Genetic Regulation of Cardiogenesis and Congenital Heart Disease

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Annu. Rev. Pathol. Mech. Dis. 2006. 1:199–213

First published online as a Review in Advance on October 24, 2005

The *Annual Review of Pathology: Mechanisms of Disease* is online at pathmechdis.annualreviews.org

doi: 10.1146/annurev.pathol.1.110304.100039

1553-4006/06/0114-0199$20.00

Key Words

cardiac development, molecular biology, signaling, transcription factors, stem cells

Abstract

Developmental heart disorders are the most common of all human birth defects and occur in nearly one percent of the population. Survivors of congenital heart malformations are an increasing population, and it is becoming clear that genetic mutations that cause developmental anomalies may result in cardiac dysfunction later in life. This review highlights the progress in understanding the underlying molecular basis for cardiac formation and how disruption of the intricate steps of cardiogenesis can lead to congenital heart defects. The lessons learned from examining the early steps of heart formation are essential for informing the prevention of malformations and their long-term consequences, as well as for approaches to guide stem cells into cardiac lineages.

INTRODUCTION

The heart has for centuries been the subject of fascination for anatomists, embryologists, biologists, and physicians. As the organ most essential for life, the heart is the first to form in an embryo and also must support the rapidly growing embryo before it has the opportunity to shape itself into a four-chambered organ. The combination of complex morphogenetic events necessary for cardiogenesis and its superimposed hemodynamic influences may contribute to the exquisite sensitivity of the heart to perturbations. This phenomenon is reflected in the estimated 10% incidence of severe cardiac malformations observed in early miscarriages. The fraction of congenital heart malformations hemodynamically compatible with intra-uterine circulation comprise the spectrum of congenital heart defects (CHDs) that are observed clinically.

Although genetic approaches have been important in understanding human CHD, detailed molecular analysis of cardiac development in humans has been difficult. Genetic pathways that dictate cardiac development are highly conserved across vastly diverse species from flies to humans, and this has resulted in a rapid expansion of information from studies in more tractable biological models (1, 2). Despite the diversity of body structures adopted by different species, a common genetic program for the early formation of a circulatory system seems to exist. Cardiovascular systems have developed increasing complexity in order to adapt to specific environments. For example, in a simplified view, higher organisms appear to have retained the morphologic steps utilized by lower organisms and built complexity into the heart as needed. In particular, the specification of chamber structures and the advent of a parallel circulation through chamber duplication and outflow-tract division by neural crest derivatives have facilitated the development of larger, air-breathing organisms that utilize complex circulatory systems.

Clinical lessons combined with experimental studies have led to a model suggesting that unique regions and segments of the heart have been added in a modular fashion during evolution. In such a scheme, defects in particular regions of the heart may arise from specific genetic and environmental effects during discrete developmental windows. To simplify the complex events of cardiogenesis and CHD, this review considers different regions of the developing heart individually in the context described above, weaving knowledge from model systems and human genetics when available.

CARDIOMYOCYTE AND HEART TUBE FORMATION

The heart is the first organ to form in vertebrates, and it arises through a complex series of morphogenetic interactions involving cells from several embryonic origins (**Figure 1**). Progenitor cells within the anterior lateral plate mesoderm become committed to a cardiogenic fate soon after gastrulation [at approximately embryonic day (E) 15 in humans] in response to an inducing signal thought to emanate from the adjacent endoderm (3). The specific signaling molecule(s) responsible for cardiogenic commitment remains to be identified, although bone morphogenetic proteins, fibroblast growth factors (Fgfs), and Wnts appear to be critical for this step (4–6). Cardiac precursors form a bilaterally symmetric cardiogenic field that develops further into parallel cardiac primordia, which fuse at the midline to form the primitive cardiac tube (7). This straight heart tube contains an outer myocardium and an inner endocardium separated by an extracellular matrix (ECM) known as the cardiac jelly. The tubular heart initiates rhythmic contractions at approximately E23.0 in humans.

