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Genetics of Cardiac Developmental Disorders: Cardiomyocyte Proliferation and Growth and Relevance to Heart Failure

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Abstract

Cardiac developmental disorders represent the most common of human birth defects, and anomalies in cardiomyocyte proliferation drive many of these disorders. This review highlights the molecular mechanisms of prenatal cardiac growth. Trabeculation represents the initial ventricular growth phase and is necessary for embryonic survival. Later in development, the bulk of the ventricular wall derives from the compaction process, yet the arrest of this process can still be compatible with life. Cardiomyocyte proliferation and growth form the basis of both trabeculation and compaction, and mouse models indicate that cardiomyocyte interactions with the surrounding environment are critical for these proliferative processes. The human genetics of left ventricular noncompaction cardiomyopathy suggest that cardiomyocyte cell-autonomous mechanisms contribute to the compaction process. Understanding the determinants of prenatal or early postnatal cardiomyocyte proliferation and growth provides critical information that identifies risk factors for cardiovascular disease, including heart failure and its associated complications of arrhythmias and thromboembolic events.

Keywords

cardiomyopathy; left ventricular noncompaction; myocardial development; trabeculation; noncompaction

INTRODUCTION

Cardiac Development and Specification of the Myocardium

Congenital heart defects occur in approximately 1% of all births and are the most common human birth defects (1). Cardiac structural abnormalities most frequently affect the valves, outflow tracts, and atrial and ventricular septae, often in combinations that define specific congenital cardiac syndromes. Defects in ventricular compaction, the process of ventricular growth and maturation, have recently been recognized as a cause of congenital heart disease (2). Initial reports of this cardiomyopathy, referred to as left ventricular noncompaction

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cardiomyopathy (LVNC), included patients with pronounced trabecular myocardium and thin compact myocardium, systolic heart failure, ventricular arrhythmia, and embolic stroke. However, increasingly sensitive imaging modalities have identified patients with LVNC who lack systolic dysfunction or rhythm abnormalities, and the likelihood of developing cardiac complications remains unclear for this subset of patients. Several genes have been associated with LVNC. Genetic mutations that alter myocardial formation during development may manifest in later life as cardiomyopathy. The process by which the ventricular myocardium forms has been molecularly dissected through gene disruption studies in mice and other model organisms, including zebrafish, a fact that emphasizes the conservation of these circuits. In this review, we briefly outline cardiac morphogenesis and describe the pathways necessary for trabeculation and compaction of the ventricular myocardium (Figure 1).

Significant progress has been made in identifying the genetics of early cardiac development, as described in many excellent reviews (3-6). Briefly, the earliest specification of cardiac progenitors begins soon after gastrulation at embryonic day (E)7 in the mouse—and, correspondingly, at E17 in humans—and requires signals from the surrounding endoderm, including those from the fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) families, as well as the expression of mesodermal transcription factors such as *Mesp1/2* and *Tbx6*. These factors coordinate gene expression programs that drive migration of cardiac mesodermal cells to the cardiac crescent, where progenitors express the transcription factors *GATA 4, 5, and 6*, as well as *Nkx2-5* and *Tbx5*. The cardiac progenitors then migrate to the midline and form the linear heart tube, a two-layer structure that comprises an inner endocardial layer and outer myocardial layer. Soon thereafter, the linear heart tube loops to the right, creating an outer curvature from which the left and right ventricles form, and this looping positions the endocardial cushions (the future atrioventricular valves) near the conotruncus (the future ventricular outflow tracts). *Nkx2-5*, *T box*, and *GATA* family members continue to be expressed throughout this process. The transcription factor *Mef2c* determines myocyte fate, which is further specified by *Hand1* and *Hand2* for the left and right ventricles, respectively. *Islet1* contributes to outflow tract development.

After cardiac looping and the establishment of left-right asymmetry, the heart undergoes significant growth that leads to septation, resulting in four chambers and enlargement of the ventricular walls, particularly in the left ventricle. During this period, which begins at about E9.5 in mice, corresponding to E26 in humans (6), considerable morphological changes occur in the ventricular walls. Initially in this heart growth phase, cardiomyocytes proliferate from the subepicardial layer as a meshwork of contractile bundles called trabecular myocardium. Trabecular cardiomyocytes are relatively large and are juxtaposed with endothelium, theoretically to provide nutrients and gas exchange before the coronary vasculature develops. The process, known as ventricular compaction, begins at approximately E11.5 in mice, corresponding to E31 in humans, and continues until birth, during which cardiomyocytes in the outer regions of the ventricles proliferate at a significantly higher rate than cardiomyocytes in the inner regions and individual cardiomyocytes adopt a smaller, denser morphology. The coronary vasculature develops in concert with compaction, and proliferation within the trabecular myocardium decreases such that the compact myocardium comprises nearly all of the left ventricular wall

thickness at birth. Gene deletion studies in mice have identified at least 60 genes from diverse molecular pathways that contribute to trabeculation and compaction (Table 1), an observation supporting the notion that these are complex processes requiring integration of multiple signals at the proper place and time.

CELL-CELL INTERACTIONS IN TRABECULAR AND COMPACT MYOCARDIAL GROWTH

Endocardial-Myocardial Interactions

Failure to initiate trabecular myocardial growth results in embryonic lethality, presumably due to insufficient contractile function and poor perfusion throughout the embryo. Furthermore, trabeculation appears to be a prerequisite for compaction, as all mouse mutations that fail to trabeculate die by E14.5 with a hypoplastic left ventricular wall; indeed, no human cardiomyopathies due to a lack of trabeculation have been described. Genetic studies in mice suggest that trabeculation requires interactions between endocardium and myocardium, and that these interactions later in development contribute to ventricular compaction. These endocardial-myocardial pathways are driven in large part by signaling in the Neuregulin (NRG1)/ErbB, Notch, and BMP10 pathways.

Neuregulin 1 and ErbB4/ErbB2.—The Neuregulin signaling pathway was the first to be recognized as a contributor to cardiac trabeculation. NRG1, which is produced in the endocardium, contains an epidermal growth factor–like (EGF) domain that binds the cardiomyocyte receptor ErbB4, an EGF-family receptor tyrosine kinase (7, 8). Upon binding of NRG1, ErbB4 dimerizes with ErbB2, which triggers autophosphorylation and activation of MAPK and PI3K/Akt second messenger pathways to promote cardiomyocyte proliferation and differentiation (9, 10). *Nrg1* expression appears to be regulated by endothelial Notch signaling pathways (described below) (11). In the absence of *Nrg1* or *ErbB2/ErbB4*, cardiomyocytes fail to proliferate and to form the trabecular myocardium, which leads to death by E11.5 due to heart failure (12-15).

Notch1.—Notch signaling pathways comprise four transmembrane receptors (Notch1–4) and five transmembrane ligands (Jagged1, Jagged2, Delta-like1, Delta-like3, and Delta-like4) that contribute to development in virtually all organ systems, including the vasculature, heart, lung, liver, intestines, and kidney (16). In this system, the receiving cell expresses Notch, whereas the signaling cell expresses the ligand. Upon ligand binding, Notch is processed into Notch extracellular domain (NECD) and Notch intracellular domain (NICD). NICD translocates to the nucleus of the receiving cell, binds the transcriptional repressor RBPJK (also known as CSL and CBF1), and converts it into a transcriptional activator (17). By driving differential gene expression between the signal and receiver cells, Notch signaling mediates the cell fate decisions that are critical for proper vasculogenesis and organogenesis.

