The Impact of Mechanical Forces in Heart Morphogenesis

Javier T. Granados-Riveron, MD, PhD; J. David Brook, PhD

Congenital heart disease (CHD) is the most common birth defect in humans and results from deficient cardiac development, a complex process that is not completely understood. The heart starts to function before its morphogenesis is complete and the increasing needs of the growing embryo for oxygen and nutrients demand a proportional increase in the performance of the organ. We review recent findings supporting the hypothesis that the activity of the developing heart influences its morphogenesis and that internal or external factors, which significantly modify its functional capability, can in turn, induce anatomic cardiac defects. We propose that a role for early cardiac contraction in heart development is supported by recent experimental evidence and that the Notch pathway acts as a key transducer between hemodynamic stimuli and cardiogenesis.

Cardiac Contraction Begins Before Convective Transport Is Necessary

Several pieces of evidence suggest that heart primordium contraction starts well before the active transport of oxygen and nutrients by the circulation is required to meet the needs of the embryonic tissues. For example, rhythmic action potentials can be detected in the chick heart primordium as early as Hamburger-Hamilton stage 9 (HH9, 7-somite stage, 29–33 hours after laying). The chick heart tube starts showing contraction as early as HH10 (10-somite, 33–38 hours), whereas effective blood flow starts during looping at HH12 (16-somite, 45–49 hours). By days 3 to 4 (HH20-23), vigorous circulation is established. However, total elimination of cardiac ejection by complete ligation of the cardiac outflow tract in 3- or 4-day chick embryos has no significant effect on O2 consumption or eye growth in the 4 hours after the procedure and no correlation between cardiac output and eye, vessel growth, or body mass accumulation was observed in 24 hours after partial conotruncal ligation. Moreover, chick embryos show no hemoglobin-mediated transport of oxygen up to about 3 days of development. Similar observations have been made in Xenopus laevis and zebrafish. In both mouse and rat the first contractions of the heart primordium occur at the 3 somites stage, before a complete heart tube has been formed. Coordinate heart contraction in the mouse producing peristaltic waves starts at the E8.25 stage in the mouse embryo; nevertheless, mice with targeted mutations in which heart beat is never observed can be viable until the E9.5 or E10 stages. Thus, although the role of early cardiac contraction is unclear, recent evidence suggests that the hemodynamic force it generates has evolved as an epigenetic factor influencing cardiogenesis, in parallel with its function in convective transport.

The Role of Intracardiac Fluid Forces

Experimental alteration of the hemodynamics during cardiogenesis can have a profound effect on the development of the embryonic heart (Table). Ligation of the right lateral vitelline vein in chick embryos decreases the cardiac preload momentarily, until blood coming from the yolk sac region, normally drained by this vein, reaches the atrial cavities via the caudal plexus and veins of the left side. This change in blood flow causes cardiac morphological defects, including ventricular septal and semilunar valve defects. Conotruncal banding causes double outlet right ventricle, persistent truncus arteriosus, ventricular septal defects, right atriocentral valve defects and thickening of the compact myocardium layer. Ligation or clipping of the left atrium induces subaortic ventricular septal defect, thinning of the ventricular trabeculae and left heart hypoplasia with dysplasia, stenosis, or atresia of the atriocentral valves. Occlusion of the inflow or outflow tracts in zebrafish embryos causes defective looping and valve and chamber formation. The mechanisms by which the alteration of the blood flow cause defective cardiogenesis are unknown. However, this phenomenon is consistent with our observation that mutations in gene encoding proteins of the sarcomere, the contractile apparatus that generates the flow, cause cardiac malformation.

