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Molecular mechanism of ventricular trabeculation/compaction and the pathogenesis of the left ventricular noncompaction cardiomyopathy (LVNC)

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Abstract

Ventricular trabeculation and compaction are two of the many essential steps for generating a functionally competent ventricular wall. A significant reduction in trabeculation is usually associated with ventricular compact zone deficiencies (hypoplastic wall), which commonly leads to embryonic heart failure and early embryonic lethality. In contrast, hypertrabeculation and lack of ventricular wall compaction (noncompaction) are closely related defects in cardiac embryogenesis associated with left ventricular noncompaction (LVNC), a genetically heterogenous disorder. Here we review recent findings through summarizing several genetically engineered mouse models that have defects in cardiac trabeculation and compaction.

Keywords

heart development; cardiac compaction; trabeculation; signaling

INTRODUCTION

Left ventricular noncompaction (LVNC, OMIM300183) is a unique type of inherited cardiomyopathy and has gained increasing attention in the past decade [Jenni et al., 2001; Towbin 2010; Oechslin and Jenni 2011]. LVNC, first described in 1926, was previously known as spongy myocardium [Grant 1926; Freedom et al., 2005]. Its prevalence was estimated from 4.5 to 26 per 10,000 adult patients referred for echocardiographic diagnosis [Ritter et al., 1997; Aras et al., 2006; Sandhu et al., 2008; Hoedemaekers et al., 2010]. In the pediatric population, LVNC is the third most common cardiomyopathy after dilated cardiomyopathy and hypertrophic cardiomyopathy [Nugent et al., 2003; Nugent et al., 2005; Daubeney et al., 2006]. A recent article by Oechslin and Jenni presented a thorough clinical view of LVNC, including the current progress of diagnostic criteria, the epidemiology, the clinical presentation, outcome and management, the use of cardiac magnetic resonance imaging (MRI) to overcome potential misdiagnosis by commonly used echocardiography, as well as the genetic heterogeneity of LVNC [Oechslin and Jenni, 2011]. However, despite these important clinical observations over the past 25 years, the etiological cause of LVNC is largely unknown. The reasons could be three-fold: 1) the majority of clinical investigation

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is based on the anatomical features of isolated LVNC in the adult patients. It is therefore impossible to retrospectively track the abnormal developmental process at the embryonic stage; 2) the poor genotype-phenotype association in LVNC patients prevents a clear understanding of specific genetic pathways relevant to the pathogenesis of LVNC; 3) there is a lack of sufficient knowledge on the molecular control of normal trabeculation and compaction during ventricular myocardial development. To overcome these challenges, it is critical to establish animal models to analyze LVNC at both genetic and molecular level and use the knowledge gained from animal studies to interpret the clinical findings and eventually to find preventative and therapeutic measures for LVNC.

THE DEVELOPMENT OF VENTRICULAR WALL

During mammalian heart development, the ventricles undergo a series of morphogenetic events [Taber, 1998; Bartman and Hove, 2005; Moorman et al., 2007]. Ventricular trabeculation and compaction are two of the many steps among those events essential for creating a functionally competent ventricular wall [Sedmera et al., 2000]. The development of ventricular wall can be viewed as a 4-step process. The first step involves the formation of a single cell layer of myocardium at an early developmental stage. Following induction via adjacent endoderm, the lateral mesoderm gives rise to an early tubular heart, which is composed of one cell layer of myocardium and one cell layer of endocardium lining the lumen with extracellular matrix (cardiac jelly) in between (Fig 1) [Brutsaert and Andries, 1992; Bartman and Hove, 2005]. The second step involves the formation of a trabeculated and compact myocardium at early midgestation stage. As the myocardium thickens, 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 trabecular myocardium, while the outside layer of myocardium becomes the base for forming the compact myocardium later in development (Fig 1). Ventricular trabeculation has been suggested to facilitate oxygen and nutrient exchange in the heart muscle and to enhance heart muscle force generation to match the increasing blood and oxygen demand in developing embryos [Sedmera et al., 2000]. The third step regards myocardial compaction at late midgestation stage. As development proceeds, the trabecular myocardium collapses towards the myocardial wall in a process termed compaction, which contributes to forming a thicker, compact ventricular wall. The majority of trabeculae have become compacted after E14.5 in mouse embryos (Fig 2) [Sedmera et al., 2000; Risebro and Riley, 2006]. The fourth step is the formation of a mature and multilayered spiral myocardium during late fetal and neonatal stage [Taber, 1998]. Unlike the skeletal muscle, the ventricular myocardium consists of aggregated myocytes within a three-dimensional mesh. A series of elegant studies by Anderson and colleagues demonstrated that myocytes in the subendocardial and subepicardial layers of the myocardium form helical sheets with reciprocal angulation, whereas the myocytes at the middle layer form a circular sheet [Torrent-Guasp et al., 2005; Anderson et al., 2006; Lunkenheimer et al., 2006; Lunkenheimer et al., 2006; Anderson et al., 2007; Dorri et al., 2007; Schmid et al., 2007; Anderson et al., 2008]. This unique myocardial structure is believed to be essential for normal contractile function of the heart. Whether ventricular trabeculation and compaction is part of this dynamic process is unknown and requires further investigations.

Following the formation of primitive trabecular ridges (at about E9.5 in mouse embryo), the myocardium undergoes extensive expansion by either recruiting cardiomyocytes from myocardial wall into the trabecular ridges or via cellular proliferation within the trabecular cardiomyocytes. In support of the cellular recruitment mechanism, proliferative activity is consistently higher within the compact myocardium, and there is a gradient of decreasing proliferation and increasing differentiation of myocytes from the compact towards the trabecular zone of the myocardium [Icardo and Fernandez-Teran 1987; Icardo, 1988;

Rumyantsev and Krylova, 1990; Pasumarthi and Field, 2002]. $P57^{kip2}$, a cyclin-dependent kinase inhibitor of the p21 family, is specifically expressed in the trabecular myocardium, suggesting an active process to suppress cell proliferation in the trabeculae [Kochilas et al., 1999]. This balance of proliferation and differentiation is critical to the formation of a functionally competent ventricular wall. Fate mapping experiments using retroviral tagging with β-galactosidase in developing chick hearts provided important insights into the interplay between cellular proliferation and differentiation during ventricular wall formation [Mikawa et al., 1992; Mikawa et al., 1992]. Upon myogenic differentiation, single labeled cardiomyocytes give rise to transmural and cone-shaped growth units. This pattern of growth is closely associated with the formation of trabecular myocardium [Gourdie et al., 1999; Meilhac et al., 2003].