Fruitflies have a primitive heart-like structure known as the dorsal vessel, which is analogous to the straight heart tube of the vertebrate embryo. It contracts rhythmically and pumps hemolymph through an open

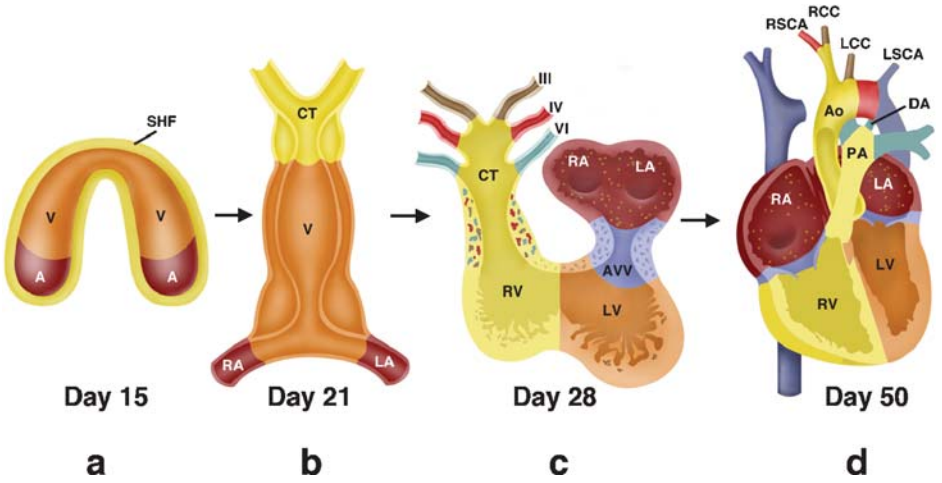

Figure 1

Schema of cardiac morphogenesis. Illustrations depict cardiac development, with morphologically related regions color-coded, seen from a ventral view. (*a*) Two distinct cardiogenic precursor fields form a crescent that is specified to form specific regions of the heart tube (A, artery; V, ventricle), which is patterned to form the various regions and chambers of the looped and mature heart. (*b*) The secondary heart field (SHF) contributes to much of the right ventricle and outflow tract as the heart loops. (*c*) Each cardiac chamber balloons from the outer curvature of the looped heart tube in a segmental fashion. Neural crest cells populate the bilaterally symmetric aortic arch arteries (III, IV, and VI) and aortic sac that together contribute to specific segments of the mature aortic arch, also color-coded. (*c*, *d*) Mesenchymal cells form the cardiac valves from the conotruncal (CT) and atrioventricular valve (AVV) segments, which divide into separate left- and right-sided valves. Corresponding days of human embryonic development are indicated. RV, right ventricle; LV, left ventricle; RA, right atrium; LA, left atrium; PA, pulmonary artery; Ao, aorta; DA, ductus arteriosus; RSCA, right subclavian artery; RCC, right common carotid; LCC, left common carotid; LSCA, left subclavian artery.

circulatory system. Formation of the dorsal vessel in flies is dependent upon a protein, tinman, whose name is based on the Wizard of Oz character that lacks a heart (8). Tinman belongs to the homeodomain family of proteins and was described initially to play a role in establishing regional identity of cells and organs during embryogenesis.

In contrast to the requirement of *tinman* gene for heart formation in flies, its mammalian orthologue, *Nkx2.5*, is not essential for specification of the cardiac lineage in mice, which suggests that either other genes may share functions with *Nkx2.5* or cardiogenesis in flies and vertebrates differs with respect to its dependence on this family of homeobox genes (9, 10). The possibility of functional redundancy between *Nkx2.5* and other cardiac-expressed homeobox genes in vertebrates is supported by the ability of dominant negative versions of Nkx2.5 transcription factor to block cardiogenesis in frog and zebrafish embryos (11, 12). Similarly, the transcriptional coactivator, myocardin, is necessary and sufficient in frogs for cardiac gene expression, likely through activation of serum response factor–dependent genes (13, 14).

