

GENETIC ASSEMBLY OF THE HEART: Implications for Congenital Heart Disease

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■ **Abstract** More children die from congenital heart defects (CHD) each year than are diagnosed with childhood cancer, yet the causes remain unknown. The remarkable conservation of genetic pathways regulating cardiac development in species ranging from flies to humans provides an opportunity to experimentally dissect the role of critical cardiogenic factors. Utilization of model biological systems has resulted in a molecular framework in which to consider the etiology of CHD. As whole genome sequencing and single nucleotide polymorphism data become available, identification of genetic mutations predisposing to CHD may allow preventive measures by modulation of secondary genetic or environmental factors. In this review, genetic pathways regulating cardiogenesis revealed by cross-species studies are reviewed and correlated with human CHD.

INTRODUCTION

The heart has captured the imagination of mankind for centuries because of its elegant simplicity yet relentless capacity to support a living organism. As the organ most essential for life, the heart is the first organ to form in an embryo and must function to 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 the 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 that are hemodynamically compatible with the intrauterine circulation compose the spectrum of congenital heart defects (CHD) that is observed clinically.

The anatomic features of most CHD in humans have been carefully catalogued. Although CHD was classified in the 18th and 19th centuries based upon

embryologic considerations, the advent of palliative procedures and clinical management led to a descriptive nomenclature founded on anatomic and physiologic features that governed surgical and medical therapy. However, seemingly unrelated CHD could be argued to share common embryologic origins from a mechanistic standpoint, suggesting that the etiology of CHD may be better understood by considering their developmental bases. Recent advances in genetics and molecular biology have stimulated a renaissance in seeking an embryologic framework for understanding CHD as genetically based alterations. Null mutations in genes that function during cardiac development established that abnormalities in cardiovascular ontogeny can be a primary cause of embryonic demise. The ability to go beyond descriptions of the anatomical defects to developing an understanding of the genes responsible for distinct steps of cardiac morphogenesis is necessary for more directed therapeutic and preventive measures.

Although human genetic approaches have been important in understanding CHD, detailed molecular analysis of cardiac development in humans has been difficult. The recognition that cardiac genetic pathways are highly conserved across vastly diverse species from flies to humans has resulted in an explosion of information from studies in more tractable and accessible biological models. The fruit fly (*Drosophila*) has been a source of discovery for genes involved in early cardiac determination events. Although no biological system is ideal for studying human disease, *Drosophila* has several advantages. It has a simple genome and usually has a single copy of genes that often have three or four orthologues in vertebrates; genetic studies are facilitated by rapid breeding times; and, most importantly, its DNA can be chemically mutated in a random fashion followed by phenotypic analysis and reverse genetics to identify the DNA mutation associated with distinct developmental defects. Similar chemical mutagenesis efforts have been successful in another model system, the zebrafish. Zebrafish have the added advantages of being vertebrates. They have a more complex two-chambered heart, and because the embryos grow in water, they have a heart that is easily visible and not necessary for survival during the period of cardiac development. Although genetic approaches are not feasible in chick embryos, they have four-chambered hearts, and the embryos are easily accessible within the egg for surgical and molecular manipulation during cardiogenesis. The chick has thus been useful in cell fate analyses and defining the role of populations of cells during development. Finally, use of the laboratory mouse, a mammal with a cardiovascular system nearly identical to humans, has been invaluable in understanding the mechanisms underlying human disease through direct gene targeting. Thus each biological system offers unique opportunities to develop a deeper understanding of cardiogenesis.

Despite the diversity of body plans adopted by different species, a genetic program for the early formation of a circulatory system has remained of central importance. The essential conservation of these programs is reflected in their repeated use of core elements like the *tinman/Nkx2.5/Csx* homeobox gene, whose expression first specifies the precardiac lineage in *Drosophila*, zebrafish, *Xenopus*, chick, mouse, and human (1). The cardiovascular systems of these organisms have

evolved with increasing complexity in order to adapt to specific environments. In a simplified view, it appears that higher organisms have retained the morphologic steps utilized by lower organisms and have built complexity into the heart in a modular fashion (2–4). 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 utilizing complex circulatory systems.

In this review, anatomic, molecular, and clinical aspects of cardiac embryology are interwoven to develop a framework in which to consider the etiology of human congenital cardiac defects. Clinical lessons combined with experimental studies in mice, chick, fish, and flies have led to a model suggesting that unique regions and segments of the heart have been added in a modular fashion during evolution. In this model, defects in particular regions of the heart may arise from unique genetic and environmental effects during specific developmental windows of time. To simplify the complex events of cardiogenesis, unique regions of the developing heart are considered individually in the context described above.

Cardiomyocyte Differentiation 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 (5) (Figure 1). Beginning soon after gastrulation (about embryonic day 18 in humans), progenitor cells within the anterior lateral plate mesoderm become committed to a cardiogenic fate in response to an inducing signal thought to emanate from the adjacent endoderm (6). The specific signaling molecule(s) responsible for cardiogenic commitment remains to be identified, although members of the transforming growth factor β (TGF- β) family, including bone morphogenetic protein-2 (BMP-2), appear to be critical for this step (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 about day 21 in humans.

Fruitflies have a primitive heart-like structure known as the dorsal vessel that is analogous to the straight heart tube of the vertebrate embryo. It contracts rhythmically and pumps hemolymph through an open circulatory system. In flies, a member of the TGF- β family, decapentaplegic (*dpp*), is essential for the initial determination of a cardioblast (8). Formation of the dorsal vessel in flies is dependent on a protein, Tinman, whose name is based on the Wizard of Oz character that lacks a heart (9, 10). Tinman belongs to the homeodomain family of proteins that was initially described to play a role in establishing regional identity of cells and organs during embryogenesis. Tinman is necessary for specification of the cardiac lineage and directly activates transcription of the *Mef2* gene, encoding a transcription factor that controls myocyte differentiation (11). Tinman and Nkx2.5,

its mammalian orthologue, directly interact with zinc-finger transcription factors of the GATA family to activate cardiac gene expression (12, 13).

In mice, Nkx2.5 is not required for initial cardiac specification, suggesting that some degree of genetic redundancy exists for the early but not later functions of Nkx2.5 (14, 15). This hypothesis is supported by the observations that dominant-negative versions of Nkx2.5 and its relative, Nkx2.3, are able to prevent early cardiogenesis in frog and zebrafish embryos (16, 17).

Bilaterally symmetric cardiac primordia converge along the ventral midline of the embryo to form a beating linear heart tube composed of distinct myocardial and endocardial layers (Figure 1). Mutations of GATA proteins in mice and zebrafish have demonstrated a critical role for this family of transcription factors in midline fusion of the heart tube (18–20). In an example of the power of zebrafish genetics, positional cloning of a gene responsible for a cardiac bifida phenotype, *miles apart*, has revealed a role for lysophospholipids in vertebrate development (21). The *miles apart* gene encodes a novel sphingosine 1-phosphate receptor that may be mediating a midline signal to attract cardiomyocytes from the lateral aspect of the early embryo, although the mechanisms of cell movement and fusion are yet to be determined.

As the straight heart tube takes shape, five distinct tubular segments form in a temporal sequence, along the anterior-posterior (AP) axis (7). The primitive right and left ventricles are the first to be distinguished, followed by the atrioventricular canal segment. The sino-atrial segment forms most caudally and has distinct left-right (LR) asymmetry with the right and left limbs of this segment later contributing to the right and left atria, respectively. The conotruncus is the last segment to form and lies in the most anterior portion of the heart tube. As the heart tube loops to the right, the cardiac chambers begin to become distinguished morphologically (Figure 1).

