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Development Gone Awry: Congenital Heart Disease

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This Review is part of a thematic series on **Genetics of Cardiovascular Development**, which includes the following articles:

Transcriptional Regulation of Vertebrate Cardiac Morphogenesis

Cardiac Septation: A Late Contribution of the Embryonic Primary Myocardium to Heart Morphogenesis

Early Signals in Cardiac Development

Development of the Coronary Vessel System

Development Gone Awry: Congenital Heart Disease

Left/Right Patterning

Christine E. Seidman, Guest Editor

Development Gone Awry Congenital Heart Disease

Peter J. Gruber, Jonathan A. Epstein

Abstract—Significant advances in the understanding of the molecular and genetic basis of congenital heart disease have emerged from gene inactivation studies in mice and from human genetic investigations. However, the ability to utilize information gleaned from animal models to inform clinical care of patients depends on an accurate anatomic analysis and presentation in terms that are meaningful to the clinical pediatric cardiologist. Likewise, the enormous depth and breadth of accumulated clinical experience can inform the developmental biologist and can highlight the importance and interrelationships of particular phenotypes. The explosion of potentially informative genetic tools demands that basic scientists and clinicians concerned with congenital cardiac disease enhance the ongoing bidirectional dialogue. In some cases, categories of congenital disease familiar to clinicians are not recognized by developmental biologists, and mechanisms accepted by the biologist seem inconsistent with clinical experience. In this review, we summarize some of the more clinically significant forms of congenital heart disease, and we highlight relevant genetic and developmental pathways. (*Circ Res.* 2004;94:273-283.)

Key Words: congenital heart disease ■ developmental biology ■ animal models

Congenital heart disease (CHD) affects over 1 out of every 100 live births^{1,2} and is responsible for the vast majority of prenatal losses. Additionally, 3 per 1000 live births will require an intervention (either catheter-based or surgical) during the first year of life. Despite the importance of this devastating complex of diseases, the causes are largely unknown. What are the obstacles to unraveling the molecular controls of heart morphogenesis? A confluence of recent advances suggests that we are in the midst of a period of significant discovery that will reshape our understanding of congenital cardiac disorders. These advances include the description of an increasing number of

gene-targeted mouse models of human cardiac disease, the availability of nearly complete genome sequence for multiple organisms, and increasingly sophisticated bioinformatics tools with which to utilize this data. In addition, the rapidly expanding single nucleotide polymorphism density map will soon make whole genome scans for the genetic causes of rare diseases more fruitful. Once specific causative genes are identified or implicated, the analysis of gene function and the mechanisms underlying the development of cardiovascular disorders can be analyzed in animal models. The manipulation of gene expression is now possible not only in mouse models, where homo-

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TABLE 1. Incidence of Congenital Heart Disease

Lesion	Frequency
Ventricular septal defect (VSD)	1:280
Atrial septal defect (ASD)	1:1062
Atrioventricular canal (AVC)	1:1372
Tetralogy of Fallot (TOF)	1:2375
Transposition of the great arteries (D-TGA)	1:3175
Tricuspid atresia	1:12 658
Ebstein's anomaly	1:8772
Pulmonary atresia	1:7576
Hypoplastic left heart syndrome	1:3759
Truncus arteriosus	1:9346
Double-outlet right ventricle (DORV)	1:6369
Total anomalous pulmonary venous connection (TAPVC)	1:10,638

Adapted from Hoffman and Kaplan.¹

gous recombination can be used to inactivate or modify a gene, but also in organisms such as zebrafish and *Xenopus* (frogs) where morpholino antisense nucleotides can be used to efficiently impair gene expression.

Despite the availability of these powerful tools, a significant challenge remains to forge a meaningful connection between developmental mechanisms deduced from experimental systems and the etiology of human congenital heart disease. Human disorders are classified according to historical, anatomic, and clinically relevant criteria that do not always correlate with genetic or molecular classifications. In fact, anatomic classifications may obscure developmental relationships. In order to take full advantage of new insights, these classifications will need to be reconciled and both clinicians and biologists will need to speak a more common language. In this review, we pursue a discussion of the most clinically significant types of structural congenital heart disease in terms of emerging molecular data (Tables 1 and 2).

Common Congenital Heart Malformations

Atrial Septal Defects

Atrial septal defects (ASDs) are a common form of CHD, affecting over 1 in 1000 live births, and often appearing in association with many other types of more complex CHD.^{1,2} Once identified, surgical- or catheter-based closure of an ASD is generally straightforward. The atrial septum is a heterogeneous structure separating two atrial chambers and forms through a complex series of events that is far more detailed than simple fusion of the septa primum and secundum. In actuality, reorientation of venous tributaries of the heart through the interaction of multiple structures (some transitory) results in a substantial restructuring of the atrial compartment.³ Deficiencies of any of these structures can lead to ASDs. Although ASD refers to a communication between the right and left atria, primum, secundum, or sinus venosus ASDs bear very few anatomic similarities outside of chamber communication.