At E9.5 in the mouse heart, Notch1 is expressed throughout the ventricular endocardium with comparable expression levels from the trabecular crypts near the compact myocardium to the tips of the trabeculae (11). However, the Notch1 intracellular domain (NICD) protein

as well as the endocardial ligands *Delta11* and *Delta4* strongly localize to endocardial nuclei at the bases of trabeculae only, which suggests that Notch signaling is concentrated in the region of trabecular myocardium that is closest to the thin compact myocardium (11). Global deletion or early-stage endothelial deletion of *Notch1* and *RBPJK* using Tie2-Cre produces the same lethal phenotype—with a lack of trabeculation and a thick, ragged endocardium—by E11.5 (11). Expression analyses in *RBPJK*-null mice have demonstrated reduced expression of endocardial EphrinB2 and NRG1 as well as myocardial BMP10. It was shown that N1ICD activates the transcription of EphrinB2 directly, and EphrinB2 is known to drive expression of NRG1. However, BMP10 expression was observed to be independent of the EphrinB2-NRG1 pathway. Thus, endocardial Notch1 signaling mediates cardiomyocyte proliferation through the EphrinB2-NRG1 pathway and differentiation through the BMP10 pathway and therefore functions as a master regulator of trabecular growth and maturation (Figure 2).

In the developing myocardium, endocardial Notch1 signaling also contributes to the compaction process, and alterations in this pathway can disrupt compaction through at least two mechanisms. Global or endothelial-specific deletion of *Fkbp1a* leads to ventricular noncompaction and embryonic lethality at E14.5 (18, 19). In endothelial-specific *Fkbp1a*-null E12.5 embryonic hearts, N1ICD is found throughout the endocardium rather than being restricted to the trabecular bases (18). Myocardial *Bmp10* expression and cardiomyocyte proliferation are significantly increased in *Fkbp1a*-mutant hearts, which suggests that unrestricted Notch1 signaling at E12.5 may drive inappropriate proliferation that either increases trabecular myocardium or prohibits the proper formation of compact myocardium (18).

A second Notch1-mediated mechanism also contributes to ventricular compaction, as recently demonstrated in murine models and two families with LVNC (20). The Notch pathway regulator mindbomb homolog 1 (*Mib1*) is an E3 ubiquitin ligase expressed in myocardium that triggers endocytosis of myocardial Jagged1 after it binds the endocardial Notch1 ECD. In mice, cardiomyocyte-specific deletion of *Mib1* with *cTnt*-Cre interferes with dissociation of ligand-bound N1ECD from the N1ICD and thereby inhibits activated Notch1 signaling in the endocardium. In contrast to mice with early endocardial Notch1 deletion, which fail to trabeculate, animals with myocardial *Mib1* deletion demonstrate ventricular noncompaction and increased cardiomyocyte proliferation (20). Dominant *MIB1* mutations were identified in two unrelated Spanish families, each with multiple family members with LVNC (20). In one family, a premature stop codon at position 530 in the ankyrin repeat domain region was described, and the other family was found to carry a missense variant (V943F) in the coiled-coil domain. Further studies are required to determine how *Mib1* deletion, which inhibits endocardial N1ICD production, leads to the same phenotype as *Fkbp1a* deletion, which increases endocardial N1ICD production. However, in accordance with these results, loss of TACE (TNF- α ADAM metalloprotease-converting enzyme), a protease that cleaves ligand-bound NECD from the NICD, also promotes increased cardiomyocyte proliferation and ventricular noncompaction (21). Finally, induction of endocardial *Notch1* deletion between E9 and E12 phenocopies the loss of myocardial *Mib1* deletion (20). Together, these findings suggest that early endocardial Notch1 signaling drives cardiomyocyte proliferation and development of

trabeculae, whereas Notch1 signaling later in development slows trabecular proliferation and contributes to the transition from the trabecular morphology to the compact morphology (Figures 2 and 3).

Notch2, Numb, and Numblike.—In contrast to Notch1, Notch2 protein is found in myocardium (22). *Notch2* mRNA is expressed in both trabecular and compact myocardium, whereas Notch2 intracellular domain (N2ICD) is restricted to trabecular myocardium, suggesting that the Notch2 ligands Delta-like1, Jagged1, and Jagged2 may be specific to endocardium and/or cardiomyocytes in the trabecular region. Animals expressing a hypomorphic form of *Notch2* die from multiple developmental abnormalities, including kidney glomerular defects and the lack of cardiac trabeculation (23), whereas ectopic expression of N2ICD in compact myocardium causes increased proliferation throughout the ventricular walls and leads to ventricular noncompaction, ventricular septal defect (VSD), and lethality between E12 and E15 (22). N2ICD degradation by Numb and Numblike, which are partially redundant proteins expressed throughout the myocardium, also contributes to cardiomyocyte proliferation and ventricular morphology. Cardiac-specific *Numb* excision in *Numblike* null mice phenocopies ectopic N2ICD expression, including increased cardiomyocyte proliferation throughout the ventricular wall (22). Taken together, studies of Notch signaling during heart development illustrate a complex system in which expression of the components (ligands and receptors) at the proper place (endocardial versus myocardial tissue) and proper time (early versus mid-to-late cardiac development) must be tightly coordinated to drive the cell fate decisions that allow normal cardiac trabeculation and compaction.

BMP10, Myocardin, and Tbx20.—BMP10, a member of the TGF β superfamily, is expressed exclusively in trabecular myocytes from E9 to E13.5. *Bmp10* expression is driven by endocardial Notch1 signaling, as described above, with direct transcriptional activation in cardiomyocytes by Myocardin, although the relationship between Myocardin and Notch signaling is not well described (11, 24). BMP10 is also considered a marker of trabecular myocardium that shows an expanded expression pattern in many mice exhibiting hypertrabeculation/noncompaction (25, 26). Mice lacking BMP10 die at E10–10.5 due to a hypoplastic heart with lack of trabeculation (27). BMP10 signaling appears to mediate trabecular differentiation as well as proliferation—the latter as demonstrated by hypertrabeculation in a *Bmp10*-overexpressing mouse—via a Smad1-Tbx20-mediated pathway (26, 28).

Tbx20 mRNA shows a complex expression pattern, with transcripts initially detected in early cardiac progenitors of the cardiac crescent and later found in endocardium and myocardium, including adult myocardium (29, 30). Mutations in human *TBX20* are associated with adult congenital defects, including atrial septal defects, mitral valve abnormalities, and dilated cardiomyopathy (DCM) (31). *Tbx20*-null mice fail to trabeculate and die by E10.5, which suggests its major action in cardiac development occurs during mid-development rather than upon initial differentiation of cardiac progenitors; indeed, the proliferation pathway mediated by Tbx20 involves derepression of NMyc1, which promotes cell division and cardiac growth (32).

Additional endocardial-myocardial trabeculation and compaction pathways.—

Neurofibromin 1 inhibits Ras signaling via its GTPase-activating function and contributes to neural and musculoskeletal development; the absence of human *NFI* leads to neurofibromatosis type 1, an autosomal dominant disease of wide phenotypic variability that includes neurofibromas with the potential for malignant transformation (33). Interestingly, absence of *Nfi* in mice leads to embryonic lethality due to cardiac abnormalities, including atrioventricular canal (AVC) defects and ventricular noncompaction, the latter seen only in endothelial-specific *Nfi* deletion (33, 34). Loss of endothelial and endocardial *Nfi* does not alter endothelial NRG or myocardial ErbB2/ErbB4, but endothelial Nfatc1 (nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1) localizes primarily to the nucleus rather than the cytoplasm, as seen in wild-type cells (33). Humans with neurofibromatosis type 1 due to *NFI* mutations have few cardiac defects, and this discrepancy between human and mouse phenotypes may reflect compensation by the remaining wild-type allele or related genes in cardiac development.

Angiopoietin 1 (*Angpt1*) is the major ligand for the receptor tyrosine kinase Tek, which is expressed primarily on endothelial cells. Global deletion of *Angpt1* or Tek, as well as cardiac-specific deletion of *Angpt1*, lead to lethality between E9.5 and E12.5 owing to vascular defects and lack of cardiac trabeculation (35-37).