In addition to the AP segmentation, a discrete dorsal-ventral (DV) polarity is 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 while remodeling of the inner curvature is essential for ultimate alignment of the inflow and outflow tracts of the heart. A model in which individual chambers “balloon” from the outer curvature in a segmental fashion has been proposed (22). Consistent with this model, numerous genes are expressed specifically on the ventral and outer curvature of the heart (22, 23). Remodeling of the inner curvature allows migration of the inflow tract to the right and outflow tract to the left, facilitating proper alignment and separation of right and left-sided circulations.

Cardiac Looping and Left-Right Asymmetry

The pathways that control the direction of cardiac looping along the LR axis have recently been elucidated (Figure 2) (reviewed in 24). The heart is the first organ to break the bilateral symmetry present in the early embryo, and the rightward

direction of its looping reflects a more global establishment of LR asymmetry that 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, suggesting that pathways regulating LR asymmetry dramatically affect cardiac development.

A cascade of signaling molecules regulating the establishment of embryonic LR asymmetry has been revealed from recent studies of chick embryonic development. 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 left lateral mesoderm expression of nodal and lefty, members of the TGF- β family (25). Transfer of this signal from the node to the lateral mesoderm is mediated by the secreted molecule, caronte (26, 27). Caronte inhibits BMP on the left side, relieving BMP-mediated repression of nodal in the left lateral plate mesoderm. Left-sided expression of nodal induces rightward looping of the midline heart tube. Fibroblast growth factor and activin receptor-mediated pathways suppress caronte expression on the right side, and the resulting activity of BMP signaling results in suppression of right-sided nodal expression. Conversely, the snail-related (cSnR-1) zinc finger transcription factor is expressed in the right lateral mesoderm and is repressed by Shh on the left (28). 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 result in expression of a homoeodomain protein, Pitx2, on the left side of visceral organs and repression of Pitx2 on the right (29–31). Asymmetric expression of Pitx2 is sufficient for establishing the LR asymmetry of the heart, lungs, and gut.

The mechanisms that control directionality of cardiac looping have also been explored by genetic analysis of mouse mutants with abnormalities in LR asymmetry. Mice homozygous for mutation in the *left-right dynein* gene (*iv/iv*) display randomization of left-right orientation of the heart and viscera (32). In the situs inversus (*inv*) mouse model, there is a nearly 100% reversal of LR asymmetry, although the function of the *inv* gene remains unknown (33, 34). *Inv* mice express nodal and Pitx2 along the right lateral mesoderm rather than the left, displaying complete reversal of the LR signals (35, 36). In contrast, *iv/iv* mice display bilaterally symmetric, absent, or randomization of nodal and Pitx2 expression. Confirming a critical role for Pitx2 in LR asymmetry, *Pitx2* mutant mice have abnormal LR pulmonary asymmetry (37, 38). Oddly, the initial LR asymmetry and roles of fg8 and shh are opposite in mice and chicks; however, the LR sidedness of later events involving nodal and Pitx2 are conserved in all species studied (39).

Although the necessity of LR asymmetric gene expression is intuitive, how the early asymmetry of molecules is established remains in question. Initial clues came from studies of immotile cilia syndrome, also known as Kartagener's syndrome, in which individuals had situs inversus totalis, with mirror-image reversal of all organs (40). It was recently found that, prior to organ formation, Hensen's node contains ciliary processes that beat in a vortical fashion (41). It is currently believed that a combination of ciliary beating that moves morphogens to the left side of the embryo and establishment of a midline barrier, possibly by *lefty* gene expression along the

left midline, are responsible for subsequent asymmetric gene expression. Mice lacking ciliary movement in the node display abnormal LR patterning, consistent with this model.

It has been proposed that abnormalities in the process of cardiac looping underlie a number of CHD. Folding of the heart tube positions the inflow cushions adjacent to the outflow cushions and involves extensive remodeling of the inner curvature of the looped heart tube. In the primitive looped heart, the segments of the heart are still in a linear pattern and must be repositioned considerably for alignment of the atrial chambers with the appropriate ventricles and the ventricles with the aorta and pulmonary arteries. The atrioventricular septum (AVS) begins to divide the common atrio-ventricular canal (AVC) into a right and left AVC that subsequently shifts to the right to position the AVS over the ventricular septum. This allows the right AVC and the left AVC to be aligned with the right and left ventricles, respectively. Simultaneously, the conotruncal region becomes septated into the aorta and pulmonary trunks as the conotruncus moves toward the left side of the heart such that the conotruncal septum is positioned over the AVS. The rightward shift of the AVS and leftward shift of the conotruncus converts the single-inlet, single-outlet heart into a four-chambered heart that has separate atrial inlets and ventricular outlets (42).

Arrest or incomplete movement of the AVS or conotruncus might result in malalignment of the inflow and outflow tracts. A scenario in which the AVS fails to shift to the right would result in communication of the right and left AVCs with the left ventricle, a condition known as double-inlet left ventricle (DILV). Incomplete shifting may be the basis for unbalanced AVC defects where the right AVC only partly communicates with the right ventricle. Similarly, if the conotruncal septum fails to shift to the left, both the aorta and pulmonary artery would arise from the right ventricle causing a double-outlet right ventricle (DORV). Thus any abnormality in cardiac looping can be associated with DILV or DORV, along with other manifestations of improper alignment of specific regions of the heart.

It is likely that patients with situs inversus totalis have a well-coordinated reversal of LR asymmetry and thus have a lower incidence of defects in visceral organogenesis. However the majority of patients with LR defects have viscerotaxial heterotaxy and thus have randomization of cardiac, pulmonary, and gastrointestinal situs, similar to the *iv/iv* mouse in which coordinated signaling is absent. Such patients can have defects in almost all aspects of cardiogenesis. Often either the right or left side predominates with patients having either bilateral right-sidedness (asplenia syndrome) or bilateral left-sidedness (polysplenia syndrome). In such cases, features of the right or left side of the lungs, heart, and gut are duplicated. Disruption of cascades determining either the left or right side of the embryo might result in asplenia or polysplenia syndromes, respectively. For example, targeted deletion of the activin receptor *Iib* in a mouse model results in randomization of heart, lung, and gut placement with a predominance of right-sidedness (43). Indeed, mutations in LR pathway members are found in some patients with heterotaxy (44). Familial cases of heterotaxy have also led to

identification of mutations in a zinc-finger transcription factor, *ZIC3*, that results in LR axis abnormalities (45).