Mutations Affecting Contractile Proteins Cause Heart Malformations

The cardiac contractile apparatus starts to function when the myocardial cells cover the endocardial tube. Sarcomeric proteins are key components of the contractile apparatus (Figure 1) and their deficiency causes morphological heart defects in a variety of species (Table). For example, in zebrafish, mutation of 2 sarcomeric proteins, cardiac troponin T and sarcomeric actin, result in defects of endocardial cushion and valves, whereas mutation of the atrial myosin heavy chain affects ventricular morphogenesis. Similarly, disruption of atrial myosin heavy chain causes defective atrial septal formation in the chick and failure to develop valves or trabeculae in Xenopus tropicalis. Mice lacking atrial...
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myosin regulatory light chain (MLC2a) display enlarged and amorphous heart tubes, defects in looping architecture and abnormalities of cardiac chamber morphology. Mouse embryos with targeted mutations of cardiac troponin T and the Na\(^+\)-Ca\(^{2+}\) exchanger, which prevent the onset of heart beating, display looping and endocardial cushion defects as well as thin ventricular walls with underdeveloped trabeculae. The higher vertebrate models mentioned previously are limited in so far as they contribute to the understanding of the consequences of haploinsufficiency of sarcomeric genes but do not address the mechanisms by which the most common missense mutations of these genes, can cause CHD in humans.

We and others have shown that in humans, mutations of genes encoding contractile proteins expressed in the developing heart and great vessels can cause a wide spectrum of CHD (Table). Mutations of the \(\alpha\)-cardiac actin (ACTC1) and \(\alpha\)-cardiac myosin heavy chain (MYH6) genes have been found in families with autosomal dominant forms of atrial septal defect and in sporadic cases of other cardiac malformation. Changes of the \(\beta\)-cardiac myosin heavy chain (MYH7) and ACTC1 genes have been reported in families with Ebstein anomaly and ventricular noncompaction and in cases of septal defects with noncompaction. Although myosin-based contraction affects ventricular morphogenesis and endocardial cushion formation, it does not appear to affect other aspects of cardiogenesis such as c-looping. As contraction of the heart begins at the heart tube stage and continues throughout life, functional deficit of its contractile apparatus can have an impact, not only during the entire developmental process but also during postnatal life. Mutations of CHD-causing genes, ACTC1, MYH6, and MYH7 can also cause hypertrophic and dilated cardiomyopathy in the adult heart, and we propose that other genes encoding sarcomeric proteins should be analyzed in patients with CHD. A mutation in a structural protein may affect developmental growth but not contractile function due to the context and timing of protein expression. For example,

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**Table. Continued**

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VSD indicates ventricular septal defect; DORV, double-outlet right ventricle; PTA, persistence of trucus arteriosus; HLH, hypoplastic left heart; LVNC, left ventricular noncompaction; TA, tricuspid atresia; TGA, transposition of great arteries; AS, aortic stenosis; BAV, bicuspid aortic valve; AVSD, atrioventricular septal defects; TOF, tetralogy of Fallot; and EMT, epithelial-mesenchymal transition.
Figure 1. Diagram summarizing the molecular pathways involved in generation, sensing, transduction, and response to blood flow and shear stress in the developing heart. Cardiac contraction by interaction between sarcomeric proteins such as myosin heavy chain (MHC) and myosin light regulatory chains (MRLC), actin, and troponin initiate blood flow, which generates shear stress over the luminal surface of endocardial cells. PKD2 acts as a mechanosensitive calcium channel activated by shear stress. KIF3A and KIF3B participate in anterograde intraciliary transport. On mechanical stimulation, PECAM1 recruits VE-cadherin. The complex activates VEGFR2, which...

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human MYH6 is expressed in the primitive atrium, the embryonic left and right ventricle and the outflow tract during Carnegie stage 14 (31–35 days), a crucial stage of cardiac development, when chamber formation, trabeculation, and cushion formation are occurring. By Carnegie stage 15 (35–38 days), expression of MYH6 is still strong in the atria, the wall of the atrioventricular canal, and, to a lesser extent, in the ventricular trabeculae and in the cranial segments of the ventricles. By Carnegie stage 17, MYH6 expression is restricted to the atria and MYH7 is expressed mainly in the ventricles.59,60 This pattern is maintained through the rest of intrauterine development and normally remains the same from infancy to adulthood.61 During postnatal life MYH6 is expressed at a low level in comparison to MYH7 in the ventricles, which may explain the apparent lack of evidence for compromised contractile function in the majority of CHD patients harboring MYH6 mutations.