Nonendothelial cells produce vascular endothelial growth factor (VEGF) to promote sprouting angiogenesis at the hypoxic front of hypoperfused tissue (38). In the developing heart, trabeculation and compaction require a tight balance of myocardial VEGF. Specifically, *Vegf* haploinsufficiency leads to lethality at E9.5 due to impaired cardiovascular development, including an absence of trabeculation (39, 40); by contrast, stabilization of the *Vegf* transcript equivalent to a threefold increase in VEGF protein leads to abnormal coronary artery maturation and ventricular noncompaction with death by E14.5 (41). Similarly, deletion of the homeobox gene *Hhex*, which either directly or indirectly represses *Vegf* transcription, leads to upregulation of VEGF protein levels and ventricular noncompaction (42). These findings demonstrate that in addition to the endocardial-to-myocardial signaling systems described above, myocardial-to-endocardial pathways contribute to trabecular and compact growth. Additional studies are required to determine whether and how myocardial VEGF promotes endocardial proliferation as a prerequisite for trabeculation—as has been suggested for endocardial-to-mesenchymal transformation in the AVC (43)—and whether excessive endocardial proliferation contributes to hypertrabeculation and/or noncompaction.

Epicardial-Myocardial Interactions

The epicardium proliferates from the proepicardial organ at the venous pole of the heart starting at E9.5 in mice, and the entire myocardial surface is covered by E11 (44). In parallel fashion, ventricular compaction begins as early as E10.5, and the compact myocardium is recognizably thicker by ~E11.5–12.5. Epicardial-myocardial interactions appear to be a major driver of the compaction process. Indeed, mechanical disruption of the nascent epicardium in chick embryos prevents normal compaction, as does genetic disruption of vascular cell adhesion molecule-1, which is required for adhesion of the proliferating

epicardium to the underlying myocardium (45, 46). The epicardial-myocardial pathways that support compaction include retinoic acid (RA), erythropoietin (Epo), insulin-like growth factor, and FGF signaling.

Retinoid X receptor α .—Retinoid X receptor α (RXR α) is a member of the RXR family of nuclear receptors that binds retinoids and appears to function primarily as a heterodimeric partner of retinoic acid receptor (RAR) family members (47). RXR α -deficient mice show mid-to-late gestational lethality beginning at E12.5, with living embryos detectable at E16.5 but none left by E18.5. Cardiac defects in the mutants include noncompaction and VSD, which are presumed to be the cause of embryonic lethality (48, 49). By contrast, developmental cardiac defects have not been described in *RAR α* -, *RAR β* -, or *RAR γ* -deleted mice, although ventricular noncompaction was noted in a portion of *RAR α* ;*RAR γ* double-null mice (50). Mitotic index analyses revealed decreased proliferation in the subepithelial cardiomyocytes of both *RXR α* -null and *RAR α* -null animals. In wild-type E14.5 embryos, highly organized sarcomeres and myofibrils are a common feature in trabecular myocardium but are rare in compact myocardium, where sarcomere assembly is at an early stage. The subepithelial cardiomyocytes from both *RXR α* -null and *RAR α* -null mice contain well-developed sarcomeres at E14.5 and as early as E8.5 (48, 51). The observation of highly structured sarcomeres in the subepithelial myocardium with a decreased mitotic index in the absence of *RXR α* suggests that RA signaling contributes to ventricular compaction by delaying differentiation and promoting proliferation.

Although *RXR α* mutants express a clear noncompaction phenotype, the source of RA, site of receptor activity, and precise role of the epicardium in this signaling pathway remain somewhat enigmatic. Embryonic chick heart explant studies demonstrated that RA does not directly stimulate cardiomyocyte growth in the absence of epicardium but rather promotes myocardial proliferation in an epicardial-dependent fashion, which implies production of a growth factor by the epicardium after activation by RA (52). Retinaldehyde dehydrogenase 2 catalyzes production of RA during early embryonic development, but its expression appears to be limited to the splanchnic mesoderm at E9.5–10.5 and restricted from the epicardium until E12.5 (after the initiation of compaction), which suggests a nonepicardial source of RA during early compaction (53). Conditional deletion of *RXR α* using *Gata5*-Cre, which excises in epicardium, indicates an epicardial cell-autonomous role for RXR α in compaction. A caveat of this approach is that *Gata5*-Cre is also active in hepatic capsule epithelium, which lies adjacent to the heart before the development of the diaphragm between E10.5 and E15.5. Therefore, the hepatic capsule may contribute as a source of RXR α activity to promote ventricular compaction (54, 55). Additional studies suggest that the hepatic epithelium is a major site of RA and RXR α signaling during early compaction and that this pathway triggers the release of hepatic Epo, which diffuses into the nascent epicardium to stimulate the release of additional mitogens (53) (Figure 3).

Erythropoietin and erythropoietin receptor.—Epo is a glycoprotein that stimulates erythropoiesis, and the embryos of mice lacking either *Epo* or its receptor *EpoR* die at ~E13.5 from failed hematopoiesis, liver abnormalities, and ventricular noncompaction (56). The Epo receptor is expressed in the heart by E10.5 and localizes to epicardium

and endocardium, but not myocardium, at E12.5 (56). In a signaling pathway parallel to RA, cultured embryonic chick explants respond to Epo in an epicardial-dependent fashion to stimulate cardiomyocyte proliferation, potentially via a growth factor produced by the epicardium (Figure 3) (52). Taken together, these studies suggest a model in which hepatic epithelial RA/RXR α signaling stimulates the production and release of Epo, which diffuses into the newly formed epicardium and activates epicardial production of growth factors that trigger myocardial proliferation and growth. Further studies—such as conditional excision of RXR α and EpoR using epicardial Cre drivers that do not excise in the hepatic epithelium—are needed to confirm aspects of this model.

Insulin-like growth factor 2 and fibroblast growth factors 9, 16, and 20.—

Insulin-like growth factor 2 (IGF2) is expressed in epicardium and endocardium at E11.5–14.5, whereas its primary receptor, IGFR1, is expressed in myocardium at E12.5–14.5; the insulin receptor also binds IGF2 and is expressed at low levels in myocardium (57). Mice lacking functional IGF2 and cardiac-specific-*Igfr2;Insr* double mutants demonstrate decreased proliferation in the compact zone at E11.5 and noncompaction at E12.5. However, the proliferative rate recovers by E14.5, and compaction is largely restored by birth, which suggests that diffusion of the epicardially derived IGF trophic signal becomes rate limiting and that additional trophic signals after E14.5 are delivered by the nascent coronary vasculature (57).

Likewise, FGFs 9, 16, and 20 appear to contribute to the early compaction process. *Fgf9* mRNA is expressed in right ventricular (RV) epicardium and endocardium at E10.5, but it is restricted to endocardium by E12.5, with highest expression at the RV apex near the septum. *Fgf9*-null mice demonstrate impaired proliferation and abnormal compaction at E12.5, but only at the apicolateral RV and septum; this type of mutant mouse is born with a normally compacted heart (58). *Fgf16* transcripts are expressed in basal LV epicardium and endocardium at E10.5 and expand to basal LV, RV, and septal endocardium by E12.5. Disruption of *Fgf16* exon 1 led to lack of trabeculation and embryonic lethality by E14.5, whereas disruption of *Fgf16* exon 2 decreased cardiomyocyte proliferation at E14.5 but did not produce embryonic lethality (58-60). *Fgf20* mRNA is expressed in the basal and apical LV epicardium and endocardium at E10.5 and is largely restricted to the RV endocardium by E12.5, and *Fgf20*-null mice show no cardiac defects (58, 61). Cardiomyocytes express the redundant FGF receptors Fgfr1 and Fgfr2, and *Fgfr1;Fgfr2* double-knockout mice have small hearts with decreased proliferation throughout the apex and base of both LV and RV at E13.5, but compaction recovers later in development (58). Taken together, these data indicate that several FGFs in the developing heart have both overlapping and unique expression patterns, but with the exception of *Fgf16*, none appear to be absolutely necessary for compaction. It is possible that FGF9/FGF16/FGF20 and FGFR1/FGFR2 signaling pathways contribute to early compaction at E12.5, perhaps initiating compaction or maintaining myocytes in a proliferative or nondifferentiated state, but that other signaling networks can compensate for their loss.

Extracellular Matrix-Myocardial Interactions

The extracellular matrix (ECM) plays a significant role in both trabeculation and compaction. Shortly after heart tube formation, a relatively thick layer of ECM called the cardiac jelly lies between the endocardium and myocardium and is necessary for proper initiation and progression of trabeculation. In the compact layer, cardiac fibroblasts proliferate in concert with cardiomyocytes and deposit ECM, which appears to be required to support compaction.