Several growth factors and their intracellular signaling pathways have been demonstrated to be critical to ventricular growth and trabeculation. Neuregulin 1 is produced in endocardial cells and acts through myocardial receptors ErbB2 and ErbB4. Mice deficient in either neuregulin 1 or ErbB2/4 develop hypoplastic wall lacking normal trabeculation [Gassmann et al., 1995; Lee et al., 1995; Kramer et al., 1996; Lai et al., 2010]. A recent study of the highly trabeculated zebrafish heart demonstrates that the endocardial neuregulin 1, in addition to its role in promoting cell proliferation, has another important function in regulating cardiomyocyte delamination to initiate ventricular trabeculation [Liu et al., 2010]. Bone morphogenetic protein 10 (BMP10), enriched in trabecular myocardium, is identified as a potent growth factor for maintaining cardiomyocyte proliferation via its activity in inhibiting $p57^{kip2}$ (Fig 1) [Chen et al., 2004; Pashmforoush et al., 2004].Vascular endothelial growth factor (VEGF) and angiopoietin likely signal from myocardium to endocardium to regulate ventricular trabeculation [Sato et al., 1995; Ferrara et al., 1996; Suri et al., 1996]. For a proper interaction between endocardium and myocardium, the acellular cardiac jelly plays a key role in modifying these growth signals to be transmitted between these cell layers. Cardiac jelly is rich in extracellular matrix. Mice-deficient in either hyaluronan synthase-2 (Has-2, an enzyme required for production of the mucopolysaccharide hyaluronan) or versican (a chondroitin sulfate proteoglycan) reduce the level of trabeculation remarkably [Yamamura et al., 1997; Mjaatvedt et al., 1998; Camenisch et al., 2000]. This close association of reduced level of cardiac jelly with reduced trabeculation suggests an important contribution of cardiac jelly to ventricular trabeculation. The composition of cardiac jelly is controlled by chromatin-remodeling factor Brg1. Chromatin can be modified non-covalently by the rearrangement of nucleosomes catalyzed by ATP-dependent chromatin remolding complexes to facilitate both transcriptional activation and repression [Dunaief et al., 1994]. The Brm/Brg-associated-factor (BAF) complex is a ten subunit complex in which the ATPase component is encoded by either Brg1 or Brm. BAF complex was shown to be involved in cardiac development [Lickert et al., 2004]. Endocardial-restricted ablation of Brg1 leads to significant defect in ventricular trabeculation in mice [Stankunas et al., 2008]. Detailed molecular analyses have revealed that the absence of Brg1 results in the derepression of ADAMTS1 (a disintegrin and metalloproteinase with thrombospondin motif), which normally increases in expression later in development to prevent excessive trabeculation. The abnormal up-regulation of ADAMTS1 in Brg1 mutant embryos causes premature breakdown of the cardiac jelly and termination of trabeculation [Stankunas et al., 2008] and further demonstrates the critical role of cardiac jelly in ventricular wall development (Fig 1).

In addition to endocardium, epicardium is another important resource for mitogenic factors influencing ventricular wall growth and formation, which includes TGFβ/BMPs, PDGF-A, Wnts, Hh, and FGFs [Sucov et al., 2009]. The epicardium is derived from the proepicardial organ originated in the septum transversum and covers over the surface of the developing heart [Viragh and Challice, 1981; Komiyama et al., 1987; Manner, 1993]. Many of these

growth factors are involved in epicardial development and coronary vasculature development. Among them, members of fibroblast growth factors (FGFs) are closely related to ventricular wall formation [Lavine et al., 2005]. Mice deficient in FGF9 or FGF16 develop thin myocardium phenotype [Lavine et al., 2005; Lu et al., 2008]. Myocardial-specific knockout of FGF receptor s1 and 2 (*FGFR1* and *FGFR2*) results in a similar thin wall phenotype, further confirming the role of epicardial FGF-mediated signaling in ventricular wall development. Most interestingly, the epicardial-derived growth signals are particularly important to regulate compact myocardium development, while endocardial-derived growth signals are more relevant to trabecular myocardium formation. Using a neonatal cardiomyocyte cell culture system, Sucov and colleagues analyzed cardiomyocyte response to epicardial- and endocardial-derived factors [Kang and Sucov 2005]. VCAM1, a cell adhesion molecule, is specifically expressed in compact myocardium [Kwee et al., 1995; Terry et al., 1997], which is opposed to BMP10 and atrial natriuretic factor (ANF) as trabecular myocardial markers [Christoffels et al., 2000; Chen et al., 2004]. VCAM1 is specifically up-regulated in cells cultured in conditioned media containing epicardial-derived factors, whereas BMP10 and ANF are up-regulated only by endocardial growth factor neuregulin 1 [Kang and Sucov, 2005]. This finding demonstrates an interactive regulation of ventricular wall growth and development by the endocardial-, myocardial- and epicardial-derived signals (Fig 1).

VENTRICULAR COMPACTION AND LVNC

A significant reduction in trabeculation is usually associated with ventricular compact zone deficiencies (hypoplastic wall), which commonly leads to embryonic heart failure and early embryonic lethality. In contrast, abnormal trabecular remodeling (*i.e.*, compaction) during ventricular wall formation is thought to be associated with ventricular noncompaction. In the past decade, a series of genetically engineered mouse models producing ventricular noncompaction phenotypes have been generated. Although “ventricular noncompaction” has been used in describing their common abnormal ventricular phenotypes, the actual ventricular wall abnormalities are not homogeneous, and they clearly vary from model to model, which may reflect the dynamic complexity of ventricular trabeculation and compaction controlled by distinct signaling pathways. “Hypertrabeculation” is another widely used terminology to describe noncompaction, and it has been suggested as an inadequate terminology for noncompaction phenotype in clinic [Oechslin and Jenni, 2011]. However, recent analyses of various of mouse models demonstrate that some mouse models having ventricular noncompaction are associated with the increased level of trabeculation (number and thickness of the trabeculae) at early embryonic stage, while some not. This suggests that there are two independent biological events leading to abnormal trabeculation and compaction. Thus, we use “hypertrabeculation” to refer to the phenotype with increased number and thickness of the trabeculae at embryonic stage (e.g., mouse E12.5-E14.5) and “noncompaction” to refer to the lack of trabecular remodeling towards the compact wall during and after the trabeculation. Detailed molecular analyses on these mouse models have helped us in determining the genetic pathways that are involved in the ventricular wall formation and maturation as well as the potential pathogenesis pathways contributing to LVNC.

FKBP12-deficient mouse model and the role of BMP10 in ventricular trabeculation

FKBP12 (also known as *Fkbp1a*) is a ubiquitously expressed 12kDa cytoplasmic protein and belongs to the immunophilin family[Bierer et al., 1990; Schreiber and Crabtree 1995]. FKBP12 has been shown to be associated with multiple intracellular protein complexes, including BMP/activin/TGFβ type-I receptors [Wang et al., 1996] and Ca^{2+}-release channels, such as inositol trisphosphate receptor (IP3R) and ryanodine receptor (RyR) [Jayaraman et al., 1992; Cameron et al., 1995; Cameron et al., 1995; Cameron et al., 1997].

FKBP12-deficient mice die between E14.5 and birth due to severe cardiac defects including a characteristic increase in the number and thickness of ventricular trabeculae, deep inter-trabecular recesses, lack of compaction, thin ventricular wall, and a prominent ventricular septal defect (VSD) [Shou et al., 1998], representing many important clinical features of LVNC. As *FKBP12*-deficient mouse is the first mouse genetic model for ventricular noncompaction, it has been extensively used to study the underlying mechanisms regulating ventricular trabeculation and compaction.