Patterning of the Developing Heart Tube

Recent studies in numerous model organisms have begun to reveal the genetic basis of a segmental ballooning model of cardiogenesis. Separable regulatory regions of genes such as *Nkx2.5*, *GATA-5,6*, *myosin light chain 2V*, and *myosin light chain 1F* direct expression to specific chambers of the heart (reviewed in 46). Correspondingly, numerous transcription factors are expressed in a chamber-specific fashion, providing a possible mechanism to explain how distinct segments of the heart adopt their respective fates.

dHAND and eHAND are related basic helix-loop-helix (bHLH) transcription factors expressed predominantly in the primitive right and left ventricle segments, respectively, during mouse heart development (47, 48). Deletion of dHAND in mice results in hypoplasia of the right ventricular segment from a cell survival defect (49). Mice lacking eHAND die early from placental defects precluding detailed analysis of its role in left ventricular development (50, 51). However, eHAND is down-regulated in *Nkx2.5*-deficient mice, which die around the stage of cardiac looping (52). Disruption of both dHAND and *Nkx2.5* results in absence of the right and left ventricle, suggesting that the combined function of dHAND and *Nkx2.5*, possibly through its regulation of eHAND, is necessary for ventricular formation (H Yamagishi & D Srivastava, unpublished observations). In zebrafish, which has a single ventricle, only one *HAND* gene has been identified (dHAND) (53), deletion of which results in lack of a ventricular segment of the heart (54), similar to that seen in the absence of dHAND and *Nkx2.5*. Expression of the ventricular-specific homeobox gene of the Iroquois family, *Irx4*, is dependent upon both dHAND and *Nkx2.5*, and misexpression of *Irx4* in the atria is sufficient to activate ventricle-specific gene expression (55, 56). These findings suggest that HAND and *Nkx2.5* proteins may cooperate in early ventricle-specific decisions. Interestingly, deletion of *MEF2C*, one of the four *MEF2* factors in mice, results in hypoplasia of the right and left ventricles but not of the atria (57). The chamber-specific role of *MEF2C*, in spite of its homogenous expression in the heart, suggests that it might be a necessary co-factor for one or more of the other ventricular-restricted regulatory proteins.

In contrast to the ventricle-specific transcription factors, the orphan nuclear receptor, COUP-TFII, is expressed specifically in the atrial precursors and is required for atrial but not ventricular growth (58). How the segmental pattern of gene expression is established remains unclear; however, retinoid signaling has been implicated in atrial specification and regulation of the atrioventricular (AV) border along the AP axis of the heart tube (59). The recent discovery of a novel class of hairy-related transcription factors (*HRT1*, *HRT2*, *HRT3*) may also provide some insight (60). Hairy proteins often function downstream of the transmembrane receptor, Notch, in establishing boundaries of gene expression. Interestingly, *HRT1*

and HRT2, are expressed in a complementary fashion in the atria and ventricles, respectively. How the many transcription factors function in a coordinated manner to regulate chamber specification and differentiation remains to be determined.

Myocardial Growth

Mutations of a wide variety of genes in mice result in hypoplasia of the muscular wall of the heart (61). Mice homozygous for a null mutation in the *retinoid X receptor- α* (*RXR α*) gene display ventricular chamber hypoplasia and have a defect in compaction of the myocardium, although this may not be a cell-autonomous effect (62). A similar phenotype is seen in mice carrying mutations in the *N-myc*, *TEF-1* and *neurofibromatosis (NF-1)* genes (63–65). Deficiencies of the cell adhesion molecules alpha-4 integrin, VCAM, and Wilm's tumor (*WT-1*) genes result in epicardial dissolution and subsequent myocardial thinning (66, 67). The diversity of genes affecting myocardial growth suggests that this aspect of cardiac development is particularly sensitive to perturbations.

Signaling between the endocardium and the myocardium also appears to be important for ventricular growth. Neuregulin growth factors are expressed in the endocardium and are required for the development of trabeculae, the finger-like projections of the ventricular myocardium. In mice deficient in neuregulin or its receptors, erbB2 and erbB4, the ventricular trabeculae fail to form, possibly as a result of decreased endocardial signals (68–70). Similar defects in ventricular trabeculation have been observed in mice lacking angiogenic factors that are also expressed in the endocardium (71, 72). Defects in myocardial formation and contractility are also observed in the *cloche* zebrafish mutant, which lacks endocardial cells, consistent with an important role for endocardial-myocardial interactions (73).

Cardiac Valvulogenesis

Formation of cardiac valves allows for chamber septation and for coordinated flow of blood from the inflow to the outflow segments of the heart. During early heart tube formation, "cushions" of extracellular matrix between the endocardium and myocardium presage 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 ECM (74). 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.

Recent studies in mice have provided an entry to understand the transcriptional mechanisms of valvulogenesis. The transcription factor, NFATc, is expressed specifically in the forming embryonic valves, and targeted deletion of *NFATc* in mice results in absence of cardiac valve formation (75, 76). In contrast, a transcription factor that mediates TGF- β signaling, Smad6, is also expressed specifically in the cardiac valve precursors, but mutation of *Smad6* in mice leads to

abnormally thickened, gelatinous valves, similar to those seen in some human valvular disease (77). Trisomy 21 (Down syndrome) in humans is commonly associated with incomplete septation of the atrioventricular valves; however, the gene(s) on chromosome 21 responsible for valve development remains unknown. Further genetic analysis of NFATc and Smad6 and use of the Trisomy 16 mouse model of Trisomy 21 may provide insight into the molecular mechanisms of valve septation.

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 CHD. This region of the heart undergoes extensive and rather complex morphogenetic changes. The cardiac outflow tract can be divided into the muscularized conus and the adjacent truncus arteriosus, collectively termed the conotruncus, as it arises from the primitive right ventricle. The conotruncus normally shifts to the left to override the forming ventricular septum. The truncus arteriosus then becomes septated by mesenchymal cells into the aorta and pulmonary arteries, with a muscular ridge forming between the two vessels known as the conotruncal septum. However, at this stage, the aorta communicates with the right ventricle and the pulmonary artery with the left ventricle. Subsequent rotation of the two vessels in a spiraling fashion places the aorta in a more dorsal and leftward position and the pulmonary artery in a more ventral and rightward location (Figure 1). This spiraling event achieves the normal alignment of the aorta and pulmonary artery to the left and right ventricles, respectively. Abnormalities in septation or incomplete spiraling of the conotruncus result in many CHD. For example, the conotruncal septum between the aorta and pulmonary artery forms in tetralogy of Fallot (TOF), but because of mal-alignment of the great vessels, the conotruncal septum and aorta are shifted to the right. This results in an aorta that overrides the ventricular septum and failure of the conotruncal septum to connect to the muscular ventricular septum, and consequently a ventricular septal defect. Similarly, any mal-alignment of the conotruncus results in an obligatory ventricular septal defect that, unlike muscular VSDs, does not have the potential to close spontaneously after birth.

A structure referred to as the aortic sac lies rostral to the conotruncus and gives rise to six bilaterally symmetric vessels known as aortic arch arteries. The aortic arch arteries arise sequentially along the AP axis, each traversing a pharyngeal arch before joining the paired dorsal aortae. The first and second arch arteries involute and the fifth arch artery never fully forms. The third, fourth, and sixth arch arteries undergo extensive remodeling to ultimately form distinct regions of the mature aortic arch and proximal pulmonary arteries (Figure 1). The majority of the right-sided dorsal aorta and aortic arch arteries undergo programmed cell death leading to a left-sided aortic arch. The third aortic arch artery contributes to the proximal carotid arteries. The left fourth aortic arch artery forms the transverse aortic arch between the left common carotid and left subclavian arteries. Finally,

the sixth arch artery contributes to the proximal pulmonary artery and the ductus arteriosus (78). Extrapolating from their embryologic origins, it is believed that aberrant subclavian arteries and other subtle arch anomalies are the result of third or fourth aortic arch defects; interrupted aortic arch from fourth arch defects; and patent ductus arteriosus and proximal pulmonary artery hypoplasia/discontinuity from defects in sixth arch artery development.

Contribution of the Cardiac Neural Crest to Cardiogenesis

A unique population of cells along the crest of the neural folds (neural crest cells) 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. 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. Because of their migratory path this segment of the neural crest is often referred to as the cardiac neural crest (79).

Surgical, and more recently laser, ablation of the cardiac neural crest prior to migration away from the neural folds in chick embryos demonstrated a critical role for neural crest cells during cardiogenesis (80). Embryos deficient in cardiac neural crest cells displayed a variety of cardiac outflow tract and aortic arch defects similar to those seen in humans. These included 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 children.