Versican, hyaluronic acid, ADAMTS1, and Fibulin-1.—The chondroitin sulfate proteoglycan versican and the mucopolysaccharide hyaluronic acid (HA) are ECM factors that make up a significant portion of the cardiac jelly. These components are produced by the endocardium and myocardium and are well described for their contribution to atrioventricular cushion remodeling during valve morphogenesis (62-65). In addition to their atrioventricular cushion defects, mice lacking *Has-1*, the enzyme that produces HA, and *Vcan*, encoding versican, fail to trabeculate (63, 65, 66). These observations demonstrate the necessity of cardiac jelly for early heart development and proliferation of the trabecular myocardium, possibly via modulation of the diffusion of myocardial VEGF and endocardial Nrg in the cardiac jelly (67). Alternatively, HA and versican may directly or indirectly stimulate cardiomyocytes. HA is known to contribute to Ras activation during endothelial cell invasion in cultured atrioventricular explants (65), and such a signaling pathway may be at play within cardiomyocytes during trabeculation.

As trabeculation proceeds, the cardiac jelly progressively disappears until the endocardium is in contact with the myocardium, and this ECM breakdown process plays a critically important role in cardiac development. ADAMTS1 (a disintegrin and metalloproteinase with thrombospondin motif) and its cofactor Fibulin-1 cleave versican beginning around E12.5, after which the cardiac jelly is degraded and trabecular proliferation eventually declines; inappropriately early ADAMTS1 activity leads to premature breakdown of cardiac jelly and lack of trabecular cardiomyocyte proliferation (67). Conversely, attenuation of ADAMTS1 activity in mice lacking Fibulin-1 leads to trabecular hyperproliferation and ventricular noncompaction (66).

β 1 integrin.—The integrins are transmembrane proteins encoded by α and β subtypes that form functional heterodimers, bind components of the ECM, and transmit intracellular signals upon ligand binding. Ligand specificity is determined by the α and β subtypes within the heterodimer; the β 1 subunit can pair with one of multiple α subunits to form heterodimers that bind collagen, laminin, fibronectin, and vitronectin (68). The developing heart is enriched in ECM components—including fibronectin and collagen—produced by embryonic cardiac fibroblasts, and these fibroblasts proliferate in parallel with cardiomyocytes in the compact region (69). Cardiomyocyte-specific deletion of β 1 integrin, which contributes to mechanical anchoring of cardiomyocytes to the ECM via costameres in adult cardiomyocytes, has significant effects on embryonic cardiac growth. Lethality occurs in half of the mutant mice between E16.5 and P7, with significantly smaller heart size from E14.5 onward and ventricular noncompaction in all mutants (69). The rate of myocardial proliferation significantly decreases at the midpoint of compaction (E16.5) in

the absence of cardiomyocyte $\beta 1$ integrin. Taken together, these results point toward a fibroblast-ECM-cardiomyocyte signaling pathway that contributes to normal proliferation, compaction, and overall cardiac growth during development (Figure 3).

Focal adhesion kinase and focal adhesion kinase–related nonkinase.—Focal adhesion kinase (FAK) is a nonreceptor tyrosine kinase encoded by the *Ptk2* gene that relays signals from activated integrins, whereas focal adhesion kinase–related nonkinase (FRNK), the C-terminal portion of FAK, is transcribed from an intronic promoter within *Ptk2* perinatally and acts to inhibit FAK signaling (70). Cardiomyocyte-specific deletion of *Ptk2* using the *Mlc2a*-Cre driver results in decreased cardiomyocyte proliferation at E14.5, VSD, and ventricular noncompaction (71). Likewise, overexpression of FRNK early in embryonic heart development leads to decreased cardiomyocyte proliferation and ventricular noncompaction (70). These observations are in keeping with a role for $\beta 1$ integrin/FAK signaling in cardiomyocyte proliferation and compaction in the developing heart.

Other Cardiomyocyte Factors

As described above, cardiac growth and development require interactions between cardiomyocytes and the surrounding environment of endocardium, epicardium, and ECM. In addition, some gene products contribute to proliferation and growth in a cell-autonomous manner, in parallel with or independently of the interactions involving surrounding cells and matrix. Other genes play a role in cardiomyocyte proliferation, but whether or how their protein products facilitate interactions with surrounding cells remains unclear.

Yap1.—During organogenesis, the Hippo signaling pathway mediates organ size via phosphorylation of Yap1 (Yes-associated protein 1), which prevents nuclear localization and activation of TEAD-family transcription factors. Growth factor stimulation inhibits the Hippo pathway and permits Yap1- and TEAD-mediated transcription of cell cycle genes, leading to proliferation and organ growth (72). In the heart, Yap1 is highly expressed in cardiomyocytes at E17.5 and in the early postnatal period, but it is significantly downregulated between 8 and 12 weeks of age (73). *Yap1*-null mice do not survive beyond E8.5, and cardiomyocyte-specific deletion of *Yap1* is embryonically lethal by E12.5, with lack of trabeculation noted in hearts from mutant animals (73). In contrast, cardiomyocyte-specific overexpression of *Yap1* is lethal by E16.5; hearts from these animals display a moderate increase in compact myocardium and significantly increased trabecular myocardium, which together cause left ventricular cavity obliteration (73). Cardiomyocyte proliferation is enhanced in both the compact and trabecular myocardium of *Yap1*-overexpressing mutants, likely via increased expression of cell cycle genes, but the magnitude of increase is markedly higher in the trabecular myocardium (73). These observations suggest that Yap1 activity is downregulated in trabecular myocardium and either sustained or upregulated in compact myocardium during the process of ventricular compaction.

Nkx2-5.—*Nkx2-5* is required early in development for cardiac looping, and this homeobox gene continues to function in conduction system differentiation as well as trabecular cardiomyocyte maturation. Specifically, excision of *Nkx2-5* within ventricular

cardiomyocytes leads to hypoplasia of the atrioventricular node and hyperproliferation specifically within the trabecular myocardium and not the compact myocardium (26). The compact layer remains relatively normal in these mutant mice, and hypertrabeculation does not appear to impact survival. *Bmp10* expression remains high in the expanded trabecular myocardium, even in adult cardiomyocyte-*Nkx2-5*-mutant mice (26). Over 40 mutations in human *NKX2-5* have been associated with congenital heart disease, many encompassing atrial septation defects and associated cardiac conduction disease, but noncompaction has not been described (26, 74-76). These results suggest that the ventricular cardiomyocyte-specific activity of *Nkx2-5* drives trabecular myocardium toward differentiation and away from proliferation (26, 74-76).

Friend of GATA 2.—Members of the friend of GATA (FOG) transcription factor family physically interact with GATA factors to repress gene transcription activity. FOG-2 is highly expressed in the heart (77). In the absence of *Zfpm2*, the gene that encodes FOG-2, the epicardium expresses low levels of intercellular adhesion molecule (ICAM) and VEGFR2, and the coronary vasculature fails to develop despite the presence of the normal epicardium; furthermore, hearts display ventricular noncompaction (77). Cardiomyocyte-specific overexpression of *Zfpm2* in *Zfpm2*-null mice rescues coronary vascularization as well as compaction (77). Early deletion of FOG-2 using an *NKX2.5*-driven Cre recapitulates the complex congenital defects seen in global FOG-2 deletion (77-79). Cardiomyocyte-restricted deletion of FOG-2 after cardiac looping reproduces the phenotype of thinned ventricular walls and deficient vasculature, an observation that links defective cardiomyocyte proliferation to deficient coronary artery development. FOG-2 interacts with the nucleosome remodeling and deacetylase (NuRD) complex, and a point mutation that disrupts the FOG-2-NuRD complex interaction induces a proliferation defect similar to that induced by cardiac-restricted FOG-2 deletion (80).