In addition to contractile deficit and the resulting hemodynamic compromise as direct causes of cardiac malformation in patients, other pathogenic mechanisms for mutations in sarcomeric protein encoding-genes could be considered. In the case of nonsense mutations, high expression of truncated toxic peptides resulting from the translation of transcripts with premature stop codons, in the presence of a compromised nonsense-mediated decay (NMD) pathway, could also affect cardiac development. We have proposed previously that the observed individual differences in the efficiency of the NMD pathway62,63 could account for the incomplete penetration of CHD in a family with a nonsense mutation of MYH6.24 Saturation of the ubiquitin-proteosome system by highly expressed misfolded proteins translated from mutant transcripts and the resulting accumulation of proapoptotic factors, such as p53, could also be a pathogenic mechanism for nonsense mutations in contractile protein genes. This mechanism has been proposed recently for hypertrophic cardiomyopathy.64

The Role of Endothelial Shear Stress

The pulsatile blood flow caused by embryonic cardiac contraction imposes a cyclic force on the organ in 2 directions. The force parallel to the blood flow induces shear stress over the endocardium, whereas the force perpendicular to the flow causes cyclic strain over the entire wall of the developing heart.

The first functional heart primordium, the heart tube, is linear. Consequently, the shear stress on the endothelial lining remains evenly distributed along its length (Figure 2A). On cardiac looping, endothelial shear forces increase in specific regions of the developing heart, most notably the developing endocardial cushions and the prospective ventricular outflow tract. As cardiogenesis progresses, shear stress increases, particularly in the atrioventricular and arterial valves forming from the endocardial cushions, and in the lining of the trabeculae.32,65 The frictional force applied to the apical surface of the endothelial cells by blood flow, or endothelial shear stress, increases the tension of the cell-cell junctions,66 resulting in the reorganization of proteins localized in these regions67 (Figure 1). The PECAM1 protein, which in endothelial cells is specifically localized to the cell-cell interface,68 has been proposed to undergo force-induced deformation resulting in tyrosine phosphorylation.69 PECAM1 is involved in several shear stress-sensing pathways in endothelial cells. Mechanical force directly transmitted from PECAM1 can, though the recruitment of VE-cadherin, activate VEGFR2, which in turn interacts with phosphatidylinositol kinase (Figure 1). In this model of mechanosensory complex, PECAM1 acts as the primary sensor, VE-cadherin as an adaptor and VEGFR2 as the effector to transduce the shear stress signal.70 Interestingly, transgenic mice deficient in VE-cadherin show normal looping but impaired chamber development and trabeculation27 and blocking of VEGFR2 signaling affects mitral and pulmonary valve morphogenesis.71

The Gq/11-G-proteins are also known to be involved in shear stress transduction in endothelial cells.72 It has been shown that chemicals decreasing the microviscosity of the cell membrane can activate Gq/11 in the absence of G-protein–coupled receptors,73 leading to the suggestion that the hemodynamic shear decreases microviscosity of the endothelial cell membrane, thus dissociating and activating Gq subunits74 (Figure 1). Additionally, the Gq11 subunits of the Gq11 proteins form a complex with PECAM1 in the endothelial cell–cell interface and this association can be disrupted on activation of Gq11, in the presence of dynamic shear stress.75 Double knockout mice for the genes encoding the Gq (Gnaq) and Gα11 (Gna11) subunits exhibit univentricular hearts and severe underdevelopment of both the trabecular and subepicardial layers of the ventricles.78

Several genes are known to be differentially expressed in endothelial cells in response to shear stress76 (Figure 1). Three of them, endothelin 1 (ET1), endothelial nitric oxide synthase (NOS3),65 and KLF2,32 are known to mediate such responses in the endothelium of the developing heart. NOS3 and KLF2 are selectively expressed in areas of the heart subjected to high levels of shear stress, whereas ET1 shows a complementary expression pattern in areas of low shear stress.32,65 Transgenic mice null for the ET1 orthologue show ventricular septal defects, double-outlet right ventricle, delayed endocardial cushion development, hypoplasia of the ventricular compact myocardium, tubular hypoplasia of the aortic arch, and interrupted aortic arch.95 Other murine models with ablation of genes involved in ET1 activation and signal transduction show similar phenotypes. Defects such as ventricular septal defects, double outlet-right ventricle, persistent truncus arteriosus, and transposition of great arteries