Insight into the genes that regulate cardiac neural crest development has come from studies in other vertebrate models. Mice lacking endothelin-1 (ET-1) or its G protein-coupled receptor, ET_A , have post-migratory cardiac neural crest defects including cleft palate and other craniofacial anomalies reminiscent of 22q11 deletion syndrome in humans (81, 82). *dHAND* and *eHAND* are normally expressed in the neural crest-derived pharyngeal and aortic arches, but are down-regulated in *ET-1*- and *ET_A*-deficient mice, although low levels of expression are detectable. These data suggest that enhancement of *HAND* expression is regulated by ET-1 signaling (83). Complete absence of *dHAND* as observed in *dHAND* null mice results in a severe survival defect of pharyngeal and aortic arch mesenchyme (83), consistent with a critical role for *dHAND*. In a screen for genes downstream of *dHAND*, neuropilin-1, a semaphorin and VEGF receptor, was found to be down-regulated in *dHAND* mutants. Interestingly, targeted mutation of *neuropilin-1* results in conotruncal and aortic arch defects similar to that of *ET-1* mutants,

suggesting that ET-1, dHAND and neuropilin-1 may function in a common pathway regulating the cardiac neural crest (84, 85).

The aortic arch undergoes a tremendous amount of remodeling in a segmental fashion, much like the heart, and each segment of the mature aortic arch develops relatively independently. Disruption of *Mfh1*, a forkhead transcription factor, specifically affects the fourth aortic arch artery in mice and results in absence of the transverse aortic arch (86), a phenotype resembling interruption of the aortic arch seen in humans. Mice harboring mutations in the homeodomain protein, *pax3*, or mutations in retinoic acid receptors also have a variety of outflow tract and aortic arch defects (87, 88). A zebrafish mutant, *gridlock*, has been described in which coarctation (narrowing) of a discrete region of the aorta is observed. Recent positional cloning efforts have demonstrated that the *gridlock* phenotype is the result of a point mutation in a gene encoding the zebrafish orthologue of the bHLH transcription factor, *Hesr2/HRT2b/Hey2* (89). How these hairy-related transcription factors mediate Notch signaling during aortic arch patterning will be an important area for future study. Finally, evidence for independent regulation of the sixth aortic arch artery comes from the third most common CHD in which the ductus arteriosus, an embryonic vessel connecting the aorta and pulmonary artery, fails to close after birth. Heterozygous mutations of the transcription factor, *TFAP2B*, can result in familial patent ductus arteriosus, suggesting a role for *TFAP2B* in governing patency or closure of this vessel (90).

Human Genetic Studies

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. Although few CHD have known genetic etiologies, monoallelic microdeletion of chromosome 22q11 is commonly associated with a variety of cardiac neural crest anomalies (91). The 22q11 deletion, typically 3 million base pairs (Mb) in size, is the most common human gene deletion and is the second most common known genetic cause of CHD, after Trisomy 21. Patients with this deletion often have other neural crest-derived defects, including cleft palate and other typical facial features, thymic hypoplasia, and hypoparathyroidism. The phenotypic spectrum is often referred to as DiGeorge, velocardiofacial, or Shprintzen syndrome, all of which are associated with the same 22q11 deletion (92–94). Thus it is likely that one or more genes involved in neural crest development lie in the 22q11 locus.

Sequencing of the commonly deleted 22q11 region revealed nearly 30 genes, of which several are expressed in developing neural crest cells (95). However, the genetic complexity and heterogeneity of this syndrome has precluded definitive determination of the critical gene(s) in this region. Attempts to model this deletion in a mouse model have met with limited success. A large heterozygous deletion of the syntenic region of 22q11 in mice (chromosome 16) encompassing 15 genes resulted in a partial penetrance of fourth aortic arch anomalies, including interruption

of the aortic arch and aberrant right subclavian artery (96). Unfortunately, none of the other cardiac or extracardiac manifestations of 22q11 deletion in humans were observed in these mice with significant frequency.

Within the commonly deleted region, *UFD1*, *TBX1*, and *HIRA* are expressed in neural crest-derived cells and may play some role in the 22q11 deletion phenotype. *Ufd1*, involved in ubiquitin-dependent degradation of short-lived cellular proteins in yeast (97,98), is down-regulated in *dHAND*-null mice and was specifically deleted, along with a neighboring cell cycle regulator, *CDC45*, in a patient with the typical features of 22q11 deletion (99). However, heterozygous mutation of *ufd1* alone does not cause any apparent defects in mice whereas homozygosity is embryonic lethal (96). *HIRA*, a transcriptional co-repressor in yeast, physically interacts with Pax3 and may thus play a role in Pax3 regulation of the cardiac neural crest (100,101). However, similar to *ufd1*, mice heterozygous for mutation in *HIRA* are normal and homozygous deletions are embryonic lethal. Finally, *TBX1*, a transcription factor expressed in the pharyngeal arches, was specifically deleted along with *COMT* (catechol-*O*-methyltransferase), in a patient with DiGeorge-like features (102). However, heterozygosity of *Tbx1* in mice does not result in any aortic arch deformity. Thus it appears that heterozygosity of a single gene in the 22q11 locus may not be sufficient for the aortic arch phenotype, although heterozygosity of a dominant gene may be necessary.

Genetic linkage analysis of several families with atrial septal defects (ASD) and conduction defects revealed a previously unrecognized role for the homeodomain-containing transcription factor, *NKX2.5*, in later phases of cardiac development and function. Numerous point mutations, clustered in the homeodomain region, have been identified in patients with ASD, providing a genetic etiology for a subset of one of the most common CHD (103). Sporadic mutations of *NKX2.5* have also been found in patients with TOF and tricuspid valve anomalies (104), suggesting that *NKX2.5* plays multiple roles during cardiogenesis that may be impacted upon by genetic background and/or environmental effects.

Holt-Oram syndrome, an autosomal dominant disease characterized by cardiac (atrial and ventricular septal defects) and limb anomalies, is caused by mutations in *TBX5* (105, 106). The heart and limbs are derived from lateral mesoderm precursors that may share common developmental regulatory pathways. Interestingly, mutations in distinct regions of *TBX5* can result in families that have predominance of either cardiac or limb defects. This observation suggests that *TBX5* may be regulating different genetic pathways in specific tissues based on interactions with unique cofactors (107). In fact, zebrafish mutations in the *dHAND* gene result in the absence of a ventricular chamber, fin abnormalities, and a failure of *Tbx5* expression (54), raising the possibility that *dHAND* functions upstream of a *Tbx5*-dependent pathway regulating limb and heart development. Accordingly, *dHAND* is required for expression of sonic hedgehog in the zone of polarizing activity of the developing limb and misexpression of *dHAND* is sufficient to induce ectopic *Shh* expression and mirror image duplications of posterior skeletal elements in limbs of mice and chicks (108, 109).

Alagille syndrome, another autosomal dominant disorder characterized by biliary atresia and cardiac defects, typically pulmonary artery stenosis and TOF, is caused by mutations in *JAGGED-1*, a ligand for the Notch receptor (110, 111). Isolated pulmonary stenosis or TOF has also been associated with *JAGGED-1* mutations (112). The Notch signaling pathway is involved in cell fate and differentiation decisions throughout the embryo but has only recently been implicated in cardiovascular development. How Notch-related pathways function to establish the vascular connections to the heart remains to be determined.