Other genes that regulate cardiomyocyte trabeculation and compaction.—The 14-3-3 protein family in mammals comprises seven members that bind phosphoserine or phosphothreonine proteins and thereby modify multiple cell processes, including cell cycle progression (81). *14-3-3e* is highly expressed in the brain and heart during development, and mice that lack *14-3-3e* die in the perinatal period (82). Hearts from *14-3-3e*-null mice are noncompacted at E18.5, and proliferation is significantly decreased in the compact myocardium owing in part to upregulation of the cell cycle progression-inhibiting protein p27^{Kip1} (82). Although *14-3-3e* is expressed in cardiomyocytes, cell autonomy still needs to be confirmed through a cardiomyocyte-specific *14-3-3e*- $\kappa\alpha$ model.

Foxp1 belongs to the Fox family of DNA-binding proteins with a forkhead or winged-helix domain (83). *Foxp1* is expressed in endocardium and myocardium, and animals with global deletion of *Foxp1* die at E14.5 with atrioventricular cushion defects and ventricular noncompaction due to increased proliferation in trabecular myocardium as well as decreased proliferation in compact myocardium (83). As with *14-3-3e*, endocardial versus myocardial specificity for *Foxp1* activity remains to be determined.

Actin-polymerizing proteins modify the sarcomere thin filaments as well as the nonsarcomere cytoskeletal components in cardiomyocytes; one such protein, Dishevelled-

associated activator of morphogenesis 1 (*Daam1*), is a formin protein localized to the cell membrane in endocardium, epicardium, and myocardium that mediates F-actin elongation at the barbed end (84). Mice homozygous for a *Daam1* genetrapped mutation show a wide range of cardiac structural abnormalities, including outflow tract defects, VSD, and possible ventricular noncompaction (85). Interestingly, *Daam1*-mutant mice display no differences from controls in cardiac-specific gene expression (*Nkx2-5*, *Tbx20*, *BMP*, *Mlc2a*, and *Mlc2v*) at E10.5 and no differences in proliferation or apoptosis at E12.5, but these measures may change later in development (85). Sarcomere and intercalated disk abnormalities have been seen via transmission electron microscopy in *Daam1*-deficient cardiomyocytes, and these cells display poor adherence to collagen, but more studies are required to determine the mechanism underlying these cardiac defects (85).

Calreticulin is a calcium-binding protein localized to the endoplasmic reticulum (86). *crt*-null mice die during embryonic development, between E15.5 and E18.5, with ventricular noncompaction that is possibly due to decreased nuclear translocation of *Nfatc4*, a calcineurin-dependent factor (86).

Adrenomedullin is a potent vasodilator that is expressed in the adrenal medulla as well as in cardiomyocytes, vascular smooth muscle, and aortic endothelium during mouse development. The calcitonin receptor-like receptor (*Calcr1*) binds adrenomedullin. Genetic deletion of either *Adm* or *Calcr1* phenocopies deletion of the other, with embryonic lethality at E14.5, noncompaction, and hydrops fetalis that is greater in magnitude than would be expected from heart failure alone (87, 88). Indeed, subsequent studies revealed that the major defect in these mice lies in lymphatic vasculature development. Whether the cardiac effects are primary or secondary to abnormal lymphatic formation remains to be determined (89).

HUMAN GENETICS OF LEFT VENTRICULAR NONCOMPACTION

Human cardiomyopathy is classified as dilated, hypertrophic, arrhythmogenic, or noncompaction (2). There is an increasing appreciation for the clinical overlap of these forms of cardiomyopathy, and this overlap is partially explained by shared genetic predisposition. Noncompaction cardiomyopathy is seen in both children and adults as an increase in the ratio of trabeculated to compacted myocardium, typically in multiple segments of the left ventricle (Figure 1). LVNC may be seen in the setting of normal or impaired systolic function. The clinical consequences of LVNC range from no symptoms to heart failure, arrhythmias, and thromboembolic events. With the growing use of cardiac magnetic resonance imaging and its ability to delineate detailed features of the myocardium better than other imaging modalities like echocardiography, hypertrabeculated segments can be seen in nearly all hearts, and the ratio of trabeculated to compact myocardium changes with age (90). In a study of normal adults without cardiac disease, 18% of subjects were found to have 1 of the traditional criteria for LVNC (91). Notably, those with hypertrabeculation had significantly higher serum pro-BNP levels compared to the general population, consistent with functional impairment, albeit mild, of the heart. LVNC is known to be heritable and is most commonly autosomal dominant, although X-linked recessive and autosomal recessive forms also make important contributions to inheritance (92). Family

members of those with LVNC have a higher incidence of LVNC, and a higher incidence of premature death, than what is seen in the general population (93).

Genetic testing for inherited cardiomyopathy now relies on the use of gene panels in which 10–100 genes are sequenced simultaneously (94). As whole-exome and whole-genome analyses become more routine, it is likely that gene panels will be replaced by exome/genome analysis (95, 96). Many genes implicated in the development of LVNC are also implicated in the genesis of DCM and hypertrophic cardiomyopathy (HCM), and this observation has promoted the use of large gene panels covering sarcomere, cytoskeletal, metabolic, mitochondrial, and other genes for human genetic testing.

Sarcomere Gene Mutations that Correlate with Human LVNC

The sarcomere is made up of thin and thick filaments, and mutations in sarcomeric genes are among the most frequent causes of inherited cardiomyopathies. Although these mutations are often considered in the context of HCM and DCM, mutations in genes encoding sarcomere proteins have also been linked to LVNC.

β myosin heavy chain.—An individual myosin subunit contains two heavy chains and two each of the regulatory and essential light chains. In the human adult myocardium, β myosin heavy chain contributes to the majority of the thick filament protein component of the sarcomere. Mutations in *MYH7*, the gene encoding β myosin heavy chain, have been identified in families with LVNC (97-105). *MYH7* mutations are more commonly associated with HCM and DCM, where the majority of mutations are missense variants that fall into the subfragment 1 “head” or motor domain region of myosin. Most subfragment 1 mutations map within the functionally significant portions of the protein, including the regions that regulate actin binding and ATPase activity, although more rare, missense mutations in the rod region can lead to cardiomyopathy. The first report of LVNC-associated *MYH7* mutations identified splice site variants within the head domain, which in principle could act through either a dominant negative or a haploinsufficient mechanism (99). However, since that time, additional missense variants, including those that extend into the rod region, have been described in LVNC. Missense variants within the head region may act by reducing force production by the sarcomere. Missense variants that perturb thick filament formation may reduce force transmission within the sarcomere.

In general, an attractive hypothesis has been that HCM-associated variants in *MYH7* are activating mutations linked to increased contractility and the hypercontractile state seen in HCM, whereas DCM- or LVNC-associated variants are linked with reduced function. However, this may be an oversimplification, and the effect of any given variant on the myocardium may actually produce a range of phenotypic outcomes throughout the lifetime of the myocardium, depending on heart morphology and the presence of alternative sarcomere proteins generated through expression or splicing events. For example, the *MYH7* mutation Arg234His has been associated with both LVNC and HCM, which supports the notion that sarcomere gene mutations manifest a clinical spectrum of phenotypes (99, 106). How the same mutation can cause different cardiomyopathies requires further study, but this clinical spectrum likely involves additional polymorphisms and epigenetic and/or

environmental interactions that sensitize the sarcomere and/or cardiomyocyte to a particular remodeling pathway.

Myosin-binding protein C3.—During systolic cardiac contraction, proper force generation depends on the rate and extent of crossbridge cycling between actin and myosin filaments, and myosin-binding protein C3 (MYBPC3) constrains this cycling to appropriately limit the systolic phase (107). As with *MYH7*, many mutations in *MYBPC3* have been associated with HCM. *MYBPC3* mutations make up the second-most common cause of LVNC after *MYH7* mutations (100, 108-110). Notably, compared with *MYH7* mutations, a greater proportion of HCM-associated *MYBPC3* mutations are frameshifting or truncating. Early-onset cardiomyopathy associated with features of hypertrabeculation and noncompaction can be seen with compound heterozygous mutations in *MYBPC3* (100, 110, 111). Mouse models support the idea that *MYBPC3* causes disease, as *Mybpc3*-null animals lack M lines; a patient with a compound heterozygous *MYBPC3* mutation was also found to have disrupted and in some areas absent M lines (100, 112). The observation that mutations in both *MYH7* and *MYBPC3* lead to noncompaction supports the idea that normal sarcomere function is required for the transition from trabeculated to compacted myocardium in humans, as it is in mice.