Using a dual fluorescence imaging technique, we have established a method to quantify the thickness of trabecular ridges and sheets, the overall thickness of the compact wall, and the ratio of trabecular myocardium to compact wall thickness [Chen et al., 2009]. *FKBP12*-deficient mice are abnormal in both the processes of trabeculation and compaction. The thickening of trabecular myocardium become apparent at E11.5 (hypertrabeculation), followed by the thinning of the compact wall at later stage (noncompaction) (Figs 3A and 3B). There are two major altered cellular phenotypes associated with FKBP12-deficient hearts: 1) increased level of cardiomyocyte cell cycle activity in mutant myocardium, particularly remarkable in trabecular myocardium (Figs 3C and 3D); 2) disrupted cardiomyocyte polarization and myofibrillogenesis in mutant myocardium (Figs 3E & 3F). In normal early developing ventricles, cardiomyocytes are polygonal-shaped [Hirschy et al., 2006; Henderson and Chaudhry, 2011]. Cardiomyocytes are elongated in the trabecular myocardium to become polarized cardiomyocytes with clear structure of myofibrils (Fig 2 and Fig 3E), while cardiomyocytes in compact zone remain polygonal-shaped without distinct structure of myofibril [Hirschy et al., 2006; Henderson and Chaudhry 2011]. In FKBP12-deficient hearts, the majority of trabecular cardiomyocytes remain sphere-shaped, and the myofibrillogenesis is markedly impaired (Fig 3F).

By transcriptome profile analysis, *BMP10* is identified as one of the genes dramatically up-regulated in *FKBP12*-deficient hearts [Chen et al., 2004]. BMP10 is a peptide growth factor that belongs to the TGF-β superfamily [Chen et al., 2004] and is expressed transiently in the ventricular trabecular myocardium from E9.0 to E13.5, a critical time window when cardiac development shifts from patterning to growth and chamber maturation. By E16.5-E18.5, BMP10 is only detectable in the atria, and then is retracted to the right atria in postnatal hearts. Interestingly, the up-regulation of *BMP10* expression in developing myocardium is associated with hypertrabeculation phenotype on other genetic mouse models, which include the Nkx2.5-myocardial specific knockout [Pashmforoush et al., 2004] and the Numb/Numblike-deficient mice [Yang et al., 2012]. This suggests that *BMP10* is a key morphogenetic growth factor involved in the regulation of cardiac trabeculation and/or compaction.

To determine whether up-regulated BMP10 expression would directly impact trabeculation and/or compaction in the developing myocardium, the human atrial natriuretic factor (hANF) promoter is used to drive exogenous *BMP10* expression in mouse embryonic hearts (hANF-BMP10). The transgene positive hearts have a significantly increased thickness of trabecular myocardium [Pashmforoush et al., 2004]. These data demonstrate that overexpression of BMP10 alone in the embryonic heart is sufficient to cause cardiac trabeculation abnormality. Consistently, *BMP10*-deficient embryos die *in utero* at E10.5 display cardiac dysgenesis with profound hypoplastic ventricular walls and an absence of ventricular trabeculae. Further analyses demonstrate that there is a marked reduction of proliferation in *BMP10*-deficient cardiomyocytes [Chen et al., 2004; Chen et al., 2009] and suggest that *BMP10*, although not critical for the initiation of cardiac trabeculation, is essential for subsequent trabecular growth. Consistent with this finding, cardiomyocyte proliferation is profoundly increased in *FKBP12*-deficient hearts and Nkx2.5 mutant hearts [Chen et al., 2004; Pashmforoush et al., 2004; Chen et al., 2009].

$p57^{kip2}$ is a critical negative cell cycle regulator [Besson et al., 2008]. Interestingly, $p57^{kip2}$ expression is first detectable in the developing mouse heart at E10.5 and is restricted to the ventricular trabecular myocardium [Kochilas et al., 1999]. Therefore, $p57^{kip2}$ is considered a key negative regulator involved in cardiac cell cycle exit within the developing ventricular trabeculae during cardiac chamber maturation. Immunohistochemistry staining reveals that $p57^{kip2}$ is up-regulated and ectopically expressed throughout the ventricular wall in BMP10-null hearts at E9.5 compared to wild type littermate controls. Conversely, *FKBP12*-deficient hearts exhibit significantly lower $p57^{kip2}$ levels in trabecular myocardium [Chen et al., 2004]. Taken together, the analysis of FKBP12-deficient hearts demonstrated the first time that the increased level of cardiomyocyte proliferation is closely associated with the genesis of ventricular hypertrabeculation and noncompaction.

Notch signaling in venricular trabeculation and LVNC

Earlier work by Watanabe et al used an inducible transgenic system to demonstrate that overexpression of activated Notch 1 (N1ICD) in early cardiac cell lineages led to several abnormal cardiac phenotypes, including abnormal ventricular morphology similar to ventricular noncompaction [Watanabe et al., 2006], suggesting that Notch signaling is a potential contributor to ventricular hypertrabeculation and/or noncompaction. Interestingly, while *Notch2* and *Jag1* expression are restricted to the developing trabecular myocardium, Notch1, *Notch4 and Delta4* are transcribed in the endocardium from gastrulation onward [Del Monte et al., 2007]. Consistently, activated Notch1 protein (N1ICD) is found predominantly in the endocardial cells proximal to the base of the developing trabeculae [Del Monte et al., 2007; Grego-Bessa et al., 2007]. Endothelial/endocardial restricted ablation of Notch 1 or its co-factor RBPjk lead to a defect in ventricular trabeculation, strongly supporting the role of Notch signaling in the development of ventricular wall. Molecular analysis suggests that Notch–dependent signaling regulates trabeculation via neuregulin 1, EphrinB2, and BMP10 [Grego-Bessa et al., 2007]. Subsequent analysis using embryo cultures with conditioned media supplemented with either neuregulin 1 or *BMP10* further demonstrate that *BMP10* is required for Notch-mediated regulation of cardiomyocyte proliferation, while neuregulin 1 likely mediates ventricular trabeculation independent of cell proliferation [Grego-Bessa et al., 2007], which is consistent with other published work [Chen et al., 2004; Liu et al., 2010]. In addition, our recent work demonstrated that *FKBP12* is a novel regulator for endothelial Notch1 activity [Chen et al., 2013] and that the upstream regulator for BMP10 expression lies within the developing endocardium [Chen et al., 2009]. Endothelial-restricted ablation of FKBP12 enhanced N1ICD activity; the inhibition of Notch activity partly normalizes abnormal hypertrabeculation phenotype in FKBP12-deficient hearts [Chen et al., 2013]. Interestingly, *Tbx20* and *Hey2* expression are expanded from compact wall to trabecular myocardium in FKBP12 mutant hearts, demonstrating a loss of trabecular/compact myocardium patterning (Fig 4) [Chen et al., 2013]. Consistently, earlier work also shows that BMP10 regulates Tbx20 myocardial expression [Zhang et al., 2011]. These findings implie that Notch signaling has a critical role in regulating trabecular/compact myocardium patterning. Other mouse models with enhanced Notch activity are also associated with hypertrabeculation and/or noncompaction, including *Jarid2*/*Jumonji* mutants [Lee et al., 2000; Mysliwiec et al., 2011; Mysliwiec et al., 2012], and the *numb*/*numblike* compound mutant mice [Yang et al., 2012]. Collectively, these data strongly support that Notch signaling is essential for normal ventricular wall development (Fig 1).