SUMMARY

Complementary studies utilizing model organisms and those primarily involving human genetics have revealed some of the complex molecular steps necessary for formation of the heart. The preliminary outlines of the mechanisms underlying cardiogenesis will provide a foundation for further dissection of the molecular pathways governing development of individual regions of the heart. This effort should be greatly facilitated by emerging technologies that capitalize on high throughput strategies in the area of genomics and proteomics. Complete sequencing of the human genome will allow for identification of single nucleotide differences in critical genes in those with or without heart disease. The combination of such approaches may ultimately identify those individuals or their progeny who have increased genetic risk for heart disease.

Discovery of the causes of complex genetic traits, such as CHD, has been difficult. However, the observation that secondary factors, be they genetic or environmental, contribute to CHD provides hope for the treatment and prevention of CHD. While prospects for gene therapy remains in the distant future, knowledge of the genetic pathways regulating cardiogenesis should lead to some of the secondary factors that may be modulated during the period of embryonic heart development. Given the rapid pace of discovery and the ever-increasing tools available to scientists and clinicians, the hope of translating genetic information regarding heart formation into tangible benefits for families with CHD has never been brighter.

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LITERATURE CITED

1. Harvey RP. 1996. NK-2 homeobox genes and heart development. *Dev. Biol.* 178:203–16
2. Srivastava D, Olson EN. 2000. A genetic blueprint for cardiac development. *Nature* 407:221–26
3. Fishman MC, Olson EN. 1997. Parsing the heart: genetic modules for organ assembly. *Cell.* 91(2):153–56
4. Fishman MC, Chien KR. 1997. Fashioning the vertebrate heart: earliest embryonic decisions. *Development* 124:2099–117

5. Harvey R, Rosenthal N. 1999. *Heart Development*. San Diego: Academic. 530 pp.
6. Schulthesis TM, Xydias S, Lassar AB. 1995. Induction of avian cardiac myogenesis by anterior endoderm. *Development* 121:4203–14
7. DeHaan RL. 1965. Morphogenesis of the vertebrate heart. In *Organogenesis*, ed. RL DeHaan, H Ursprung, pp. 377–420. New York: Holt, Reinhart & Winston
8. Frasch M. 1995. Induction of visceral and cardiac mesoderm by ectodermal Dpp in the early Drosophila embryo. *Nature* 374(6521):464–67
9. Bodmer R. 1993. The gene *tinman* is required for specification of the heart and visceral muscles in Drosophila. *Development* 118:719–29
10. Azpiazu N, Frasch M. 1993. *Tinman* and *bagpipe*: two homeo box genes that determine cell fates in the dorsal mesoderm of Drosophila. *Genes Dev.* 7:1325–40
11. Gajewski K, Kim Y, Lee YM, Olson EN, Schulz RA. 1997. D-mef2 is a target for *Tinman* activation during Drosophila heart development. *EMBO J.* 16:515–22
12. Gajewski K, Fossett N, Molkentin JD, Schultz RA. 1999. The zinc finger proteins Pannier and GATA4 function as cardiogenic factors in Drosophila. *Development* 126:5679–88
13. Durocher D, Charron F, Warren R, Schwartz RJ, Nemer M. 1997. The cardiac transcription factors Nkx2-5 and GATA-4 are mutual cofactors. *EMBO J.* 16:5687–96
14. Lyons I, Parsons LM, Hartley L. 1995. Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeobox gene Nkx2-5. *Genes Dev.* 9:1654–66
15. Tanaka M, Chen Z, Bartunkova S, Yamasaki N, Izumo S. 1999. The cardiac homeobox gene *Csx/Nkx2.5* lies genetically upstream of multiple genes essential for heart development. *Development* 126(6):1269–80
16. Fu Y, Yan W, Mohun TJ, Evans SM. 1998. Vertebrate tinman homologues XNkx2-3 and XNkx2-5 are required for heart formation in a functionally redundant manner. *Development* 125:4439–49
17. Grow MW, Kreig PA. 1998. Tinman function is essential for vertebrate heart development: elimination of cardiac differentiation by dominant inhibitory mutants of the tinman-related genes, XNkx2-3 and XNkx2-5. *Dev. Biol.* 204:187–96
18. Molkentin J, Lin Q, Duncan SA, Olson EN. 1997. Requirement of the GATA4 transcription factor for heart tube formation and ventral morphogenesis. *Genes Dev.* 11:1061–72
19. Kuo CT, Morrisey EE, Anandappa R, Sigrist K, Lu MM, et al. 1997. GATA4 transcription factor is required for ventral morphogenesis and heart tube formation. *Genes Dev.* 11:1048–60
20. Reiter JF, Alexander J, Rodaway A, Yelon D, Patient R, et al. 1999. Gata5 is required for the development of the heart and endoderm in zebrafish. *Genes Dev.* 13:2983–95
21. Kupperman E, An S, Osborne N, Waldron S, Stainier DY. 2000. A sphingosine-1-phosphate receptor regulates cell migration during vertebrate heart development. *Nature* 406(6792):192–95
22. Christoffels VM, Habets PEMH, Franco D, Campione M, de Jong F, et al. 2000. Chamber formation and morphogenesis in the developing mammalian heart. *Dev. Biol.* 223:266–78
23. Thomas T, Yamagishi H, Overbeek PA, Olson EN, Srivastava D. 1998. The bHLH factors, dHAND and eHAND, specify pulmonary and systemic cardiac ventricles independent of left-right sidedness. *Dev. Biol.* 196(2):228–36
24. Capdevila J, Vogan KJ, Tabin CJ, Belmonte JC. 2000. Mechanisms of left-right determination in vertebrates. *Cell* 101:9–21
25. Levin M, Johnson RL, Stern CD, Kuehn M, Tabin CJ. 1995. A molecular pathway