Other sarcomere genes in LVNC.—Mutations within the thin filament genes *ACTC1*, *TNNT2*, and *TPM1*, as well as the Z-disk gene *ACTN2*, have all been associated with LVNC (99, 108, 113, 114, 115). Of note, the cardiac α -actin mutation Glu202Lys and *TNNT2* mutation Arg131Trp have been described in HCM and DCM as well (99, 106, 113, 116). *CasZ1* is a recently identified zinc finger protein that falls within the human congenital heart disease-associated deletion region of chromosome 1p36, and the gene product either directly or indirectly stimulates transcription of many sarcomere components—including *TNNI2*, *TNNT1*, and *ACTA1* (117).

LDB3 protein, also known as Cypher/ZASP, contributes to the sarcomere Z disk as one of several proteins that mediate physical interactions between α -actinin and actin (118). Mutations within LDB3 are associated with DCM, arrhythmia, and LVNC (119). Among mice, the global-*Lbd3*-null animals die perinatally owing to muscle weakness and heart failure. In the diaphragm muscle, Z lines are seen via transmission electron microscopy during embryonic development, but the electron-dense Z-line region disappears after birth; in the heart, Z lines are abnormally wide during development and progressively widen until death (120). These observations suggest that *Lbd3* is not required for Z-line or sarcomere assembly, but rather is necessary for Z-line and/or sarcomere maintenance during the mechanical process of contraction.

Alpha-dystrobrevin, encoded by *DTNA*, is a member of the dystrophin complex, which associates with costameres to link sarcomeres at the myocyte periphery with the cardiomyocyte membrane and ECM. Mutations in *DTNA* were identified early in the study of genetic causes of LVNC (121). Whereas mutations in other components of the dystrophin complex are known to cause skeletal and cardiac myopathy, the mechanism of α -dystrobrevin-mediated LVNC remains unknown.

X-Linked LVNC

Tafazzin (*TAZ*), also known as G4.5, was one of the first genes connected with human LVNC (121, 122). This X-linked gene is associated with Barth syndrome, is highly expressed in cardiac and skeletal muscle, and encodes a phospholipid-lysophospholipid transacylase that catalyzes the final step in cardiolipin synthesis; the unbalanced cardiolipin profile in *Taz* mutants adversely affects mitochondrial function (123). *Taz* knockdown in mice leads to ventricular noncompaction due to decreased cardiomyocyte proliferation at E13.5. These observations suggest a link between mitochondrial bioenergetics and cardiomyocyte proliferation that deserves further investigation.

Arrhythmias and LVNC

The relationship between arrhythmia risk and LVNC is not well understood and requires further study. The risk of ventricular arrhythmias is increased in LVNC, and in first-degree relatives the risk of premature death is heightened (93). This overlap between arrhythmias and LVNC may reflect the concomitant development of the cardiac conduction system and the maturing myocardium in the same developmental windows, such that perturbation of the myocardium may have a secondary adverse effect on the developing conduction system. However, in some cases, the responsible gene may be leading to independent pathologies in the cardiac conduction system and the myocardium. Recently, the *HCN4* gene, which encodes the potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4, was linked to LVNC in multiple families. *HCN4* is known to be expressed highly in the sinus node and to contribute to pacemaker activity. Mutations in *HCN4* have now been associated with LVNC and concomitant bradycardia (124, 125). Whether noncompaction is a primary effect of *HCN4* mutation or a secondary effect of decreased cardiac output/force generation from bradycardia requires further investigation.

The nuclear envelope proteins lamin A and lamin C are produced from a single gene, *LMNA*. Lamins A and C are found in most postmitotic cell types, including heart, muscle, and brain. Mutations in *LMNA* lead to a constellation of phenotypes. Among *LMNA*-associated diseases, cardiomyopathy—with or without associated skeletal muscle disease—is a frequent consequence (126, 127). The mechanism by which *LMNA* mutations cause disease is not fully known and likely employs multiple pathways, including the stability of chromatin structure (128). An early study identified a link between LVNC and *LMNA* mutations (129). In *LMNA*-associated cardiomyopathy, cardiac conduction system disease is common and can include atrial- and ventricular-associated abnormal rhythms (130).

Genetic Assessment of LVNC: Lessons from Mouse Models

In mouse models, many of the genes linked to cardiomyocyte proliferation—and therefore noncompaction-like phenotypes—yield broad effects necessitating tissue-specific genetic analysis. Cell type-specific deletion of many genes studied in mice was required to dissect the genes' roles in proliferation, trabeculation, and compaction. Because the products of many of these genes perform functions in other cells, the absence of the genes would be expected to cause embryonic lethality. By contrast, many of the mutations linked to human LVNC alter the resultant protein rather than eliminating the protein's function. Many of the genes implicated through genetic deletion in mice should likely be included in gene panel

testing for cardiomyopathy. Mutations in two genes implicated by mouse studies, *Mib1* and *Nkx2-5*, have been associated with human LVNC as disease-associated variants, and *Nkx2-5* is a cardiomyocyte-specific gene (20, 76). In fact, the majority of human LVNC cases with an established genetic diagnosis are due to mutations in cardiomyocyte-specific genes, particularly those that encode components of the sarcomere, the basic myocyte contractile unit (108).

Large databases of human genetic variation now emerging in the form of the 1000 Genomes Project, the NHLBI Exome Variant server, and the Exome Aggregation Consortium have yielded information that supports a higher-than-expected prevalence of pathogenic mutations, including those that are anticipated to cause cardiomyopathy and LVNC (131, 132) (<http://exac.broadinstitute.org>). The observation that hypertrabeculation and LVNC can be found in seemingly normal individuals is consistent with the genetic landscape of the overall population.

EPIGENETICS OF LEFT VENTRICULAR NONCOMPACTION

Epigenetics refers to features of DNA other than the sequence itself that affect gene expression. These features generally relate to chromatin-level regulation—particularly DNA methylation, ATP-dependent chromatin remodeling that changes nucleosome structure, and covalent histone modifications that alter DNA-histone interactions (reviewed in 133). Chromatin-level regulation contributes to gene expression in cardiac development, including the expression of genes involved in cardiomyocyte proliferation, trabeculation, and compaction.

Jarid2

Jarid2 (Jumonji, AT-rich interactive domain 2) is a nuclear factor found in both endocardium and myocardium; whereas most members of the Jumonji family function as histone demethylases, Jarid2 appears to promote histone H3-K9 methylation via recruitment of G9a and GLP methyl-transferases (134). Global *Jarid2* deletion leads to ventricular noncompaction, VSD, double outlet right ventricle, and perinatal lethality (135). Reporter gene studies demonstrate that *Jarid2* expression begins in the trabecular cardiomyocytes at E10.5, as the mitotic index in this region declines, and that expression expands to the compact myocardium at low levels beginning at E12.5 and then dramatically increases in this region at postnatal day 7. These findings suggest that Jarid2 promotes cardiomyocyte cell cycle exit, an idea supported by the observation of increased proliferation in *Jarid2*-null hearts (136). Indeed, Jarid2 appears to act as a transcriptional repressor of the cell cycle progression gene *cyclin D1* via both cardiomyocyte cell-autonomous and non-cell-autonomous mechanisms: Jarid2 directly binds the *cyclin D1* promoter to repress expression in cardiomyocytes, and it also binds an intronic *Notch1* repressor element in endocardium to repress Nrg and ErbB2/ErbB4 signaling, as previously described (136, 137).

Brg1

Brg1 is a member of the SWI/SNF (BAF) chromatin-remodeling family that is expressed in endocardium and myocardium and mediates associations between DNA and histones/

nucleosomes as well as interacts with transcription factors to affect promoter and repressor functions (reviewed in 133, 138). In endocardium, Brg1 acts as a transcriptional repressor of ADAMTS1, a matrix metalloprotease that cleaves versican in trabecular ECM, as described above; the absence of endocardial Brg1 leads to lack of trabeculation due to inappropriately early derepression of *Adams1* expression with resultant premature degradation of the cardiac jelly (64).