Noncanonical Wnt/PCP signaling and ventricular noncompaction

Planar cell polarity (PCP) signaling is an essential molecular mechanism by which epithelial cells establish polarity in planes orthogonal to apical-basal axis [Wang and Nathans, 2007; Simons and Mlodzik, 2008]. The key features for PCP signaling include the control of cellular alignment and orientation in polarized tissues [Wang and Nathans, 2007; Simons

and Mlodzik, 2008]. The major PCP signaling components are mainly demonstrated in the Drosophila by using genetic mutagenesis and clonal analysis, which has revealed an essential role of noncanonical Wnt signaling, independent of β-catenin, is critical for PCP [Wang and Nathans, 2007; Simons and Mlodzik, 2008]. This Wnt/PCP signaling pathway has an essential function in embryonic patterning and organogenesis, more specifically in vertebrates including the regulation of gastrulation, neurulation, and hair cell polarity within the inner ear in mouse and within the lateral line in zebrafish [Wang and Nathans, 2007; Simons and Mlodzik, 2008]. This noncanonical Wnt/PCP signaling pathway in Drosophila is relatively well understood [Wang and Nathans, 2007; Simons and Mlodzik, 2008], which is composed of core signaling components [*e.g.*, Frizzled (Fz), Dishevelled (Dvl), Prickle (Pk), Vangl, Celsr1], PCP regulators [e.g., Casein kinase 1ε (CK1ε)] and a large number of PCP effectors (e.g., Daam1, Rac1, PhoA) [Simons and Mlodzik, 2008]. Interestingly, many of the core signaling components are well conserved in vertebrates and mammals. However, the degree of mechanistic signaling conservation between flies and vertebrates and mammals is still unclear. Cardiomyocytes are not a type of epithelial cells, thus they may not have a classic PCP phenotype. But the mature cardiomyocytes are highly polarized with the majority of cell-cell junction proteins located at intercalated disc, joining cell end to end, while the attachment to the extracellular matrix are on their lateral surface [Hirschy et al., 2006; Henderson and Chaudhry, 2011]. Interestingly, the immature cardiomyocytes at early embryonic hearts are mostly polygonal or spherical [Hirschy et al., 2006; Henderson and Chaudhry, 2011]. The mechanism of cardiomyocytes polarization during cardiac development, and more specifically whether Wnt/PCP signaling has a role in cardiomyocyte polarization, is unknown.

Interestingly, mutant mouse models deficient in several key Wnt/PCP signaling components develop ventricular noncompaction. *Vangl2* is expressed in the developing myocardium and adult myocardium [Phillips et al., 2005]. Mice-deficient in *Vangl2* (loop-tail mutant, *lp*/*lp*) develop thin ventricular wall with noncompaction characteristics [Phillips et al., 2008]. *lp*/*lp* cardiomyocytes are spherical in shape, whereas normal cardiomyocytes are elongated [Phillips et al., 2008]. Another key Wnt/PCP molecule *Scrib* may also contribute to cardiomyocyte polarization [Murdoch et al., 2003; Phillips et al., 2007]. Examination of *Scrib* mutant mice (Circletail mutant, *Crc*/*Crc*) demonstrate altered ventricular chamber formation that resembles ventricular noncompaction. Importantly, the mutations in both of these genes result in abnormalities in the polarization and organization of cardiomyocyte in ventricular myocardium as well as outflow myocardium; the latter gives rise to outflow defects such as Double Outlet Right Ventricle (DORV). This strongly suggests that cardiomyocyte polarization is critical for cardiac development and that Wnt/PCP signaling is a key signaling pathway in regulating cardiomyocyte polarization (Fig 2). In addition, other recent experimental evidence for a role of Wnt/PCP signaling in ventricular development comes from the studies of *dishevelled* (*Dvl*, the core Wnt/PCP signaling element) and disheveled-associated activator of morphogenesis 1 (*Daam1*, the potential effector of Wnt/PCP signaling) mutant mice (Figure 2). Similar to *Vangl2* and *Scrib* mutants, mutant mice-deficient in *Dvl1/2* develop severe defects in both outflow tract development and ventricular structure [Sinha et al., 2012]. *Daam1* is expressed in the developing hearts and adult hearts. The first indication of *Daam1* has a role in ventricular wall development comes from our initial screening of differentially expressed genes in FKBP12-deficient hearts when compared to normal control hearts. Daam1 is markedly down-regulated in FKBP12-deficient ventricles (Fig 5). Thus, we have generated Daam1-deficient mice to explore the potential function of *Daam1* in ventricular formation [Li et al., 2011]. In most part, Daam1-deficient mice resemble abovementioned mutant mice and have defects in cardiomyocyte polarization, alignment, and junction, which results in ventricular noncompaction, DORV, and ventricular septal defect (VSD) [Li et al., 2011]. Importantly, Daam1-deficient hearts have normal cardiomyocyte proliferation and BMP10 expression, suggesting that altered cell

cycle activity is not required for ventricular noncompaction phenotype [Li et al., 2011] and that two independent signaling pathways underlie the pathogenesis of hypertrabeculation and noncompaction (Fig 6). A recent case report revealed that a human copy number variation (CNV) in *DAAM1* gene was associated with multiple cardiac congenital defects (CHDs) and embryonic heart failure [Bao et al., 2012], further suggesting the role of Wnt/PCP signaling in the pathogenesis of CHDs and inherited cardiomyopathies.

LVNC mouse models for human mutations

In the past decade, genetic screening of LVNC patients (mostly isolated form) identified a handful of gene mutations, mostly sarcomere proteins, such as β-myosin heavy chain (β*MHC/MYH7*), α-cardiac actin (*ACTC*), cardiac troponin T (*TNNT2*), Tafazzin (*TAZ/G4.5*), α-dystrobrevin, lamin A/C (*LMNA*), ZASP/LBD3, and dystrophin [Xing et al., 2006; Klaassen et al., 2008]. Interestingly, most of these genes are part of myofibril structure and are associated with contractile function. However, it is puzzling how these genetic variations and mutations lead to ventricular noncompaction that is presumably developed at embryonic stage. One recent report suggests that the presence or absence of a sarcomere gene mutation in LVNC does not predict the clinical phenotypes [Probst et al., 2011], further clouding the genetic etiology and the pathogenesis pathways for LVNC in patients.