- determining left-right asymmetry in chick embryogenesis. *Cell* 82:803–14
26. Yokouchi Y, Vogan KJ, Pearse RV II, Tabin CJ. 1999. Antagonistic signaling by Caronte, a novel Cerberus-related gene, establishes left-right asymmetric gene expression. *Cell* 98:573–83
 27. Rodriguez-Esteban C, Capdevila J, Economides AN, Pascual J, Ortiz A, Izpisua-Belmonte JC. 1999. The novel Cer-like protein Caronte mediates the establishment of embryonic left-right asymmetry. *Nature* 401:243–51
 28. Isaac A, Sargant MG, Cooke J. 1997. Control of vertebrate left-right asymmetry by a snail-related zinc finger gene. *Science* 275:1301–4
 29. Piedra ME, Icardo JM, Albajar M, Rodriguez-Rey JC, Ros MA. 1998. Pitx2 participates in the late phase of the pathway controlling left-right asymmetry. *Cell* 94(3):319–24
 30. Logan M, Pagan-Westphal SM, Smith DM, Paganessi L, Tabin CJ. 1998. The transcription factor Pitx2 mediates site-specific morphogenesis in response to left-right asymmetric signals. *Cell* 94:307–17
 31. Ryan AK, Blumberg B, Rodriguez-Esteban C, Yonei-Tamura S, Tamura K, et al. 1998. Pitx2 determines left-right asymmetry of internal organs in vertebrates. *Nature* 394:545–51
 32. Supp DM, Witte DP, Potter SS, Brueckner M. 1997. Mutation of an axonemal dyenin in the left-right asymmetry mouse mutant *inversus viscerum*. *Nature* 389:963–99
 33. Mochizuki T, Saijoh Y, Tsuchiya K, Shirayoshi Y, Takai S, et al. 1998. Cloning of *inv*, a gene that controls left/right asymmetry and kidney development. *Nature* 395:177–81
 34. Morgan D, Turnpenny L, Goodship J, Dai W, Majumder K, et al. 1998. *Inversin*, a novel gene in the vertebrate left-right axis pathway, is partially deleted in the *inv* mouse. *Nat. Genet.* 20:149–56
 35. Campione M, Steinbeisser H, Schweickert A, Deissler K, van Bebber F, et al. 1999. The homeobox gene Pitx2: mediator of asymmetric left-right signaling in vertebrate heart and gut looping. *Development* 126:1225–34
 36. Yoshioka H, Meno C, Koshiba K, Sugihara M, Itoh H, et al. 1998. Pitx2, a bicoid-type homeobox gene, is involved in a left-signaling pathway in determination of left-right asymmetry. *Cell* 94:299–305
 37. Lin CR, Kioussi C, OConnell S, Briata P, Szeto D, et al. 1999. Pitx2 regulates lung asymmetry, cardiac positioning and pituitary and tooth morphogenesis. *Nature* 401:279–82
 38. Lu MF, Pressman C, Dyer R, Johnson RL, Martin, JF. 1999. Function of Rieger syndrome gene in left-right asymmetry and craniofacial development. *Nature* 401:276–78
 39. Meyers EN, Martin GR. 1999. Differences in left-right axis pathways in mouse and chick: functions of FGF8 and SHH. *Science* 285:403–6
 40. Ajzelius BA. 1976. A human syndrome caused by immotile cilia. *Science* 193:317–19
 41. Nonaka S, Tanaka Y, Okada Y, Takeda S, Harada A, et al. 1998. Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell* 95(6):829–37
 42. Mjaatvedt CH, Yamamura H, Wessels A, Ramsdell A, Turner D, Markwald RR. 1999. Mechanisms of segmentation, septation, and remodeling of the tubular heart: endocardial cushion fate and cardiac looping. In *Heart Development*, ed. RP Harvey, N Rosenthal. San Diego: Academic. 530 pp.
 43. Oh SP, Li E. 1997. The signaling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. *Genes Dev.* 11:1812–26
 44. Kosaki R, Gebbia M, Kosaki K, Lewin

- M, Bowers P, et al. 1999. Left-right axis malformations associated with mutations in ACVR2B, the gene for human activin receptor type IIB. *Am. J. Med. Genet.* 82(1):70–76
45. Gebbia M, Ferrero GB, Pilia G, Bassi MT, Aylsworth A, et al. 1997. X-linked situs inversus and situs ambiguous result from mutations in the zinc-finger transcription factor ZIC3. *Nat. Genet.* 17:305–8
 46. Schwartz RJ, Olson EN. 1999. Building the heart piece by piece: modularity of cis-elements regulating Nkx2.5 transcription. *Development* 126:4187–92
 47. Srivastava D, Cserjesi P, Olson EN. 1995. A new subclass of bHLH proteins required for cardiac morphogenesis. *Science* 270:1995–99
 48. Srivastava D. 1999. HAND proteins: molecular mediators of cardiac development and congenital heart disease. *Trends Cardiovasc. Med.* 9(1–2):11–18
 49. Srivastava D, Thomas T, Lin Q, Kirby ML, Brown D, Olson EN. 1997. Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND. *Nat. Genet.* 16:154–60
 50. Firulli AB, McFadden DG, Lin Q, Srivastava D, Olson EN. 1998. Heart and extra-embryonic mesodermal defects in mouse embryos lacking the bHLH transcription factor Hand1. *Nat. Genet.* 18(3):266–70
 51. Riley P, Anson-Cartwright L, Cross JC. 1998. The Hand1 bHLH transcription factor is essential for placenta and cardiac morphogenesis. *Nat. Genet.* 18:271–75
 52. Biben C, Harvey RP. 1997. Homeodomain factor Nkx2-5 controls left/right asymmetric expression of bHLH gene eHand during murine heart development. *Genes Dev.* 11:1357–69
 53. Angelo S, Lohr J, Lee KH, Ticho BS, Breitbart RE, Hill S, et al. 2000. Conservation of sequence and expression of Xenopus and Zebrafish dHAND during cardiac, branchial arch and lateral mesoderm development. *Mech. Dev.* 95:231–37
 54. Yelon D, Ticho B, Halpern ME, Ruvinsky I, Ho RK, et al. 2000. Parallel roles for the bHLH transcription factor HAND2 in zebrafish and pectoral fin development. *Development* 127:2573–82
 55. Bruneau BG, Bao ZZ, Tanaka M, Schott JJ, Izumo S, et al. 2000. Cardiac expression of the ventricle-specific homeobox gene *Irx4* is modulated by *Nkx2-5* and *dHand*. *Dev. Biol.* 217:266–77
 56. Bao Z, Bruneau BG, Seidman JG, Seidman CE, Cepko CL. 1999. Regulation of chamber-specific gene expression in the developing heart by *Irx4*. *Science* 283:1161–64
 57. Lin Q, Schwarz J, Bucana C, Olson EN. 1997. Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. *Science* 276:1404–7
 58. Pereira FA, Qui Y, Zhou G, Tsai M, Tsai S. 1999. The orphan nuclear receptor COUP TFII is required for angiogenesis and heart development. *Genes Dev.* 13:1037–49
 59. Dyson E, Sucov HM, Kubalak SW, Schmid-Schonbein GW, DeLano FA, et al. 1995. Atrial-like phenotype is associated with embryonic ventricular failure in retinoid X receptor alpha *-/-* mice. *Proc. Natl. Acad. Sci. USA* 92:7386–90
 60. Nakagawa O, Nakagawa M, Richardson JA, Olson EN, Srivastava D. 1999. HRT1, HRT2, and HRT3: a new subclass of bHLH transcription factors marking specific cardiac, somitic, and pharyngeal arch segments. *Dev. Biol.* 216(1):72–84
 61. Rossant J. 1996. Mouse mutants and cardiac development: new molecular insights into cardiogenesis. *Circ. Res.* 78(3):349–53
 62. Sucov HM, Dyson E, Gumeringer CL, Price J, Chien KR, et al. 1994. RXR α mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis. *Genes Dev.* 8:1007–18