The cardiac outflow tract (conotruncus) and parts of the right ventricle, which arise from a distinct population of cardiac precursors, are the last segments to form and appear to be added to the most anterior portion of the heart tube. A group of cells that lie dorsal and anterior to the cardiac precursors described above ultimately become part of the pharyngeal mesoderm (15–17). These cells appear to migrate to the conotruncus between E8.0 and E9.5, prior to neural crest invasion, and

give rise to the outflow-tract myocardium and much of the right ventricle (**Figure 1**). Secreted signals from the conotruncus apparently induce the migration of cells from the pharyngeal region, although neither the nature of the signals nor the transcriptional regulation of these cells has been established. Fgf10 is expressed in this population of cells, but its role in their development remains unknown (17).

The pharyngeal mesoderm cells described above represent a second cardiac progenitor pool that differentiates into cardiomyocytes in the arterial pole of the heart tube and is therefore often referred to as a secondary heart field. The transcription factor Tbx1, which appears to be a cause of cardiac and craniofacial disorders in humans (18–21), is a major transcriptional regulator of the secondary heart field and is necessary for proper development of conotruncal myocardium and fibroblast growth factor secretion (22–24). Islet1, a basic helix-loop-helix (bHLH) transcription factor involved in pancreatic development, also marks this population and is necessary for its development (25). Interestingly, these cells mark niches of cardiac progenitor cells in the postnatal heart (26), which suggests that understanding the regulation of secondary heart field–derived progenitor pools may be useful in developing approaches for cardiac repair.

In addition to anterior-posterior (AP) patterning, a discrete dorsal-ventral polarity is also present in the primitive heart tube. As the heart tube loops to the right, the ventral surface of the tube rotates, becoming the outer curvature of the looped heart with the dorsal surface forming the inner curvature. The outer curvature becomes the site of active growth, and remodeling of the inner curvature is essential for ultimate alignment of the inflow and outflow tracts of the heart. Researchers have proposed a model in which individual chambers balloon from the outer curvature in a segmental fashion (27). Consistent with this model, numerous genes, including the transcription factor Hand1, are expressed on specific sites of the ventral and outer curvature of the heart (28, 29). Remodeling of the inner curvature occurs, allowing migration of the inflow tract to the right and outflow tract to the left. This process facilitates proper alignment and separation of right- and left-sided circulations (**Figure 2**). Defects of inner curvature remodeling may underly a host of human congenital heart malformations that involve improper alignment of the atria, ventricles, and outflow tract and are often observed in the setting of left-right (LR) asymmetry abnormalities, whereas other cardiac defects are a result of the disruption of discrete developmental events (**Table 1**).

CARDIAC LOOPING AND LEFT-RIGHT ASYMMETRY

The pathways that control the direction of cardiac looping along the LR axis are now well-established (30). The heart is the first organ to break the bilateral symmetry that characterizes the early embryo, and the rightward direction of its looping reflects a more global establishment of LR asymmetry, which affects the lungs, liver, spleen, and gut. Defects in establishment of LR asymmetry in humans are associated with a wide range of cardiac alignment defects.

A cascade of signaling molecules that regulate the establishment of embryonic LR asymmetry was revealed initially from studies of chick embryonic development (31). Before the formation of organs in the developing embryo, asymmetric expression of the morphogen Sonic hedgehog (Shh) on the left side of Hensen's node leads to the expression of nodal and lefty, members of the transforming growth factor-β (TGF-β) family, in the left lateral mesoderm. Left-sided expression of nodal induces the rightward looping of the midline heart tube. Fibroblast growth factor and activin receptor–mediated pathways suppress right-sided nodal expression. The above signaling pathways are active in the lateral plate mesoderm, but not in the heart or other organs that actually display LR asymmetry. Ultimately, the nodal-dependent pathways