Several attempts have been made to mimic human LVNC in mice. Barth syndrome (OMIM302060) is a rare-X-linked multisystem genetic disorder due to mutations in G4.5 (*tafazzin*) gene [Schlame et al., 2002; Towbin 2010]. *Tafazzin* catalyzes cardiolipin biosynthesis [Xu et al., 2006; Xu et al., 2006]. Normal cardiolipin is critical to mitochondrial bioenergetic function. LVNC is one of several clinical hallmarks of Barth syndrome. Phoon and his colleagues used an inducible shRNA transgenic approach to knock-down *tafazzin* in mice (TAZKD) and studied the pathogenesis of LVNC [Phoon et al., 2012]. *Tafazzin* knockdown leads to altered cardiolipin and mitochondrial ultrastructure, as well as prenatal and perinatal lethality due to altered cardiac function at embryonic stage [Phoon et al., 2012]. Importantly, the defects of TAZKD hearts mimic clinical phenotype of ventricular noncompaction, which can be seen as early as E13.5, thus providing an important animal model for Barth syndrome and the LVNC [Phoon et al., 2012].

Another interesting missense mutation at codon 96 (from GAG to AAG) in exon 10 of the cardiac troponin T gene (TNNT2) is found to associate with a familial case of LVNC. Mouse transgenic approach was used to mimic the clinical condition by introducing this mutation into the mouse heart using the αMHC promoter [Luedde et al., 2010]. Although the transgenic overexpression of the mutant TNNT2 leads to cardiomyopathy and severely altered cardiac function, histological findings have excluded LVNC phenotype [Luedde et al., 2010]. This discrepancy is likely due to transgenic overexpression system and/or the genetic difference between humans and mice. The highly variable outcomes of cardiomyopathy phenotypes in association with mutations of TNNT2 and other genes critical to cardiac contractile function strongly suggest the genetic modifiers are involved in the pathogenesis of LVNC. Excitingly, a recent report describes a human mutant in mindbomb homolog 1 (*MIB1*) is associated with a LVNC familial case. Myocardial specific knockout of *Mib1* apparently recapitulates the clinical hallmarks of LVNC [Luxan et al., 2013]. MIB1 is an E3 ubiquitin ligase that regulates the endocytosis of the Notch ligands Delta and Jagged. Affected individuals show altered NOTCH1 activity and altered expression of target genes, which leads to the loss of trabecular/compact myocardial patterning similarly seen in FKBP12 mutant hearts (Figure 4 and Figure 6). This mutation and the subsequent confirmation using a mouse model further strengthen the notion that Notch signaling is a critical contributor to the pathogenesis of LVNC, and it may also reflect the complexity of Notch signaling pathways in which the right combination of spatial, temporal and dosage is essential for regulating ventricular wall formation.

In addition to abovementioned mouse models, there are many other mouse models having LVNC phenotypes, such as 14-3-3ε/Ywhae knockout mouse [Kosaka et al., 2012; Chang et al., 2013], transgenic mice overexpressing SHP2-Q79R (the Noonan Syndrome SHP2 gain-of-function mutation) [Nakamura et al., 2007], and transgenic mice overexpressing βMHC-Met531Arg [Kaneda et al., 2008]. However, the complexity of genetic heterogeneity and clinical phenotypes in LVNC patients suggest that multiple mechanisms at molecular and cellular levels are involved in the pathogenesis of LVNC. Figure 6 provides our currently understanding and hypothesis of two independent signaling pathways associated with ventricular hypertrabeculation and noncompaction using FKBP12 mutant mice as a model. It is understandable that the current study may have only revealed a subset of signaling pathways and networks. Thus, continued efforts to model clinical and genetic findings in animals will advance our understanding of the molecular mechanisms by which regulate ventricular trabeculation and compaction, and will eventually provide solutions to the medical challenges of LVNC.

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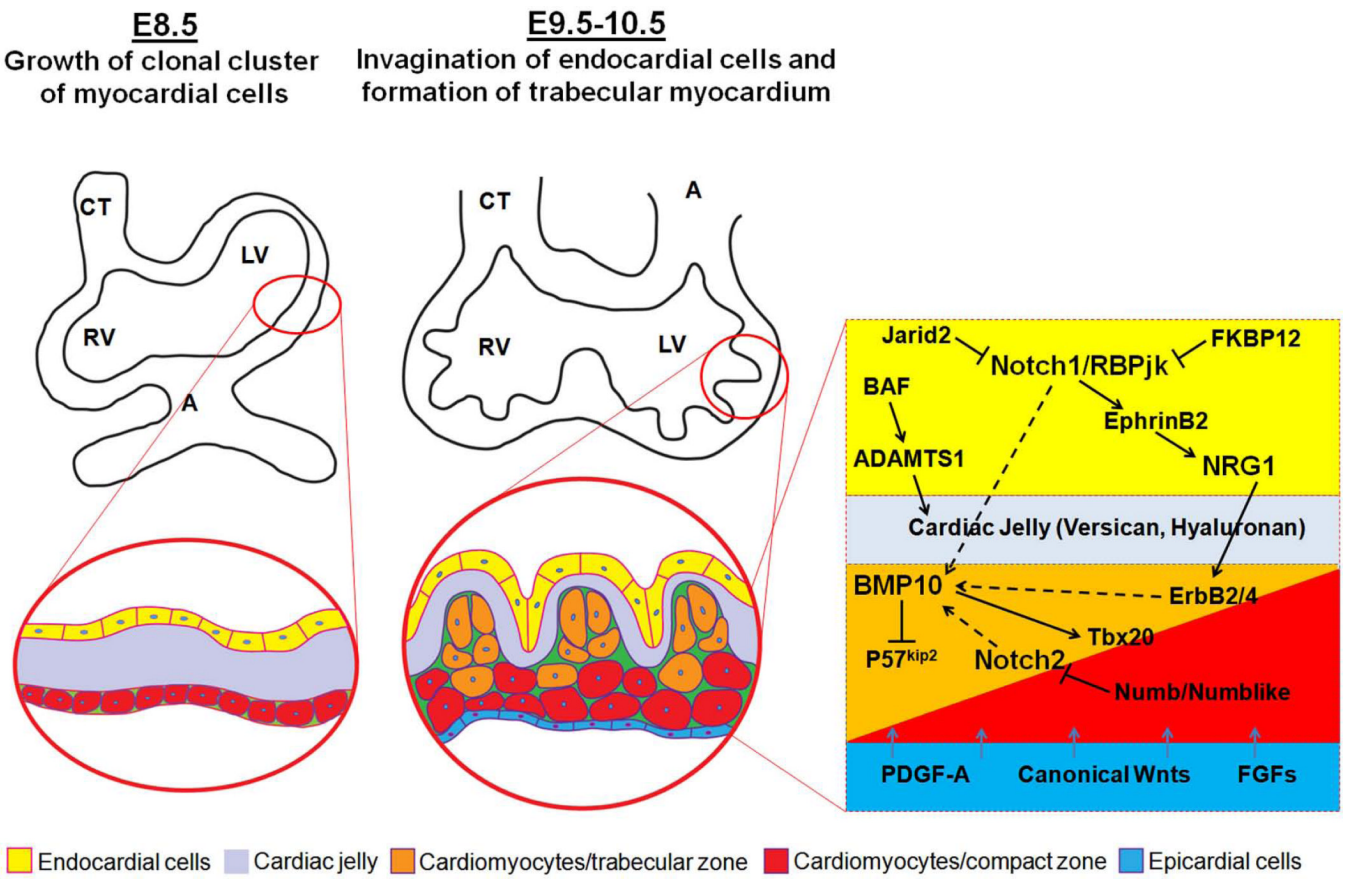

Figure 1.
Early growth and development of ventricular wall. The interactive regulation of endocardial-, myocardial- and epicardial-derived growth factors and signaling networks is critical to the ventricular wall growth and trabeculation.