63. Charron J, Malynn BA, Fisher P, Stewart V, Jeannotte L, et al. 1992. Embryonic lethality in mice homozygous for a targeted disruption of the N-myc gene. *Genes Dev.* 6:2248–57
64. Chen Z, Friedrich GA, Soriano P. 1994. Transcriptional enhancer factor 1 disruption by a retroviral gene trap leads to heart defects and embryonic lethality in mice. *Genes Dev.* 8:2293–301
65. Brannan CI, Perkins AS, Vogel KS, Rattner N, Nordlund ML, et al. 1994. Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. *Genes Dev.* 8:1019–29
66. Kwee L, Baldwin HS, Shen HM, Stewart CL, Buck C, et al. 1995. Defective development of the embryonic and extraembryonic circulatory systems in vascular cell adhesion molecule (VCAM1) deficient mice. *Development* 121:489–503
67. Kreidberg JA, Sariola H, Loring JM, Maeda M, et al. 1993. WT-1 is required for early kidney development. *Cell* 74:679–91
68. Meyer D, Birchmeier C. 1995. Multiple essential functions of neuregulin in development. *Nature* 378:386–90
69. Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C. 1995. Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature* 378:394–98
70. Gassmann M, Casagrande F, Orioli D, Simon H, Lai C, et al. 1995. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature* 378:390–94
71. Carmeliet P, Ferreira V, Breier G, Polteyft S, Kieckens L, et al. 1996. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 380:435–39
72. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, et al. 1996. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell* 87:1171–80
73. Parker L, Stainier DY. 1999. Cell-autonomous and non-autonomous requirements for the zebrafish gene *cloche* in hematopoiesis. *Development* 126(12):2643–51
74. Brown CB, Boyer AS, Runyan RB, Barnett JV. 1999. Requirement of type III TGF beta receptor for endocardial cell transformation in the heart. *Science* 283:2080–82
75. Ranger AM, Grusby MJ, Hodge MR, Gravalles EM, de la Brousse FC, et al. 1998. The transcription factor NF-ATc is essential for cardiac valve formation. *Nature* 392:186–90
76. De la Pompa JL, Timmerman LA, Takimoto H, Yoshida H, Elia AJ, et al. 1998. Role of the NF-ATc transcription factor in morphogenesis of cardiac valves and septum. *Nature* 392:182–86
77. Galvin KM, Donovan MJ, Lynch CA, Meyer RI, Paul RJ, et al. 2000. A role for smad6 in development and homeostasis of the cardiovascular system. *Nat. Genet.* 24:171–74
78. Sadler TW. 2000. Cardiovascular system. In *Langman's Medical Embryology*, ed. TW Sadler, pp. 208–59. Baltimore, MD: Williams & Wilkins
79. Kirby ML, Waldo KL. 1990. Role of neural crest in congenital heart disease. *Circulation* 82:332–40
80. Kirby ML, Waldo KL. 1995. Neural crest and cardiovascular patterning. *Circ. Res.* 77:211–15
81. Kurihara Y, Kurihara H, Oda H, Maemura K, Nagai R, et al. 1995. Aortic arch malformations and ventricular septal defect in mice deficient in endothelin-1. *J. Clin. Invest.* 96:293–300
82. Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, et al. 1998. Cranial and cardiac neural crest defects in endothelin-A receptor-deficient mice. *Development* 125:813–24
83. Thomas T, Kurihara H, Yamagishi H, Kurihara Y, Yazaki Y, et al. 1998. A signaling cascade involving endothelin-1, dHAND

- and Msx1 regulates development of neural crest-derived branchial arch mesenchyme. *Development* 125:3005–14
84. Yamagishi H, Olson EN, Srivastava D. 2000. The bHLH transcription factor, dHAND, is required for vascular development. *J. Clin. Invest.* 105:261–70
 85. Kawasaki T, Kitsukawa T, Bekku Y, Matsuda Y, Sanbo M, et al. 1999. A requirement for neuropilin-1 in embryonic vessel formation. *Development* 126:4854–902
 86. Iida K, Koseki H, Kakinuma H, Kato N, Mizutani-Koseki Y, et al. 1997. Essential roles of the winged helix transcription factor MFH-1 in aortic arch patterning and skeletogenesis. *Development* 124:4627–38
 87. Li J, Liu KC, Jin F, Lu MM, Epstein JA. 1999. Transgenic rescue of congenital heart disease and spina bifida in Sp1otch mice. *Development* 126:2495–503
 88. Gruber PJ, Kubalak SW, Pexieder T, Suvov HM, Evans RM, et al. 1996. RXR α deficiency confers genetic susceptibility for aortic sac, conotruncal, atrioventricular cushion, and ventricular muscle defects in mice. *J. Clin. Invest.* 98:1332–43
 89. Zhong TP, Rosenberg M, Mohideen MA, Weinstein B, Fishman MC. 2000. Gridlock, an HLH gene required for assembly of the aorta in zebrafish. *Science* 287:1820–24
 90. Satoda M, Zhao F, Diaz GA, Burn J, Goodship J, et al. 2000. Mutations in TFAP2B cause Char syndrome, a familial form of patent ductus arteriosus. *Nat. Genet.* 25:42–46
 91. Goldmuntz E, Clark BJ, Mitchell LE, Jawad AF, Cuneo BF, et al. 1998. Frequency of 22q11 deletions in patients with conotruncal defects. *J. Am. Coll. Cardiol.* 32(2):492–98
 92. Wilson DI, Burn J, Scambler P, Goodship J. 1993. DiGeorge syndrome: part of CATCH 22. *J. Med. Genet.* 30:852–56
 93. Driscoll DA, Salvin J, Sellinger B. 1993. Prevalence of 22q11 microdeletions in DiGeorge and velocardiofacial syndromes: implications for genetic counseling and prenatal diagnosis. *J. Med. Genet.* 30:813–56
 94. Burn J, Takao A, Wilson D. 1993. Conotruncal anomaly face syndrome is associated with a deletion within chromosome 22q11. *J. Med. Genet.* 30:822–24
 95. Emanuel BS, Budarf ML, Scambler PJ. 1998. The genetic basis of conotruncal cardiac defects: the chromosome 22q11.2 deletion. In *Heart Development*, ed. RP Harvey, N Rosenthal, pp. 463–78. New York: Academic
 96. Lindsay EA, Botta A, Jurecic V, Carattini-Rivera S, Cheah YC, et al. 1999. Congenital heart disease in mice deficient for the DiGeorge syndrome region. *Nature* 401:379–83
 97. Johnson ES, Ma PC, Ota IM, Varshavsky A. 1995. A proteolytic pathway that recognizes ubiquitin as a degradation signal. *J. Biol. Chem.* 270:17442–56
 98. Pizzuti A, Novelli G, Ratti A, Amati F, Mari A, et al. 1997. UFD1L, a developmentally expressed ubiquitination gene, is deleted in CATCH 22 syndrome. *Hum. Mol. Genet.* 6:259–65
 99. Yamagishi H, Garg V, Matsuoka R, Thomas T, Srivastava D. 1999. A molecular pathway revealing a genetic basis for human cardiac and craniofacial defects. *Science* 283(5405):1158–61
 100. Magnaghi P, Roberts C, Lorain S, Lipinski M, Scambler P.J. 1998. HIRA, a mammalian homologue of *Saccharomyces cerevisiae* transcriptional co-repressors, interacts with Pax3. *Nat. Genet.* 20:74–77
 101. Farrell MJ, Stadt H, Wallis KT, Scambler P, Hixon RL, et al. 1999. HIRA, a DiGeorge syndrome candidate gene, is required for cardiac outflow tract septation. *Circ. Res.* 84(2):127–35
 102. McQuade L, Christodoulou J, Budarf M, Sachdev R, Wilson M, et al. 1999. Patient with a 22q11.2 deletion with no overlap of the minimal DiGeorge syndrome

- critical region (MDGCR). *Am. J. Med. Genet.* 86(1):27–33
103. Schott J-J, Benson DW, Basson CT, Pease W, Silberbach GM, et al. 1998. Congenital heart disease caused by mutations in the transcription factor NKX2-5. *Science* 281:108–11
104. Benson DW, Silberbach GM, Kavanaugh-McHugh A, Cottrill C, Zhang Y, et al. 1999. Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. *J. Clin. Invest.* 104:1567–73
105. Basson CT, Bachinsky DR, Lin RC, Levi T, Elkins JA, et al. 1997. Mutations in human TBX5 cause limb and cardiac malformation in Holt-Oram syndrome. *Nat. Genet.* 15:30–35
106. Li QY, Newbury-Ecob RA, Terrett JA, Wilson DI, Curtis AR, et al. 1997. Holt-Oram syndrome is caused by mutations in TBX5, a member of the Brachyury (T) gene family. *Nat. Genet.* 15:21–29
107. Basson CT, Huang T, Lin RC, Bachinsky DR, Weremowicz S, et al. 1999. Different TBX5 interactions in heart and limb defined by Holt-Oram syndrome mutations. *Proc. Natl. Acad. Sci. USA* 96:2919–24
108. Fernandez-Teran M, Piedra MD, Kathiriya I, Srivastava D, Rodriguez-Rey JC, Ros MA. 2000. Role of dHAND in anterior-posterior polarity during limb development: implications for the Sonic hedgehog pathway. *Development* 127:2133–42
109. Charite J, McFadden DG, Olson EN. 2000. The bHLH transcription factor dHAND controls Sonic hedgehog expression and establishment of the zone of polarizing activity during limb development. *Development* 127(11):2461–70
110. Li L, Krantz ID, Deng Y, Genin A, Banta AB, et al. 1997. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat. Genet.* 16:243–51
111. Oda T, Elkahloun AG, Pike BL. 1997. Mutations in the human Jagged1 gene are responsible for Alagille syndrome. *Nat. Genet.* 16:235–42
112. Krantz ID, Colliton RP, Genin A, Rand EB, Li L, et al. 1999. Jagged1 mutations in patients ascertained with isolated congenital heart defects. *Am. J. Hum. Genet.* 84:56–60