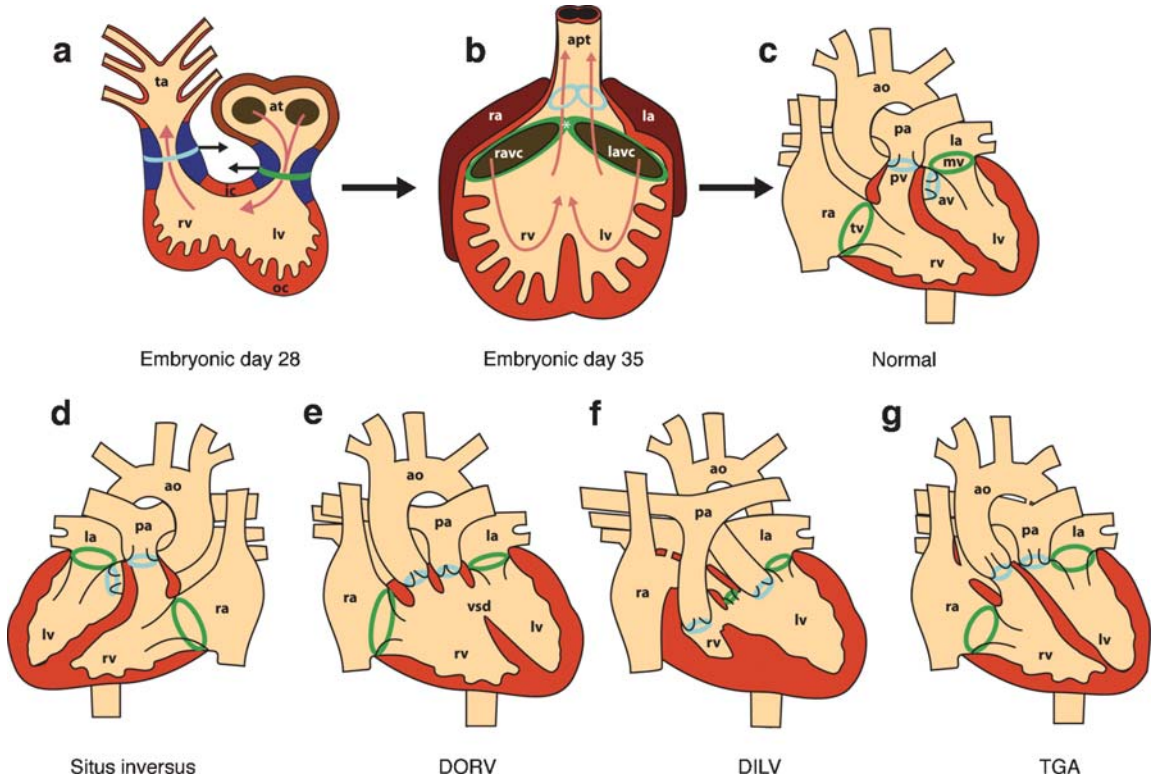

Figure 2

Normal and abnormal cardiac morphogenesis associated with left-right (LR) signaling. (*a*) As the linear heart tube loops rightward with inner curvature (ic) remodeling and outer curvature (oc) proliferation, the endocardial cushions (*dark blue*) of the inflow (*green*) and outflow (*light blue*) tracts become adjacent to one another. Subsequently, the atrioventricular septum (AVS) shifts to the right, while the aortopulmonary trunk (apt) shifts to the left. (*b*) The inflow tract is divided into the right (ravc) and left atrioventricular canal (lavc) by the AVS. The outflow tract, known as the truncus arteriosus (ta), becomes the apt upon septation. (*c*) Ultimately, the left (la) and right atrium (ra) are aligned with the left (lv) and right ventricle (rv), respectively. The lv and rv become aligned with the aorta (ao) and pulmonary artery (pa), respectively, after 180° rotation of the great vessels. (*d*) If the determinants of the LR axis are coordinately reversed, then a condition known as situs inversus results. (*e*) If the apt fails to shift to the left, then a condition known as double-outlet right ventricle (DORV) results, in which the right ventricle is aligned with both the aorta and pulmonary artery. (*f*) Likewise, if the AVS fails to shift to the right, both atria communicate with the left ventricle in a condition known as double-inlet left ventricle (DILV). (*g*) Transposition of the great arteries (TGA) results if the apt fails to twist, resulting in communication of the rv with ao and lv with pa. at, atrium; pv, pulmonary valve; av, aortic valve; mv, mitral valve; tv, tricuspid valve; vsd, ventricular septal defect. (Reproduced with permission from Reference 82).

result in expression of a homeodomain protein, Pitx2, on the left side of visceral organs and repression of Pitx2 on the right (32). Asymmetric expression of Pitx2 is sufficient for establishing the LR asymmetry of the heart, lungs, and gut, and this may occur via differential regulation of Wnt-dependent cell cycle pathways downstream of Pitx2 (33).