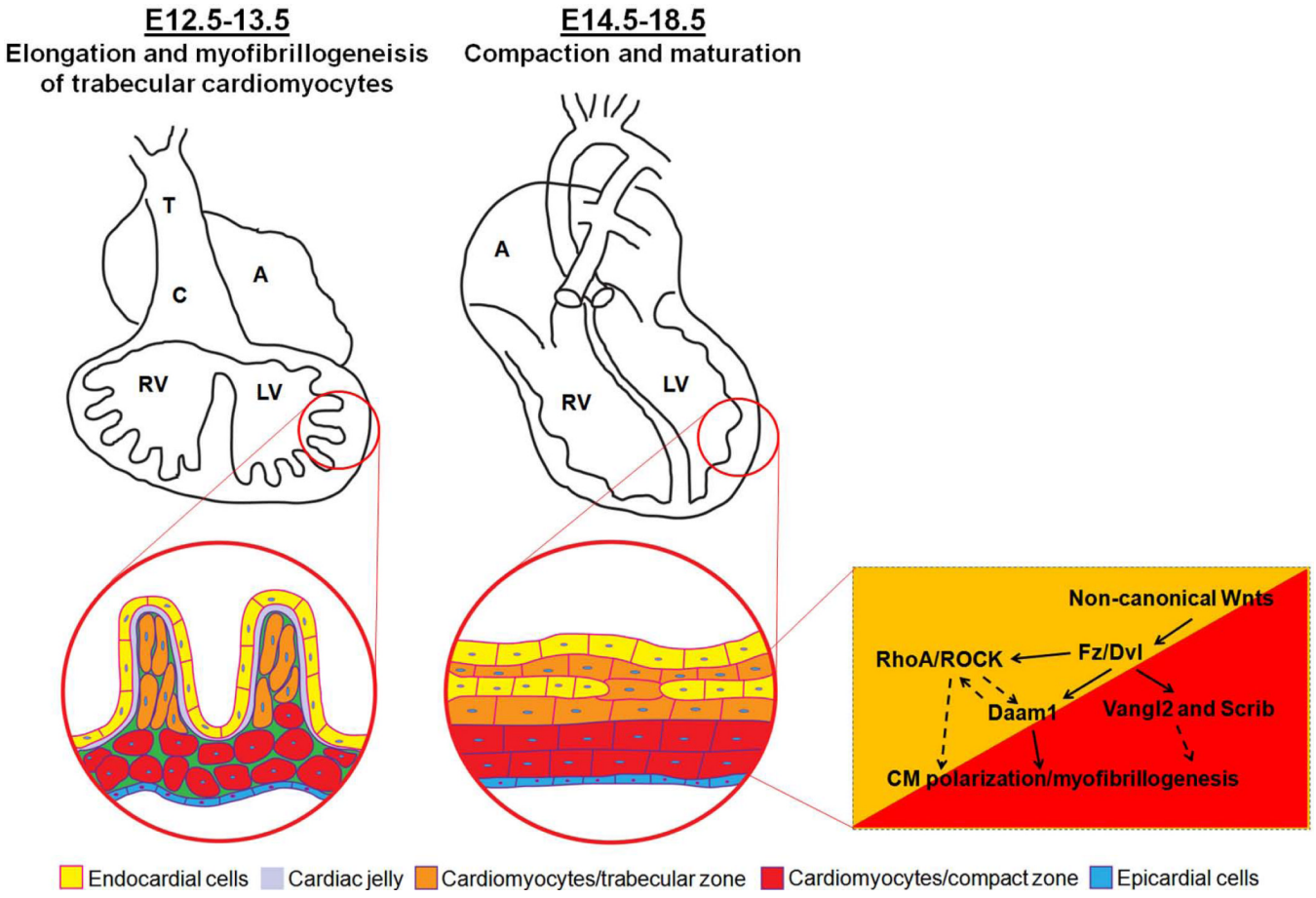

Figure 2.
Ventricular wall trabeculation and compaction. Wnt/PCP signaling components are important in cardiomyocyte polarization and myofibrillogenesis and ventricular compaction.

