-specific iPSCs derived cardiomyocytes, mice are more commonly used to genetically engineer LVNC phenotypes as shown in **Table 1** [75, 76].

3.1 Animal models related to the Notch signaling pathway

The Notch signaling pathway is a highly conserved intercellular pathway involved with multisystem differentiation. Particularly in the cardiovascular system, Notch1 mediates ventricular morphogenesis, coronary vessel development, and communication between the endocardium and myocardium for cardiomyocyte proliferation and differentiation. Mutations in the Notch signaling pathway cause congenital heart disease [77]. Mammals have Notch 1–4, a group of transmembrane receptors with an extracellular domain and an intracellular domain. The Notch extracellular domain (NECD) interacts with ligands of the Delta and Jagged family, and these receptor-ligand interactions are modulated by manic fringe (Mfng) glycosyltransferase. Delta and Jagged ligands are ubiquitinated by Mib1, an E3 ubiquitin ligase, and trigger endocytosis of the ligand. Mib1 activity exposes the receptor to ADAM metalloproteases for Notch cleavage. In response, the Notch intracellular domain (NICD) translocates to the nucleus and acts as a transcription factor. The NICD is comprised of the RBPJ domain and ANK repeats [77].

The first murine model for LVNC was the Fkbp1a (or FKBP12)-deficient mouse. Deficiency in FKBP1a, a binding protein of the immunophilin family, causes ventricular noncompaction, thin ventricular walls, hypertrabeculation, and ventricular septal defects [20, 78]. Fkbp1a is a negative modulator of activated Notch 1. In Fkbp1a-deficient mice, activated Notch1 is upregulated. In Fkb1a-deficient mice, Fkbp1a overexpression significantly reduced activated

Notch1 [79]. Fkbp1a deficiency upregulates BMP10, a peptide growth factor in the TGF-β family involved in the cardiac compaction process. BMP10 upregulation in Fkbp1a-deficient mice indicates the importance of this gene in the trabeculation and compaction process.

In mouse embryogenesis, Bmp10 is transiently expressed in ventricular myocardium at E9.0–13.5 during myocardial maturation and in the atria E16.5–18.5 [74]. BMP10-deficient mice embryos appear unaffected at E8.5–9.0, arrest at E9.0–9.5, and die at E10.5. Immunostaining of mutant embryos exhibited thin ventricular walls and primitive trabecular ridges. The localization of BMP10 to the ventricular myocardium for the brief period at E9.0–13.5 suggests that BMP10 is crucial for continued myocardial development. Postnatal cardiac-specific BMP10 overexpression compromised cardiac growth, caused subaortic narrowing and concentric myocardial thickening [80]. Human atrial natriuretic factor (hANF) promoter can be used to overexpress BMP10 in mice. Overexpression of BMP10 demonstrated hypertrabeculation and severe heart failure [81]. Like in Fkbp1a-deficient mice and cardiac overexpression models, BMP10 is also upregulated in NUMB/NUMBL-deficient mice (myocardial double-knockout mice) [82]. NUMB and NUMBL proteins of the NUMB family are cell fate determinants for hemopoietic stem cells, muscle satellite cells, cancer stem cells, and hemangioblast progenitor cell types and maintain the fate of neural stem cells as well as regulate their differentiation [83]. Both NUMB and NUMBL inhibit Notch1 signaling and are crucial for trabeculation, cardiomyocyte proliferation and differentiation, and trabecular thickness [84]. On the other hand, inhibitory intracellular transducers such as Smad6 and Smad7 negatively regulate the BMP/ TGF-β signaling pathway [85]. Smad7 is expressed by endothelial cells in the major arteries in mice, and Smad7 deficiency causes increased Smad 1, 5, and 8 in the endocardial endothelium [81, 86]. Unsurprisingly, Smad7 mutant mice demonstrate ventricular noncompaction, thin ventricular walls, and ventricular septal defect. One of the key mediators of BMP10 signaling in ventricular myocardial development and maturation is TBX20, a member of the TBX1 subfamily of the T-box family transcription factors [87]. In murine embryos, Tbx20 can be detected in the cardiac precursor cells at E7·5 and the developing myocardium and endocardium at E8·0 [88]. Cardiac-specific overexpression of TBX20 results in severe DCM, ventricular hypertrabeculation, and abnormal muscular septum, consistent with the DCM type of LVNC [87].

Another Notch pathway element, Mib1, is associated with the LVNC phenotype of biventricular noncompaction with dilation and heart failure [20]. Genetic sequencing of 100 European patients revealed two autosomal dominant mutations—V943F and R530X. Injection of Mib1-mutated mRNA, corresponding to the V943F and R530X mutations, into zebrafish embryos disrupts Notch signaling [89]. Inactivation of Mib1 reduces Notch1 signaling and myocardial arrest. Mutant Mib1 mice produce an LVNC phenotype of immature trabeculae and noncompaction [89].

Vascular endothelial growth factor (VEGF) is produced by the myocardium and plays a role in endocardium-myocardium communication by binding to endocardial receptor Flk-1 [20]. Overexpression of VEGF-A in mice causes hypertrabeculation, abnormalities in cardiac morphology and coronary vessels, and embryonic lethality at E12.5–14.0 [90].