Figure 1 (Continued). Interacts with PI3K. Dynamic shear stress disrupts the interaction between PECAM1 and the α-Gq/11 proteins. Decreased microviscosity of the membrane can dissociate the interaction between the α and βγ subunits of Gαq/11. On dissociation, the α-Gq11 subunit translocates to the perinuclear area. Trans activation of the Notch receptors by the DELTA or JAGGED ligands releases the Notch intracellular domain (NICD), which translocates to the nucleus, whereby interaction with RBP-JK promotes the transcription of Notch target genes. Shear stress upregulates the NOS3 and KLF2 genes and downregulates the ET1 gene. Connexins allow propagation of the shear stress stimulus between endothelial cells and coordinate contraction of individual cardiomyocytes. PIP2 indicates Phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate.
Monocilia and Shear Stress Transduction

Monocilia play a dual role during morphogenesis of the heart. Their activity is crucial in left-right determination of the viscera and importantly to internal cardiogenesis, as shear stress-sensing elements in endothelial cells (Figure 1). Both motile and nonmotile monocilia are present on the epithelium of the heart tube, endothelial monocilia are located in the center of the Hensen node, whereas nonmotile monocilia are present in the periphery of the node.81 Both populations express polycystin-2 (PKD2), a mechanosensitive calcium channel that activates the production of nitric oxide in response to shear stress87 as well as KIF3A and KIF3B, 2 subunits of the trimeric kinesin complex, essential for anterograde intraciliary transport.51,83 The mechanical activity of the motile monocilia has been shown to produce a laminar leftward flow of the fluid in the amniotic cavity immediately surrounding the node.84 This nodal flow is thought to be sensed by the nonmotile primary cilia in a subset of nodal cells, which in turn induce asymmetrical expression of other signals.81 Mice with mutations of Lrd have morphologically normal but nonfunctional motile monocilia of the node. They show randomized development of visceral left-right orientation and a wide range of cardiac malformations, including heterotaxy, anomalous venous drainage, common atrioventricular canal, double-outlet right ventricle, and transposition of great arteries.33 Ablation of Pkd2 in mice causes right pulmonary isomerism, randomization of embryonic turning and heart looping, and abdominal situs as well as cardiac septation defects.34,35 Mice subjected to targeted mutation of Kif3a or Kif3b show complete absence of cilia, randomization of left-right visceral development, and incomplete or retarded cardiac looping.57–59

In the developing mouse heart, after looping of the heart tube, endothelial monocilia are found in the endocardium lining the common atria and the forming endocardial cushions and ventricular trabeculations. During septation, the endothelial monocilia persist in the ventricular trabeculations and the endocardial cushions.36,85 Randomization of the direction of looping in Lrd, Pkd2, Kif3a, or Kif3b mutants mice occurs by disruption of the system described above, which normally induces cardiac looping and rotation of the viscera to the right. However, when only embryos that undergo adequate rightward looping are analyzed, Pkd2 or
**Kif3a** deficient mice show marked thinning of the ventricular compact myocardium, decreased or absent trabeculations, and reduced cellularity of the endocardial cushions. In contrast, Lrd mutant embryos with rightward looping show normal compact myocardium and trabeculations. These data support the hypothesis that sensory monocilia have an important role in cardiogenesis independent of left-right development, through sensing of intracardiac endothelial shear stress.

### The Role of Action Conduction

Conduction of action potentials through the developing myocardium ensures a coordinated contraction of independent cardiomyocytes, which in turn induces early hemodynamically effective cardiac activity. Connexins form gap junctions that mediate ionic currents involved in the propagation of electric stimuli through the cardiac tissue (Figure 1). At least three of the connexins, Cx40, Cx43, and Cx45, are expressed in the mammalian developing heart. Mouse embryos deficient for Cx40 display endocardial cushion defects, ventricular septal defects, tetralogy of Fallot, double-outlet right ventricle, and biventricular appendages. Cx43-null mouse embryos show obstruction of the right ventricular outflow tract by hypertrophy of the trabeculae, formation of abnormal septae, intertrabecular pouches and tricuspid valve abnormalities. Additionally, transgenic mice with loss of Cx45 have looping and endocardial cushion defects as well as decreased trabeculae formation (Table).