Genetic analysis of mouse mutants with abnormalities in LR asymmetry have illuminated some of the mechanisms controlling directionality of cardiac looping. Mice homozygous for mutation in the *LR dynein* gene

Table 1 Developmental pathway disruptions that may contribute to congenital heart defects in humans

Disruption of neural crest and SHF[1] contribution to the aortic arch
Right aortic arch
Interrupted aortic arch
Aberrant left subclavian artery origins
Vascular "rings" and "slings"
Disruption of neural crest and SHF contribution to the outflow tract
Tetralogy of Fallot
Persistent truncus arteriosus
Membranous ventricular septal defects
Double-outlet right ventricle
Disruption of aortic valve development
Aortic valve stenosis
Aortic valve atresia
Hypoplastic left heart syndrome
Calcific aortic stenosis
Disruption of pulmonary valve development
Pulmonary valve stenosis
Pulmonary valve atresia
Hypoplastic right ventricle with PA and an intact ventricular septum
Disruption of atrioventricular valve development
Complete or partial atrioventricular septal defects
Primum atrial septal defects
Cleft mitral valves
Tricuspid atresia
Mitral stenosis/insufficiency
Tricuspid stenosis/insufficiency
Ebstein's anomaly
Disruption of right ventricular development
Tricuspid atresia with ventricular septal defect
Ebstein's anomaly
Disruption of left ventricular development
Mitral atresia with ventricular septal defect
Left ventricular noncompaction
Disruption of left-right asymmetry pathways affecting alignment
Double-outlet right ventricle
Double-inlet left ventricle
Tetralogy of Fallot
Atrioventricular septal defects, particularly unbalanced
Anomalous pulmonary venous return

[1]SHF, secondary heart field.

(*iv/iv*) display random LR orientation of the heart and viscera (34, 35). In the situs inversus (*inv*) mouse model, there is nearly a 100% reversal of LR asymmetry, although the function of the *inv* gene remains unknown. *Inv* mutant mice express nodal and Pitx2 along the right lateral mesoderm rather than the left, displaying complete reversal of the LR signals (32, 36, 37). In contrast, *iv/iv* mutant mice display bilaterally symmetric, absent, or random nodal and Pitx2 expression. *Pitx2* mutant mice have abnormal LR asymmetry of the lungs and a low penetrance of reversed cardiac looping, similar to *Shh* and *Fgf8* mutant mice (38). Oddly, the initial LR asymmetry and roles of Fgf and Shh are reversed in mice and chicks; however the LR sidedness of later events involving nodal and Pitx2 are conserved (39). Additional understanding of the early events will likely lead to convergence of LR mechanisms across species.

Although the necessity of LR asymmetric gene expression is obvious, how the initial asymmetry of gene expression is established remains in question. Initial clues came from studies of immotile cilia syndrome, also known as Kartagener's syndrome, in which individuals expressed situs inversus totalis, a condition causing mirror-image reversal of all organs (40). It has been described that, prior to organ formation, Hensen's node contains ciliary processes that beat in a counter-clockwise vortical fashion (41). Whether ciliary beating moves morphogens to the left side of the embryo or if bending of cilia induced by directional flow causes subsequent asymmetric gene expression remains controversial (42). In either case, mice lacking ciliary movement in the node display abnormal LR patterning, which is consistent with that notion that nodal cilia plays a critical role in LR asymmetry (35, 43–45).