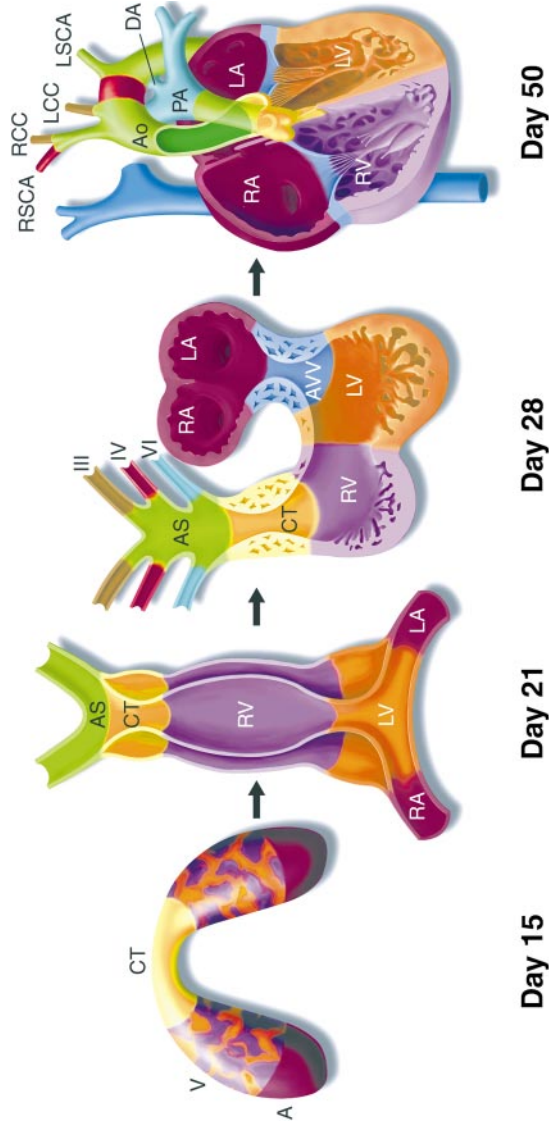


Figure 1 Schematic of cardiac morphogenesis. Illustrations depict cardiac development with color coding of morphologically related regions, seen from a ventral view. Cardiogenic precursors form a crescent (*far-left panel*) that is specified to form specific segments of the linear heart tube, which is patterned along the AP axis to form the various regions and chambers of the looped and mature heart. Each cardiac chamber balloons out 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 (AS) that together contribute to specific segments of the mature aortic arch (color-coded). Mesenchymal cells form the cardiac valves from the contruncal (CT) and atrioventricular valve (AVV) segments. 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; LSCA, left subclavian artery; RCC, right common carotid; LCC, left common carotid. (Reproduced with permission from Reference 2.)

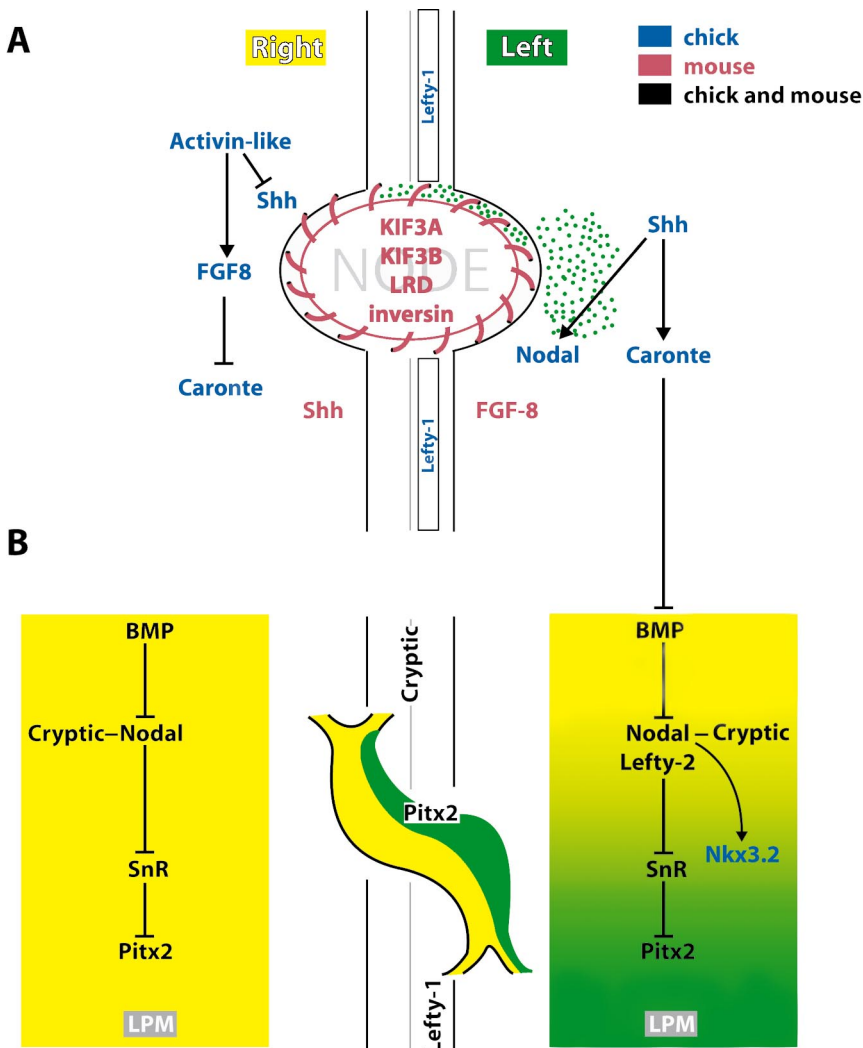


Figure 2 Cascades regulating left-right (LR) asymmetry. Early asymmetrical gene expression around the node (A) results in activation or repression of sonic hedgehog (Shh) or fibroblast growth factor (FGF) 8-dependent pathways on the right or left (ventral view). Early roles of Shh and FGF8 are reversed in mouse and chick, as indicated by color. Leftward flow of morphogens (green dots) by nodal cilia establishes the asymmetric gradient around the node in mice. Expression of Lefty-1 near the midline may serve as a barrier to maintain left-sided asymmetry of morphogens. At later stages of organogenesis, the nodal LR asymmetric information is transferred to the lateral plate mesoderm (LPM) by caronte. Caronte relieves BMP inhibition of the left, initiating a cascade of events culminating in expression of Pitx2 in the left LPM and in the left side of the heart tube (B).



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