Connexin gene mutations could cause abnormal cardiogenesis due to deficient communication between flow-stimulated endothelial cells or by contractile dysfunction secondary to deficient propagation of the action potentials through the developing myocardium. Also, the extent to which cardiac conduction per se is required for adequate cardiogenesis is developing myocardium. Also, the extent to which cardiac conduction per se is required for adequate cardiogenesis is unclear. However, studies of the zebrafish mutant dco (cx46) provide some insight. This gene is expressed in the developing heart and mutant embryos show defective outer curvature of the looped heart tube and deformed atrioventricular cardiomyocytes resulting in loss of atrioventricular canal constriction, dysmorphic ventricles, and dysynchronous ventricular contraction. Furthermore, zebrafish mutants for cardiac troponin T (sih) show cardiomyocytes with normal morphology despite their structural abnormalities and lack of heartbeat. Interestingly, dco;sih double mutants display cardiomyocyte morphology equivalent to the dco single mutant, which has led to the suggestion that electric forces per se may be necessary to preserve cardiac chamber morphology.

### The Notch Connection

As described above, data from surgical manipulation and analysis of natural and artificial mutants, suggest that formation of trabeculae and endocardial cushions are particularly sensitive changes in intracardiac hemodynamic shear stress. Trabeculae have been proposed to contribute to the formation of the papillary muscles and the conduction system as well as to ventricular septation by coalescing with the primary ventricular septum. Endocardial cushions contribute to formation of the valvular apparatus and to atrial, atrioventricular, and outflow tract septation.

Recent findings have highlighted the crucial importance of the Notch pathway in the morphogenesis of the cardiac trabeculae and endocardial cushions. In mammals, the Notch signaling pathway consists of a group of proteins that act as transmembrane ligands of the Delta and Jagged families as well as transmembrane receptors (Notch 1–4). The Notch receptors consist of both an extracellular and an intracellular domain (NICD). On activation by ligand binding to the Notch receptors, the NICD is released to the cytoplasm, from where it translocates to the nucleus and dimerizes with the recombination signal-binding protein for immunoglobulin kappa J region (RBPJ-K), which in turn activates transcription of a number of Notch target genes.

In the developing heart, Notch acts by lateral induction between neighboring endocardial cells during development. The Delta4 Notch ligand, the Notch1 receptor and the cell adhesion molecule VE-cadherin are expressed throughout the mouse endocardium before epithelial to mesenchymal transition (EMT), a crucial stage in the development of the endocardial cushions. As EMT commences in mouse at the E9.5 stage, the expression of VE-cadherin decreases in the endocardial cells lining the atrioventricular canal and the outflow tract. Some of these cells start to show high-density lipid vesicle hypertrophy, to form filopodia in the direction of the cardiac jelly and to detach from the epithelium due to the disassembly of adherens junction complexes, a consequence of the downregulation of VE-cadherin. The detached cells migrate to the cardiac jelly to form the endocardial cushion mesenchyme, which contributes to seption and formation of the atrioventricular and semilunar valves. The atrioventricular canal endocardial cells of Rbpj-k mouse mutant embryos show the ultrastructural signs of EMT but no downregulation of VE-cadherin in the atrioventricular canal and no dissolution of the adherens complexes. As a result, endocardial cells fail to migrate to the cardiac jelly and form the cushion mesenchyme, similar to the phenotype observed in Notch1 mutant embryos.

As development progresses the expression of Notch1 is highest in the prospective trabecular endocardium and, as trabeculae grow, it concentrates at the base of these structures. Notch1 and Rbpj-k mutant mouse embryos display a less dense ventricular compact zone myocardium and poorly develop trabeculae. It has been proposed that Notch activity in endocardial cells has a 2-fold role in ventricular morphogenesis. First, to promote the differentiation between compact and trabecular myocardium through the secretion of neuregulin 1 (NGR1) trough a mechanism dependent on EphrinB2, and second, to maintain the proliferating population of trabecular cardiomyocytes through a BMP10-dependent mechanism. Fine-grained patterns of cells with binary fates can be produced spontaneously by interaction between Delta4 ligands and Notch in the membrane of the same cell (cis-inhibition), in combination with transactivation of Notch receptors by ligands on the membrane of adjacent cells (Figure 2B). The binary fates of closely adjacent cells with higher or lower Notch activation are reflected in the endocardial cells lining the growing trabecular ridges (lower) compared with the cells remaining at the trabecular base (higher), and in the cells detaching from the endocardium and
undergoing EMT (higher) in contrast with those cells remaining attached to the epithelium (lower) (Figure 2C).