PATTERNING OF THE DEVELOPING HEART TUBE

Studies using model organisms have begun to reveal the genetic basis of a segmental, ballooning model of cardiogenesis. Numerous transcription factors in mice are expressed in a region-specific fashion, which provides a possible explanation for how distinct segments of the heart assume their respective fates. Two

related bHLH transcription factors, Hand2 and Hand1, are expressed predominantly in the primitive right- and left-ventricle segments, respectively, during mouse heart development (46, 47). Deletion of Hand2 in mice results in hypoplasia of the right ventricular segment and a thin left ventricular myocardium (47), suggesting a role for Hand2 in regulating derivatives of the secondary heart field. Hand1 is downregulated in Nkx2.5-deficient mice, who fail to segment the heart tube precisely and die around the stage of cardiac looping (29). Disruption of both Hand2 and Nkx2.5 results in the absence of the right and left ventricle, suggesting that the combined function of Hand2 and Nkx2.5, possibly through its regulation of Hand1, is necessary for ventricular formation (48). Indeed, compound loss of Hand1 and Hand2 in mice results in progressive ventricular defects with failure of ventricular expansion in mice lacking both genes (49). Consistent with the idea of an evolutionarily conserved role of Hand in ventricular expansion, zebrafish lacking the single fish Hand orthologue, Hand2, fail to expand the pool of ventricular precursors and do not develop a ventricular chamber (50).

Epigenetic factors may also contribute to ventricular morphogenesis as disruption of the chromatin remodeling protein Smyd1 (also known as mBop) results in a phenotype reminiscent of *Hand2* mutants, including a small right ventricular segment and poor development of the left ventricular myocardium (51). Smyd1 contains a SET domain that harbors histone methyltransferase activity and a MYND domain that recruits histone deacetylase activity that together are responsible for the transcriptional repression of target genes (51). Smyd1 activity is necessary for Hand2 expression in cardiac precursors of the primary heart field, likely through an intermediate that is unknown, consistent with the similar phenotype observed in targeted deletion of Hand2 or Smyd1 in mice. Interestingly, Smyd1 is a direct target of Mef2c (51a), a MADS box–containing transcription factor that is also required for right- and left-ventricle development. This suggests that a transcriptional cascade involving *Mef2c*, *Bop*, and *Hand* genes regulate ventricular cardiomyocyte development.

CONOTRUNCAL AND AORTIC ARCH DEVELOPMENT

Congenital cardiac defects involving the cardiac outflow tract, aortic arch, ductus arteriosus, and proximal pulmonary arteries account for 20%–30% of all CHDs. This region of the heart undergoes extensive and rather complex morphogenetic changes with contributions from the secondary heart field (discussed above) and neural crest–derived mesenchyme. Mutations in many signaling cascades affect neural crest migration or development, which includes the endothelin and semaphorin pathways, resulting in outflow-tract defects (52). Similarly, disruption of secondary heart field development by mutation of genes such as *Tbx1*, *Fgf8*, and *Islet1* results in persistent truncus arteriosus and malalignment of the outflow tract of the heart with the ventricular chambers (21, 25, 53, 54).

Neural crest cells are a unique population of cells along the crest of the neural folds that migrate away from the neural folds and retain the ability to differentiate into multiple cell types and are therefore pluripotent. Their migratory path and ultimate cell fates are dependent upon their relative position of origin along the anterior-posterior axis. Such neural crest cells differentiate and contribute to diverse embryonic structures, including the cranial ganglia, peripheral nervous system, adrenal glands, and melanocytes. Neural crest cells that arise from the otic placode to the third somite migrate through the developing pharyngeal arches and populate the mesenchyme of each of the pharyngeal and aortic arch arteries, the conotruncus and conotruncal septum (55). Because of their migratory path, this segment of the neural crest is often referred to as the cardiac neural crest.

Ablation of the cardiac neural crest prior to its migration away from the neural folds in

chick embryos demonstrates a critical role for neural crest cells during cardiogenesis. Embryos deficient in cardiac neural crest cells display a variety of cardiac outflow-tract and aortic-arch defects similar to those seen in humans. These include tetralogy of Fallot (TOF), persistent truncus arteriosus, double-outlet right ventricle, and conotruncal ventricular septal defects. Within the aortic arch, a broad spectrum of aortic arch anomalies were observed, including interruption of the aortic arch, aberrant origins of the right subclavian artery, and persistence of the right aortic arch rather than the left aortic arch. Thus, defects in neural crest migration or differentiation likely underlie the many conotruncal and aortic arch defects seen in humans. Indeed, human mutations of the neural crest–enriched transcription factor TFAP2b results in persistent patency of the ductus arteriosus, a specialized aortic-arch vessel essential for fetal cardiac physiology (56), and it is likely that other genetic mutations affect specific regions of the aortic arch.