CONCLUSIONS

The orchestration of myocardial development is under tight control and heavily influenced by the concomitant development of vascular supply of the myocardium. In early development of the myocardium, the trabeculated myocardium is thought to derive nutrients from blood within the pulsating ventricular cavity. With development of the coronary arteries, the compacted myocardium extracts oxygen from this vascular source and becomes less reliant on the trabeculated myocardium. Disruptions in the genetic pathways that specify trabeculation by and large significantly impair survival. In contrast, disruption of the compaction process, either completely or in part, may be compatible with postnatal life. However, in many cases abnormal compaction manifests later in life with impaired cardiac function, which may reflect continued molecular dysfunction. For example, in the case of *MYH7* or *TAZ* mutations, defective sarcomeres or mitochondrial function, respectively, can cause cardiomyopathy. However, the suboptimal morphology of the myocardium itself, with a reduced compacted layer, may also be a continued source of heart dysfunction.

The excessive left ventricular trabeculation observed in a measurable fraction of the normal population may be linked to an increased risk of heart failure, arrhythmias, or thromboembolic events later in life. Broader genetic and image profiling of the human population will help clarify the risks of hypertrabeculated myocardium.

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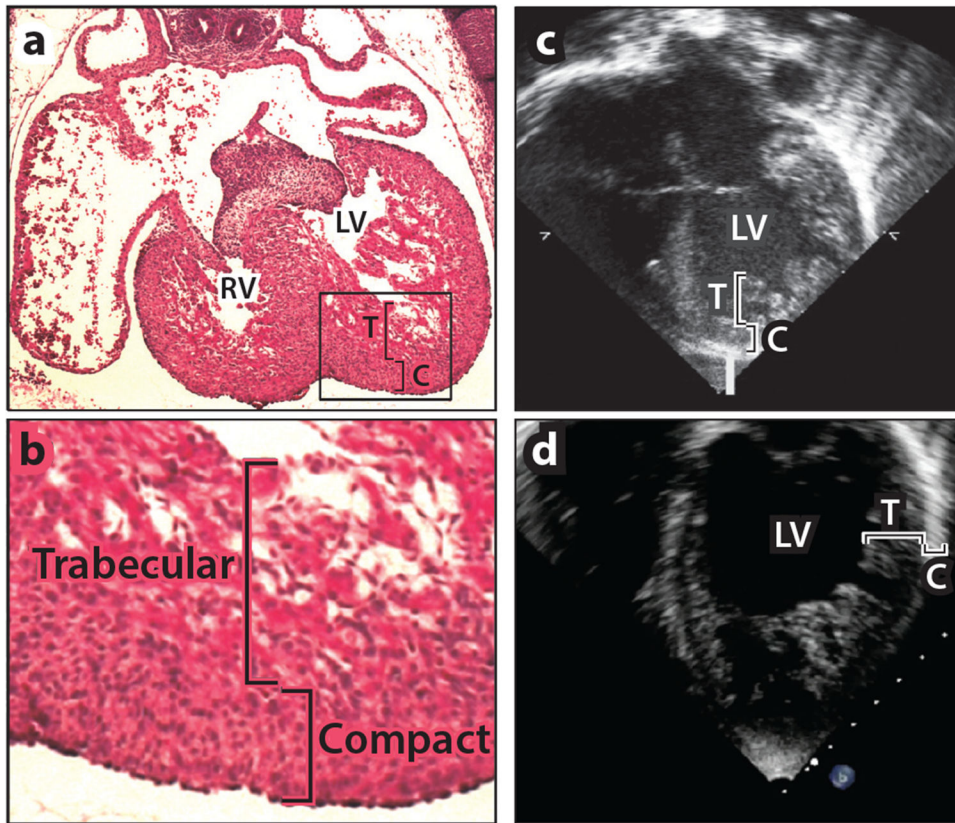


Figure 1. Myocardial trabeculation, compaction, and left ventricular noncompaction cardiomyopathy (LVNC). (a) Hematoxylin and eosin staining of the heart [right ventricle (RV) and left ventricle (LV)] from an embryonic day 16.5 (E16.5) mouse embryo demonstrates the presence of both trabecular (T) and compact (C) myocardium. (b) Higher-power image of the boxed area in panel a reveals that trabecular cardiomyocytes are larger and more loosely organized than the smaller, denser compact cardiomyocytes. (c,d) Echocardiographic images of a patient with LVNC, taken from the (c) parasternal short axis and (d) apical four-chamber views. These views show an abnormally thick trabecular layer and abnormally thin compact layer.

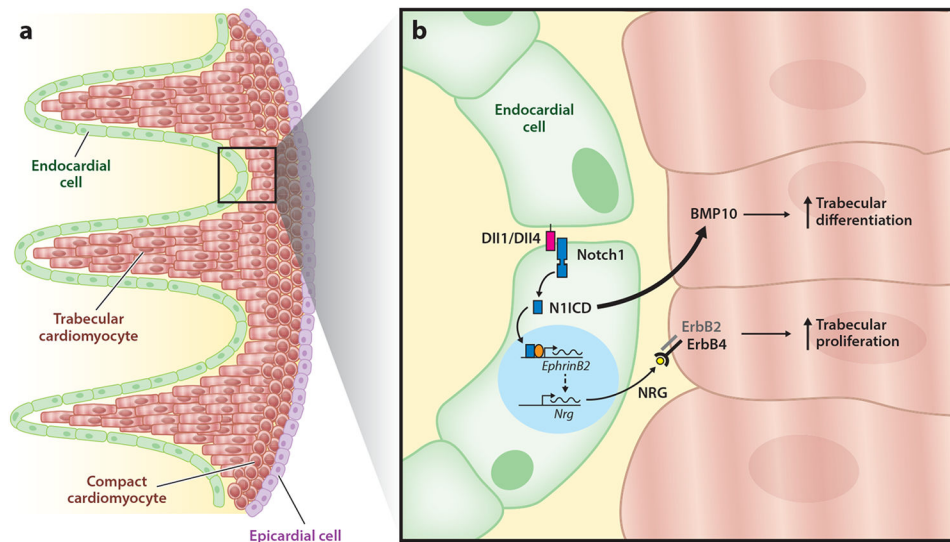


Figure 2.

Notch signaling during trabeculation. (a) The heart contains three layers: the endocardium (*green*), myocardium (*red*), and epicardium (*purple*). The myocardium is further divided into trabecular and compact cardiomyocytes. Throughout the trabecular phase of cardiac growth, the compact myocardial layer just beneath the epicardium remains thin, and bundles of trabecular cardiomyocytes are juxtaposed with endocardium. (b) During trabecular growth, endocardial Notch1 binds endocardial Delta-like ligand 1 (Dll1) and Delta-like ligand 4 (Dll4), triggering cleavage and endocytosis of the Notch1 intracellular domain (N1ICD) and promoting activation of *EphrinB2* transcription. EphrinB2 triggers *Neuregulin* (*Nrg*) transcription. NRG is released from the endocardium and binds myocardial ErbB4, which dimerizes with ErbB2 and drives trabecular cardiomyocyte proliferation. N1ICD also contributes to bone morphogenetic protein 10 (BMP10) expression in trabecular cardiomyocytes to promote differentiation.