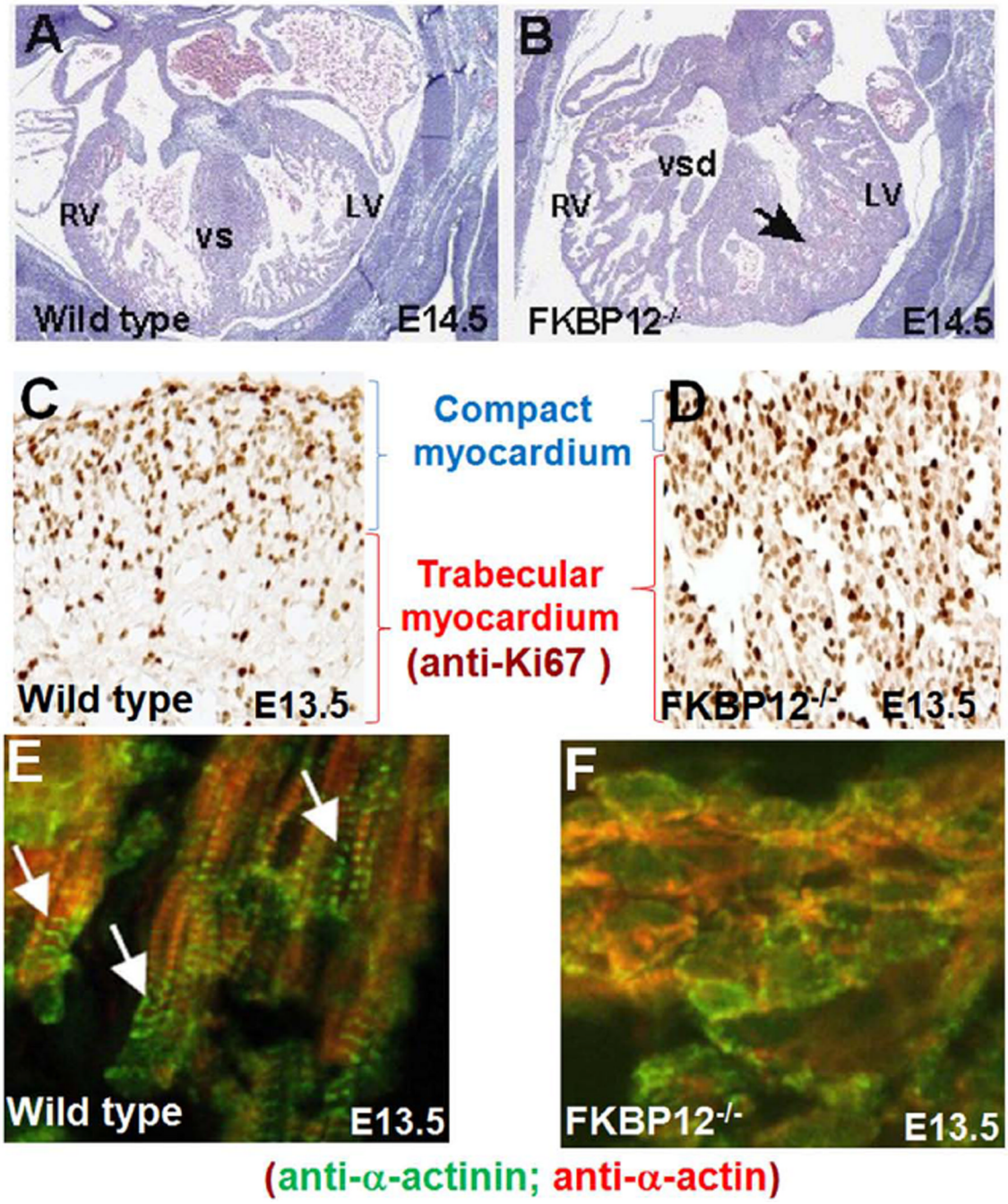

Figure 3.

Hypertrabeculation and noncompaction in FKBP12-deficient hearts. **A** and **B**, Cardiac histology of wild-type (A) and FKBP12-deficient heart (B) at E14.5. Black arrow denotes persistent trabecular myocardium in FKBP12 mutant heart. LV, left ventricle; RV, right ventricle; VS, ventricular septum; VSD, ventricular septal defect. **C** and **D**, Marked increase of cardiomyocyte proliferation in FKBP12 mutant heart. Immunohistochemical analysis of anti-Ki67 immune reactivity; the dark-brown nuclear signals are positive for Ki67, indicating proliferating cells. **E** and **F**, Disrupted cardiomyocyte polarization and myofibrillogenesis in FKBP12 mutant trabecular myocardium. Immunofluorescence

staining using anti-α-actinin and anti-α-actin antibody, white arrows denote well organized sarcomeres in elongated normal trabecular cardiomyocytes.

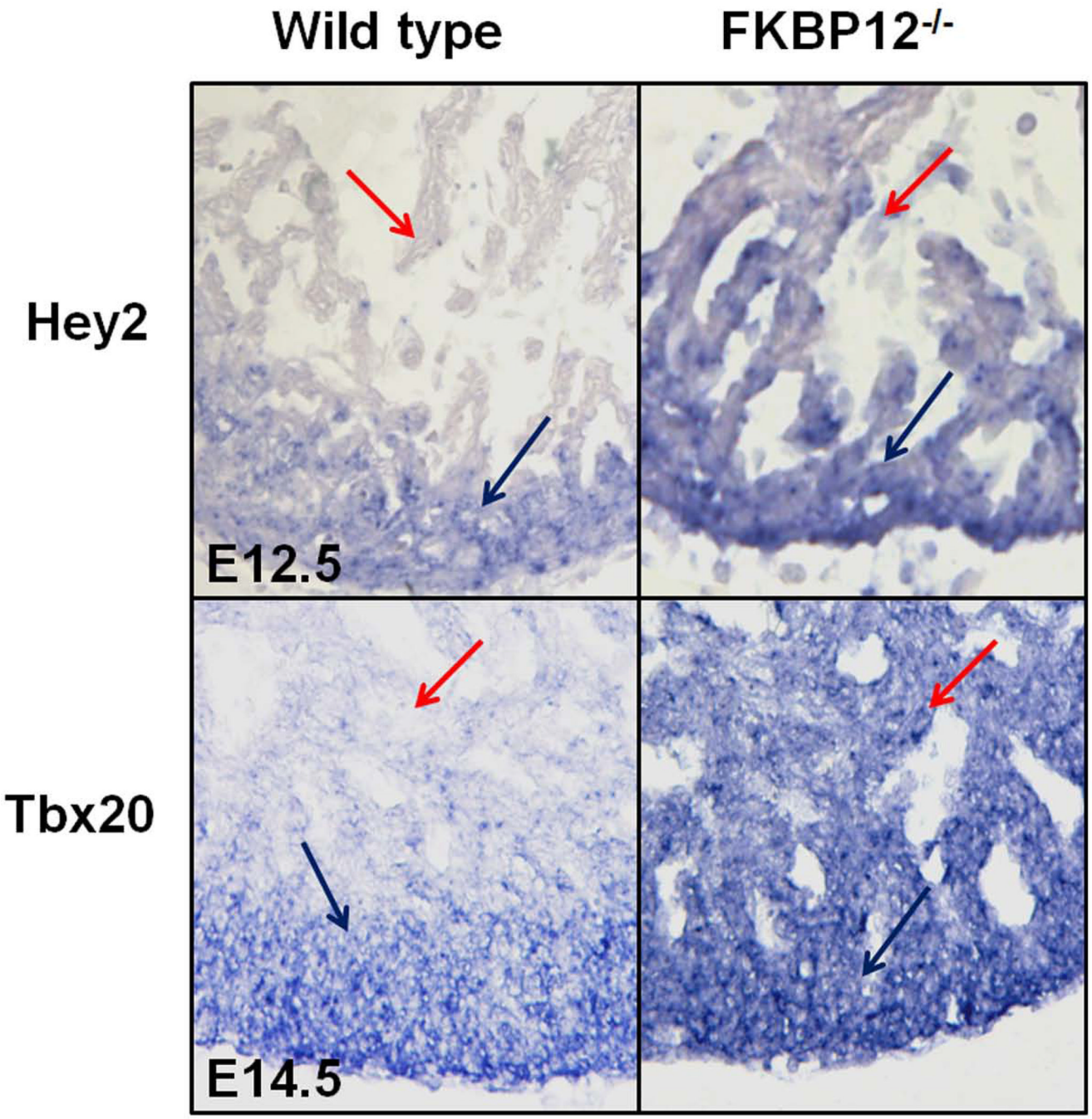

Figure 4.
Disrupted morphogenetic patterning of trabecular and compact myocardium. In situ hybridization analysis of Hey2 and Tbx20 expression; both Hey2 and Tbx20 expression are significantly higher in compact myocardium of wild-type hearts, but are expanded to trabecular myocardium in FKBP12 mutant hearts. Red arrows denote trabecular myocardium; blue arrows denote compact wall.