Interestingly, it has been shown that Notch activation and increased production of EphrinB2 occur in response to shear stress in endothelial cells. This shear stress–dependent EphrinB2 upregulation is abolished by inhibitors of the Notch pathway. Furthermore, exposure of endothelial cells to cyclic strain, the transmural direction of the hemodynamic force, upregulates Notch1 and Notch4 receptors in a temporal and dose-dependent manner.

Mutations in genes encoding factors implicated in the Notch pathway occur in monogenic forms of human CHD. For example, mutations of NOTCH1 have been reported in a family with mendelian bicuspid aortic valve with calcification and stenosis and in isolated cases of bicuspid aortic valve with and without late-onset aortic aneurism. Furthermore, mutations of JAG1, which encodes the JAGGED1 Notch ligand have been identified in a large family with autosomal dominant tetralogy of Fallot. Also, mutations of JAG1 have been identified in patients with peripheral pulmonary stenosis and with Alagille syndrome, which includes peripheral pulmonary stenosis as a feature. Mutations in NOTCH2 can also cause Alagille syndrome. These are consistent with the notion that the Notch pathway is an intermediary between hemodynamics and morphogenesis. Additionally, in the developing heart, Notch signaling has been shown to be important for other processes. Selective inhibition of Notch in cardiac neural crest derivatives in mice recapitulates several outflow tract defects observed in Alagille syndrome (aortic arch defects, pulmonary artery stenosis, and ventricular septal defects) and affects differentiation of neural crest derivatives into smooth muscle cells. Inhibition of Notch signaling in the secondary heart field results in aortic arch arteries and outflow tract abnormalities in some types of CHD. These data suggest that Notch can also act independently of mechanical factors in some aspects of cardiogenesis.

Interestedly, analysis of cardiac tissue from infants with and without 22q11.2 deletion, obtained during surgical reconstruction of tetrology of Fallot, showed a statistically significant downregulation of genes involved in the Notch pathway in comparison to tissue from developmentally normal control subjects. This has led to the suggestion that the suppression of the Notch regulatory network can result in sporadic CHD and also modify the progression of Mendelian CHD.

Conclusions

The embryonic heart starts to beat well before convective transport of oxygen, nutrients, and waste is required to sustain the tissues of the growing embryo and it has been hypothesized that cardiac activity before this stage is required for adequate morphogenesis of the heart. In the linear heart tube, shear stress is evenly distributed (Figure 2A). On looping, hemodynamic forces are redistributed to specific places of the heart primordium, which in turn induce differential expression of specific genes (Figure 1). Several factors affect this process, including mechanical manipulations that modify hemodynamics, as well as mutations in genes encoding proteins involved in cardiac contraction, sensing, and responding to shear stress (Table). These changes result in altered morphogenesis of trabeculae and endocardial cushions in particular, which, in turn, can cause defects in septation, valve formation, and ventricular wall architecture. In the early embryonic heart, shear stress and cyclic strain are the main stimuli resulting from the flow of blood over the endothelial lining, which increases the activity and expression of Notch receptors (Figure 2B). The endothelial activity of the Notch pathway is essential for epithelial-mesenchymal transition in the endocardial cushions and for trabecular formation in the developing common ventricle (Figure 2C). We propose that the contractions of the embryonic heart that occur before the requirement for convective transport, have evolved to promote proper cardiogenesis and Notch pathway activity in the embryonic endocardium is likely to be a key transducer of these hemodynamic stimuli. We also propose that the data summarized here warrants the inclusion of genes involved in the Notch pathway as well those involved in shear stress sensing and response, in future studies to detect novel mutations in CHD.

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Disclosures

None.

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