CARDIAC VALVE FORMATION

Appropriate placement and function of cardiac valves is essential for chamber septation and unidirectional blood flow through the heart. During early heart tube formation, cushions of ECM between the endocardium and myocardium precede valve formation at each end of the heart tube. Reciprocal signaling, mediated in part by TGF-β family members, between the myocardium and endocardium in the cushion region induces a transformation of endocardial cells into mesenchymal cells that migrate into the cushion of ECM. These mesenchymal cells differentiate into the fibrous tissue of the valves and are involved in septation of the common atrioventricular canal into right- and left-sided orifices. The transcription factor NFATc is expressed specifically during the formation of embryonic valves, and targeted deletion of NFATc in mice results in the absence of cardiac valve formation (57, 58).

Although lack of cardiac valve leaflets is a rare cardiac anomaly, thickened valve leaflets resulting in stenotic valves are a common form of CHD. In mouse models, the absence of the *Ptpn11* gene, which encodes the protein tyrosine phosphatase Shp-2, results in dysplastic semilunar valves via its involvement in a signaling pathway mediated by epidermal growth factor receptor (59). The importance of *PTPN11* in congenital heart disease was shown by the identification of point mutations in *PTPN11* in patients with Noonan syndrome, whose phenotype commonly includes pulmonic valve stenosis (60).

The Smad proteins are intracellular transcriptional mediators of signaling initiated by TGF-β ligands. Smad6 is expressed specifically in the atrioventricular cushions and outflow tract during cardiogenesis and is a negative regulator of TGF-β signaling. Targeted disruption of *Smad6* in mice results in thickened and gelatinous atrioventricular and semilunar valves, similar to those observed in human aortic and pulmonary valve disease (61). As with loss of Smad6 in mice, lack of Ephrin B2 also results in thickened valves, although the mechanism for this remains unclear (62).

The Notch signaling pathway is required for cell fate and differentiation decisions in the embryo (63), but has only recently been implicated in cardiovascular development. In fish and frogs, Notch appears to be involved in the development of the endocardial cushions that contribute to valve tissue (64). Consistent with this observation, the authors recently discovered that nonsyndromic, autosomal-dominant pedigrees with aortic valve disease segregate with missense mutations in *NOTCH1* (65). The *NOTCH1* mutations cause a developmental anomaly in the aortic valve in which two of the three valve leaflets fail to separate, resulting in a bicuspid rather than tricuspid aortic valve. *NOTCH1* mutations cause a wide spectrum of aortic valve diseases, ranging from severe newborn-onset cardiac disease to milder valve disease, which is initially asymptomatic but manifests

in the fourth or fifth decade of life as severe and premature valve calcification.

The finding that *NOTCH1* mutations segregate with severe aortic valve calcification led to the recognition that Notch1 negatively regulates a central regulator of osteoblast-specific gene expression, Runx2, and may do so in the valve through the physical interaction of the hairy-related transcriptional repressor Hrt with Runx2 (65–67). It is possible that *NOTCH1* variants may be predictors of individuals at higher risk for developing valve calcification, providing an opportunity to identify genetically those who may benefit from more aggressive management of other known risk factors such as hypercholesterolemia and diabetes. This is another example of how identification of genetic causes of cardiac malformations may lead to prevention of related adult-onset cardiac disease.

Genetic studies of Alagille syndrome also support an important role for Notch signaling in human disease. Alagille syndrome is an autosomal-dominant disorder characterized by biliary atresia and cardiac defects, typically in the form of pulmonary artery stenosis and TOF, which is caused by mutations in *JAGGED-1*, a ligand for the Notch receptor (68, 69). Isolated pulmonary stenosis, or TOF, has also been associated with *JAGGED-1* mutations, consistent with the notion that it plays a role in valve development (70).