3.2 Animal models related to the WNT signaling pathway

The planar cell polarity (PCP) pathway is a β-catenin-independent Wnt pathway that was first studied in *Drosophila*. The PCP pathway plays a role in the epithelial orientation of hair and sensory bristles, apical-basolateral polarity, gastrulation, and neurulation [31, 91]. Disheveled-associated activator of morphogenesis I

(Daam1) is a mediator of the PCP pathway and a formin protein found in the plasma membrane and cytoplasmic vesicles. Daam1 is involved with cardiac morphogenesis and is highly expressed in murine cardiac tissue. Daam1-deficient mice have been shown to cause an LVNC phenotype with ventricular noncompaction and the thin ventricular wall. It is likely that the abnormalities seen in Daam1-deficient mice are due to cytoskeletal dysfunction since Daam1 is involved with F-actin assembly and sarcomere organization. Interestingly, the absence of Daam1 does not alter BMP10 expression nor cardiomyocyte proliferation, which offers another pathogenic model of LVNC through disruption in myofibrillogenesis, cytoskeleton organization, and cardiomyocyte polarization [31, 92].

NUMB is also a component of the adherens junction by forming complexes with β-catenin to regulate cellular adhesion *via* Wnt signaling [82]. It also interacts with integrin-β subunits to regulate cell migration and promote their endocytosis for directional cell migration. Deletion of NUMB and NUMBL from mouse hearts results in LVNC with congenital heart disease with atrioventricular septal defects, truncus arteriosus, and double outlet right ventricle *in vivo* [82]. This model shows that NUMB family proteins regulate trabecular thickness by inhibiting Notch1 signaling and control cardiac morphogenesis in a Notch1-independent manner.

3.3 Animal models related to other signaling pathways

NKX2–5, a cardiac homeobox gene, is a transcription factor that regulates heart development, working along with MEF2, HAND1, and HAND2 transcription factors to direct heart looping during early heart development [93]. Genetic variants in *NKX2–5* are associated with progressive cardiomyopathy and conduction defects in humans [94, 95]. Ventricular-muscle-cell-restricted knockout of *NKX2–5* in mice leads to progressive atrioventricular block with conduction system cell dropout and fibrosis [96]. LVNC is a prominent feature in neonatal mice, with progressive biventricular dilation and heart failure developing early. It directly activates *MEF2* to control cardiomyocyte differentiation and operates in a positive feedback loop with GATA transcription factors to regulate cardiomyocyte formation. A high-level BMP10 expression in the adult ventricular myocardium has been also observed.

The expression of early response genes in lymphocytes is regulated by NFAT transcription factors [97]. *NFATC1* mutant mouse embryos have cardiac abnormalities including myocardial developmental abnormalities, narrowing or occlusion of the ventricular outflow tract, defective septum morphogenesis, and underdevelopment of valves [98, 99]. Ventricular hypertrophy and noncompaction with hypertrabeculation were seen in 40% of mutant mice, suggesting that NFAT signaling pathways are important for hypertrabeculation and noncompaction as well as the development of valves and the septum.

Barth syndrome is caused by mutations in the X-linked *TAZ* and is associated with LVNC and abnormal cardiolipin remodeling [12, 100]. Tafazzin catalyzes cardiolipin maturation reactions at the final stage of cardiolipin biosynthesis [101]. Inducible knockdown of *TAZ* (TAZKD) in murine models using short-hairpin RNA (shRNA) exhibited an adult-onset LVNC associated with abnormal cardiolipin profiles and mitochondrial structural abnormalities [102]. Knockout of *TAZ* at the embryonic stage leads to unique developmental cardiomyopathy characterized by ventricular myocardial hypertrabeculation and noncompaction and early lethality, suggesting that mitochondrial function is important for proper myocardial development [65].

Cytoskeletal and sarcomeric proteins encoding gene mutations have been shown to account for 20% or more of LVNC [7]. Truncating variants in *TTN* are well-documented to cause skeletal myopathies and cardiomyopathies including LVNC

[48, 49, 103]. Homozygous mouse model carrying titin A178D mutation created by using CRISPR-Cas9 gene-editing displayed features of mild DCM, but not LVNC phenotype [104]. These mice showed complete loss of telethonin from the Z-disc and induction of a proteo-toxic response in the heart upon aging and adrenergic stress.

4. Conclusions

The pathogenesis of LVNC, a recently classified cardiomyopathy, remains largely unclear. With over 40 genes linked to LVNC, both genotype variation and phenotype variation are vast. Genetic testing in families and individuals with LVNC shas proved useful in identifying disease-causing variants. While the Notch signaling pathway is implicated in the pathogenesis of LVNC, there may be other pathways leading to congenital and acquired forms of LVNC. Identifying specific pathway elements is crucial for diagnosis and treatment. Modeling LVNC variants can help us to determine the genetic basis and pathogenesis of the disease.

Conflict of interest

The authors have no conflicts of interest.

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