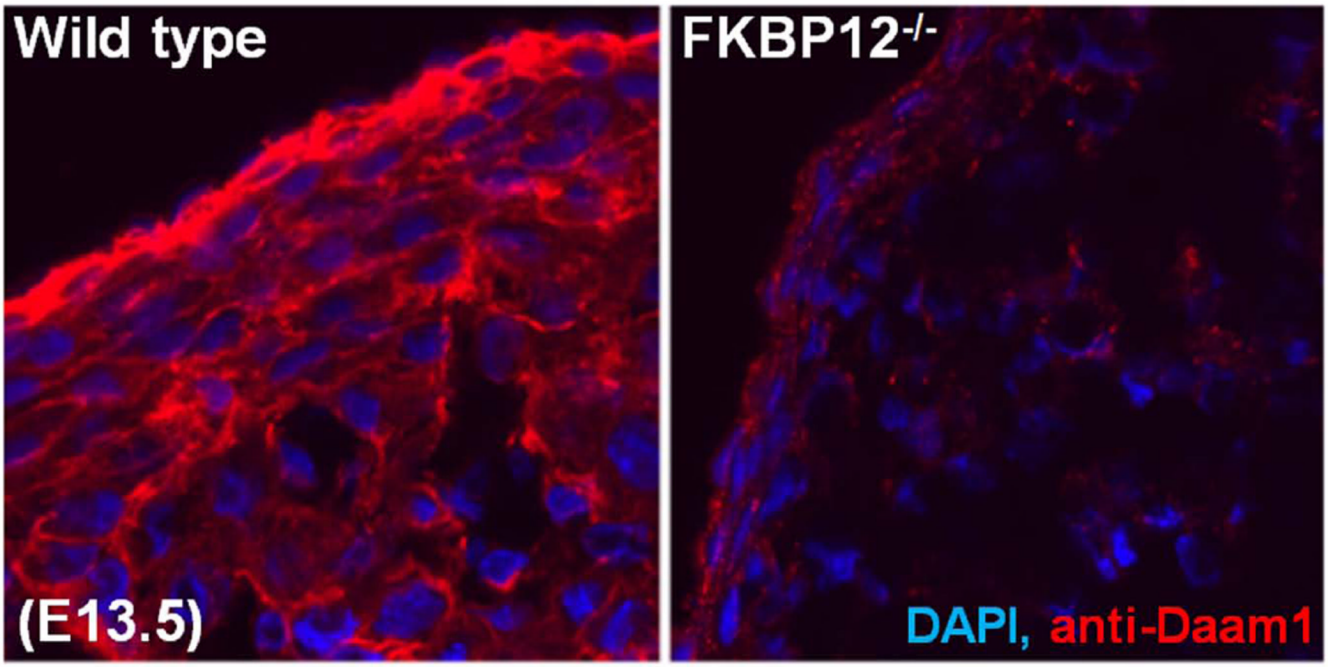

Figure 5.
Daam1 down-regulated in FKBP12 mutant hearts (E14.5). Immunofluorescence staining using anti-Daam1 antibody.

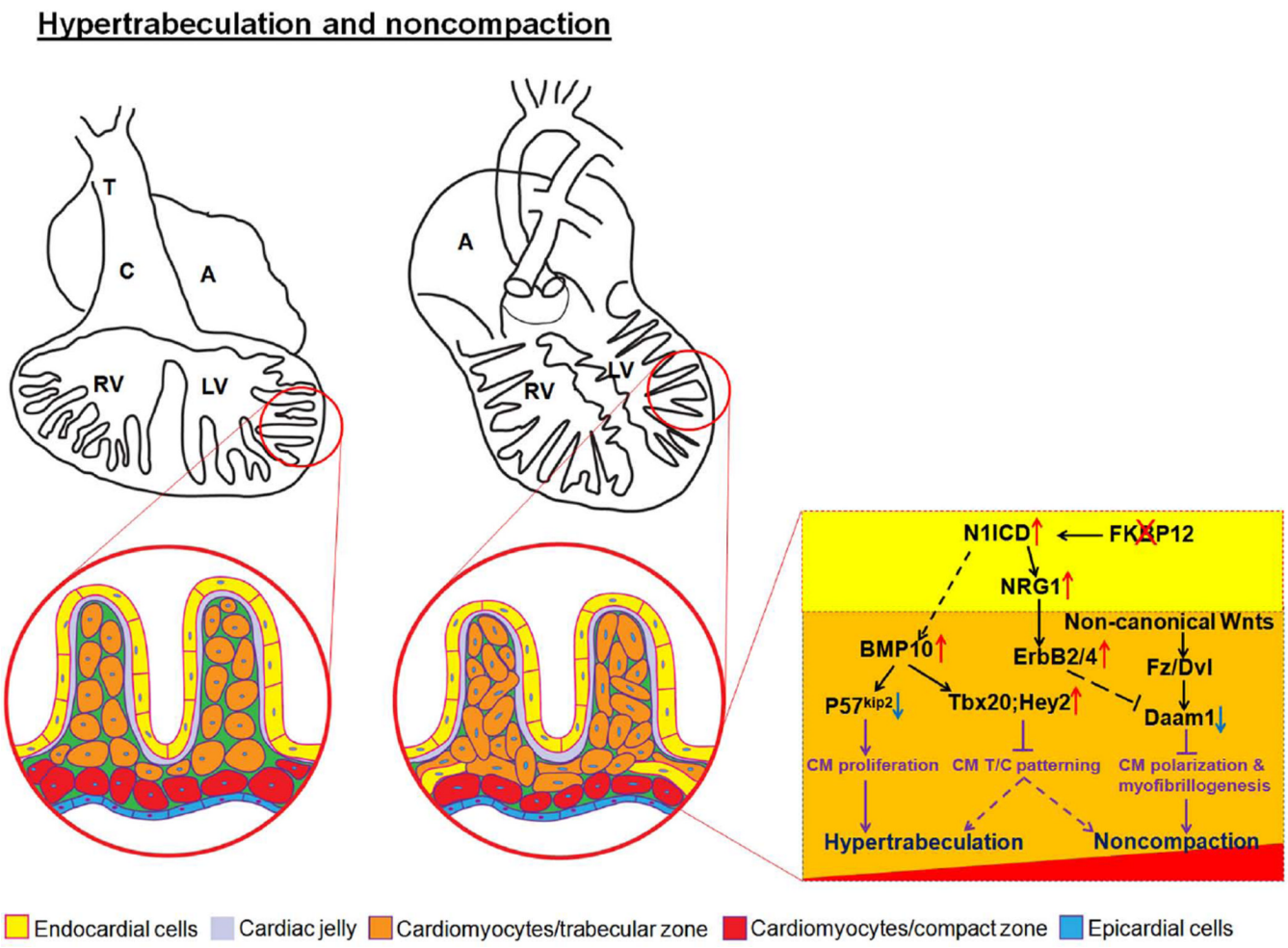

Figure 6.

Hypothetical model for the pathogenesis of ventricular hypertrabeculation and noncompaction. Two independent signaling pathways are associated with ventricular hypertrabeculation and noncompaction, in which abnormal regulation of cardiomyocyte proliferation (*i.e.*, BMP10-mediated pathway) underlies the development of hypertrabeculation, while abnormal regulation of cardiomyocyte polarity and myofibrillogenesis (*i.e.*, Daam1-mediated function) underlies the development of ventricular noncompaction.