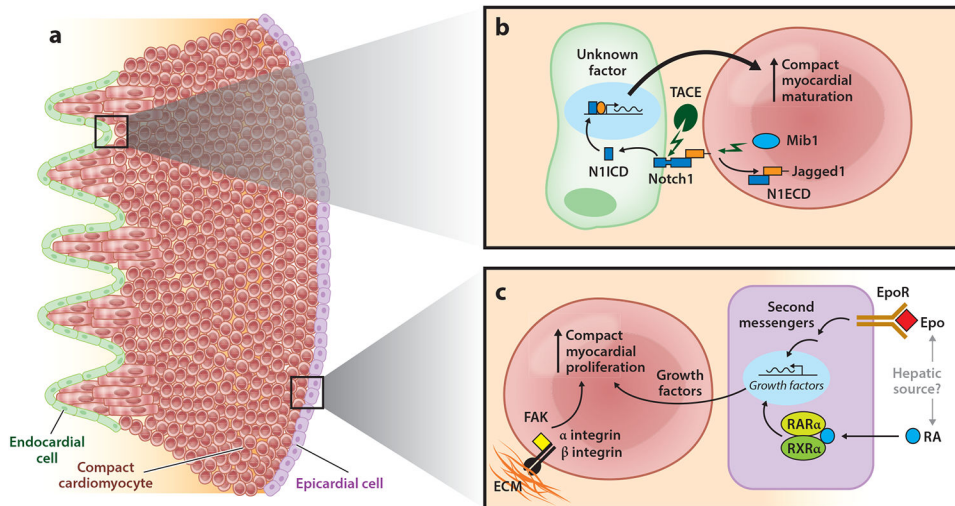


Figure 3.

Major proliferative signaling pathways that contribute to compaction. (a) The compact myocardium (*red circles*) expands dramatically via proliferative signals from the endocardium (*green*), epicardium (*purple*), and extracellular matrix (ECM) (*orange*). (b) During compact myocardial growth, endocardial Notch1 binds the myocardial ligand Jagged1, triggering cleavage by the protease TACE and myocardial internalization of Notch1 extracellular domain (N1ECD) and Jagged1 in a mindbomb homolog 1 (Mib1)-dependent pathway. Both the TACE and Mib1 steps are required for endocardial internalization of Notch1 intracellular domain (N1ICD). The endocardial N1ICD target gene(s) that promotes compact myocardial maturation remains unknown. (c) The epicardium responds to erythropoietin (Epo) and retinoic acid (RA), both possibly from a hepatic source, through the erythropoietin receptor (EpoR) and RA receptor dimer RXR α /RAR α (retinoid X receptor α /retinoic acid receptor α). Receptor binding triggers transcription of growth factors, including insulin-like growth factor and fibroblast growth factor family members, which bind myocardial receptors to promote compact myocardial proliferation. Fibroblasts in the compact myocardium also produce ECM components, including fibronectin and collagen. β 1 integrin (in concert with its α integrin heterodimer partner) binds these ECM ligands, activates focal adhesion kinase (FAK), and contributes to compact myocardial proliferation.

Table 1

Genes that contribute to cardiomyocyte proliferation and growth during trabeculation and compaction

Role	Gene	Full gene name	Stage of impact	Reference(s)
Endocardial-myocardial interactions	<i>Adam17</i>	TACE	Compaction	(21)
	<i>Bmp10</i>	Bone morphogenetic protein 10	Trabeculation	(27)
	<i>Ejnb2</i>	EphrinB2	Trabeculation	(11)
	<i>ErbB2</i>	ErbB2	Trabeculation	(12-15)
	<i>ErbB4</i>	ErbB4	Trabeculation	(12-15)
	<i>Fkbp1a</i>	FK506-binding protein 1a	Compaction	(18, 19)
	<i>Hhex</i>	Hematopoietically expressed homeobox	Compaction	(42)
	<i>Jag1</i>	Jagged 1	Compaction	(20)
	<i>Mib1</i>	mindbomb homolog 1	Compaction	(20)
	<i>Myocd</i>	Myocardin	Trabeculation	(24)
	<i>Nf1</i>	Neurofibromatosis 1	Compaction	(33, 34)
	<i>Notch1</i>	Notch 1	Trabeculation Compaction	(11, 20)
	<i>Notch2</i>	Notch 2	Trabeculation	(22, 23)
	<i>Nrg1</i>	Neuregulin 1	Trabeculation	(12-15)
	<i>Numb</i>	Numb gene homolog (Drosophila)	Compaction	(22)
	<i>Numbl</i>	Numb-like	Compaction	(22)
	<i>Rbpj</i>	Recombination signal binding protein for immunoglobulin κJ region	Trabeculation	(11)
	<i>Tek</i>	Endothelial-specific receptor tyrosine kinase	Trabeculation	(37)
<i>Tbx20</i>	T-box	Trabeculation	(32)	
<i>Vegfa</i>	Vascular endothelial growth factor A	Trabeculation Compaction	(39, 40, 41)	
Epicardial-myocardial interactions	<i>Epo</i>	Erythropoietin	Compaction	(53, 56)
	<i>Epor</i>	Erythropoietin receptor	Compaction	(56)
	<i>Fgf16</i>	Fibroblast growth factor 16	Trabeculation Compaction	(58-60)
	<i>Fgf20</i>	Fibroblast growth factor 20	Compaction	(58, 61)
	<i>Fgf9</i>	Fibroblast growth factor 9	Compaction	(58)
	<i>Igf2</i>	Insulin-like growth factor 2	Compaction	(57)
	<i>Rxra</i>	Retinoid X receptor α	Compaction	(48, 49, 51, 54)
	<i>Vcam1</i>	Vascular cell adhesion molecule 1	Compaction	(46)
Extracellular matrix-myocardial interactions	<i>Adams1</i>	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1	Compaction	(67)
	<i>Fbln1</i>	Fibulin-1	Compaction	(66)
	<i>Has1</i>	Hyaluronic acid synthase 1	Trabeculation	(65)
	<i>Itgb1</i>	Integrin β1	Compaction	(69)

Role	Gene	Full gene name	Stage of impact	Reference(s)
	<i>Ptk2</i>	PTK2 protein tyrosine kinase 2; also known as Focal Adhesion Kinase (FAK)	Compaction	(71)
	<i>Vcan</i>	Versican	Trabeculation	(63, 66)
Intracardiomyocyte interactions	<i>Adm</i>	Adrenomedullin	Compaction	(87, 88)
	<i>Calcr1</i>	Calcitonin receptor-like	Compaction	(87, 88)
	<i>Calr</i>	Calreticulin	Compaction	(86)
	<i>Daam1</i>	Dishevelled-associated activator of morphogenesis 1	Compaction	(85)
	<i>Foxp1</i>	Forkhead box P1	Compaction	(83)
	<i>Nkx2-5</i>	NK2 homeobox 5	Trabeculation	(26)
	<i>Taz</i>	Tafazzin	Compaction	(121-123)
	<i>Yap1</i>	Yes-associated protein 1	Trabeculation	(73)
	<i>Ywhae</i>	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, ϵ polypeptide; also known as 14-3-3 ϵ	Compaction	(82)
	<i>Zfp2</i>	Zinc finger protein, multitype 2; also known as Friend of GATA 2 (FOG2)	Compaction	(77)
Epigenetic interactions	<i>Jarid2</i>	Jumonji, AT-rich interactive domain 2	Compaction	(135-137)
	<i>Smarca4</i>	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4; also known as Brg1	Trabeculation	(67)
Human LVNC genes	<i>ACTC1</i>	Actin, α , cardiac muscle 1	Myofibrillogenesis	(99, 113)
	<i>ACTN2</i>	Actinin α 2	Myofibrillogenesis	(117)
	<i>CASZ1</i>	Castor zinc finger 1	Myofibrillogenesis	(117)
	<i>DTNA</i>	Dystrobrevin α	Costamere assembly	(121)
	<i>HCN4</i>	Hyperpolarization-activated, cyclic nucleotide-gated K ⁺ 4	Conduction system	(124, 125)
	<i>LDB3</i>	LIM domain binding 3; also known as Cypher and ZASP	Myofibrillogenesis	(119, 120)
	<i>LMNA</i>	Lamin A	Nuclear membrane	(130)
	<i>MYBPC3</i>	Myosin-binding protein C, cardiac	Myofibrillogenesis	(100, 108-111)
	<i>MYH7</i>	Myosin, heavy polypeptide 7, cardiac muscle, β	Myofibrillogenesis	(97-105)
	<i>TNN2</i>	Troponin T2, cardiac	Myofibrillogenesis	(99, 114)
<i>TPM1</i>	Tropomyosin 1, α	Myofibrillogenesis	(108)	