GENETICS OF HUMAN VALVULOSEPTAL DEFECTS

The study of chromosomal disorders and autosomal-dominant syndromes in the setting of CHD, and genetic linkage analysis of rare pedigrees with milder forms of CHD, have been informative, particularly in conjunction with functional studies in model organisms. Recent genetic studies of cardiac transcription factors NKX2.5, TBX5, and GATA4 exemplify the parallels in etiology between model organisms and human genetic studies of CHD. Numerous point mutations have been identified in *NKX2.5* in families with atrial septal defects and cardiac conduction abnormalities (71). Sporadic mutations of *NKX2.5* have also been found in patients with TOF and tricuspid valve anomalies (72). How these mutations result in cardiac defects is unknown, but the linkage of specific types of loss-of-function mutations with distinct abnormalities suggests that different structural determinants of NKX2.5 have unique functions in developmental decisions in the heart. Mutations in *NKX2.5* cause not only a developmental defect, but may also cause cardiac disease later in life, which highlights the notion that genetic causes of CHD may also underlie adult disease. This realization may provide an avenue to study preventive approaches for the later sequelae of congenital heart malformations and adult-onset heart disease (73, 74).

Humans with Holt-Oram syndrome, caused by mutations in *TBX5*, are characterized by cardiac anomalies similar to those with *NKX2.5* mutations (atrial and ventricular septal defects) and also limb abnormalities (75, 76). Intriguingly, mutations responsible for defects in the heart and limbs are clustered in different regions of the protein, which suggests that TBX5 engages different downstream genes or cofactors in these tissues that depend upon unique structural motifs in the protein.

Like individuals with *NKX2.5* and *TBX5* mutations, nonsyndromic, autosomal-dominant pedigrees that harbored mutations in the zinc finger–containing protein GATA4 were recently described (77). GATA4 or its relatives are essential for cardiogenesis in flies, fish, and mice (78–81). Interestingly, *GATA4* mutations cause atrial and ventricular septal defects that are very similar to those observed in the setting of *NKX2.5* and *TBX5* mutations. Correspondingly, GATA4 and TBX5 interact and cooperate physically to activate transcription (76). A familial *GATA4* point mutation disrupts GATA4's ability to interact with TBX5, and several human TBX5 mutations disrupt its interaction with GATA4, which suggests that the two cooperate in cardiac septation

events (77). A genetic interaction between the two proteins has been confirmed as mice heterozygous for *Gata4* and *Tbx5* die during embryogenesis from severe hypoplasia of the endocardial cushion tissue necessary for valuloseptal development (V. Garg & D. Srivastava, unpublished observations). The endocardial cushion defect is similar to that observed in the setting of Trisomy 21 (Down Syndrome) in humans, and it will be interesting to determine if *GATA4*, *TBX5*, or other related genes are modifier genes for the cardiac phenotype of Trisomy 21.

CONCLUSIONS

Over the past decade investigators have witnessed remarkable advances in our understanding of cardiogenesis, and the heart has become one of the most well-understood organs in biology. This knowledge is now being used to discover the underlying genetics of CHD and the basis for many adult-onset diseases that have their origin in mutations of developmental genes. What is lacking is a coherent picture of the hierarchical pathways that govern most developmental processes and the mechanisms through which such pathways regulate the cell biology of morphogenesis. Such knowledge will require a better understanding of the target genes of critical transcriptional and signaling pathways that control cardiogenesis. Research in this area will be an essential step, as the targets will be the most amenable sites of intervention, both in a therapeutic sense and for the purpose of prevention. For example, identification of dietary substances that modulate key developmental pathways such as folic acid, which prevents neural tube defects, will be necessary for preventive efforts. In addition, early genetic identification of those at risk for adult-onset disease originating from a cardiac developmental defect, such as the age-related calcification that occurs in bicuspid aortic valve, will provide ample time for intervention to slow the progression of disease. The convergence of additional developmental knowledge and improved genetic tools should make this vision a reality in the coming years.

ACKNOWLEDGMENTS

D.S. was supported by grants from the National Heart, Lung, and Blood Institute/National Institutes of Health, March of Dimes Birth Defects Foundation, and American Heart Association's Established Investigator Award.

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Annual Review of Pathology: Mechanisms of Disease

Volume 1, 2006

Contents