Atrial septal defects are anatomically classified into four categories: ostium secundum (defect in the septum primum; 85% of all ASDs and 10% of all CHD), ostium primum

(defect in septum secundum; 10% of ASDs), sinus venosus (defect in right horn of sinus venosus; 5%), and coronary sinus (defect in left horn of sinus venosus; rare).⁴ These defects result in a communication between the right and left atria and clinically result in a shunt. Early in postnatal life there is little shunting. With time, persistent left-to-right shunting of blood leads to enlargement of the right atrium and ventricle, atrial arrhythmias, ventricular dysfunction, and pulmonary overcirculation eventually leading to irreversible pulmonary vascular obstructive disease. Patients with significant shunts have an average life expectancy of 45 years. Therefore, children with ASDs should have them closed, usually by age 3 to 4 years.⁵

Genetic causes of some forms of ASD have recently been identified. For instance, patients with Holt-Oram syndrome frequently have ASDs in association with limb deformities. This disorder is due to mutations in the T-box transcription factor gene *TBX5*.⁶ Mutations in *GATA4* are found in some patients with isolated ASDs. Interestingly, *Gata4* and *Tbx5* can physically interact, suggesting that pathways regulated by the cooperative activity of these two factors within a transcriptional complex may contribute to atrial septal formation.⁷ Another transcription factor that can physically interact with *Gata4* has also been associated with ASDs in humans. *NKX2-5* encodes a homeodomain-containing DNA binding protein that is homologous to the *Drosophila* tinman protein, so named because in its absence the fly has no heart.^{8,9} Complete loss of *Nkx2-5* in mouse models results in embryonic lethality and a failure of cardiac development at the looping stage.¹⁰ However, heterozygous loss of only one copy of *Nkx2-5* results in ASD in some animals. Studies in a wide variety of organisms suggest that *Tbx5*, *Gata4*, and *Nkx2-5* function at many stages of cardiac development in a wide range of cardiac tissues. However, proper development or closure of the atrial septum must be particularly susceptible to modest impairment of transcriptional activity such that non-lethal mutations in these genes frequently result in ASD. An alternative possibility is that there is little specificity in these gene products, and that the atrial septum, with its large number of contributing tissues, happens to be the most sensitive tissue to growth inhibition through abrogation of general growth promoting factors such as *Nkx2-5*, *Tbx5*, and *Gata4*.

Interestingly, development of the atrioventricular (AV) node and cardiac conduction system is also dependent on similar transcriptional programs, and conduction defects are often found in association with ASD in these patients. Although this was commonly believed to be due to structural impairment of the conduction system in the setting of the anatomic defect of ASD, it now seems more likely that common underlying genetic and developmental processes account for the coincidence of ASD and conduction defects.¹¹

In addition to *Nkx2-5*, *Tbx5*, and *Gata4*, other genes have been implicated in the etiology of ASDs, although mutations have not been identified in human patients. Mouse models in which the transcription factor genes *Cited2* or *Fog2/Zfp2* have been inactivated result in complex structural heart disease including ASD, suggesting that these and related genes are good candidates for disease-causing genes in humans.¹²⁻¹⁵

TABLE 2. Gene Mutations Demonstrating Cardiac Phenotypes

Gene	Null Phenotype	Reference
<i>Acrv2b</i> (activin A receptor, type IIB)*	Laterality defects,* TGA, DORV, PTA	69
<i>Agpt</i> (angiopoietin 1)	Atrial dysgenesis, ASD, venous malformation (cardinal vein obstruction)	87
<i>Bmp4</i> (bone morphogenic protein 4)	AVSD	37
<i>Bmpr2</i> (bone morphogenic protein receptor, type II)	PTA, semilunar valve dysgenesis	57
<i>Cited2</i> (Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2)	ASD, VSD, DORV, PTA	12
<i>CRELD1</i> (cysteine-rich with EGF-like domains 1)*	AVSD*	41
<i>Cspg2</i> /versican/hdf (chondroitin sulfate proteoglycan 2)	CT hypoplasia	88, 89
<i>Dvl2</i> (disheveled homologue 2)	PTA, TGA	55
<i>Ece1</i> (endothelin converting enzyme 1)	IAA, VSD, DORV, PTA	65
<i>Edn1</i> (endothelin 1)	IAA, VSD	90
<i>Egfr</i> (epidermal growth factor receptor)	AS, AI	91
<i>ErbB2</i> (erythroblastic leukemia viral oncogene homologue 2)	Noncompaction, aberrant trabeculation	92
<i>ErbB3</i> (erythroblastic leukemia viral oncogene homologue 3)	AV hypoplasia	92
<i>Endra</i> (endothelin receptor type A)	IAA, VSD, DORV, PTA, TGA	66
<i>Fgf8</i> (fibroblast growth factor 8)	DORV, PTA, ASD, VSD, AV valve atresia, arch hypoplasia	93
<i>Foxc1</i> (<i>Fkh1</i> /forkhead box C1)	Aortic arch abnormalities	68
<i>Foxc2</i> (<i>Mfh1</i> / <i>Fkh14</i> /forkhead box C2)	Aortic arch abnormalities	68
<i>Gata4</i> (GATA binding protein 4)*	ASD,* VSD, cardia bifida, ventral morphogenesis	7, 94
<i>Gja1</i> (connexin43/gap junction membrane channel protein α_1)*	RVOT obstruction, aberrant coronary patterning; heterotaxy*	95, 96
<i>Hand1</i> (<i>eHand</i> /heart and neural crest derivatives expressed 1)	Looping abnormality	97, 98
<i>Hand2</i> (<i>dHand</i> /heart and neural crest derivatives expressed 2)	RV hypoplasia, Ao arch hypoplasia, trabecular abnormality	99
<i>Has2</i> (hyaluron synthase 2)	Absent AV cushions and trabeculae	100
<i>Hoxa3</i> (<i>Hox-1.5</i> /homeobox A3)	CT abnormalities	101
<i>Hspg2</i> (perlecan/heparin sulfate proteoglycan of basement membrane)	TGA/IVS, coronary anomalies	83, 84
<i>Jag1</i> (jagged 1)*	PS, VSD, TOF, Alagille's syndrome*	21, 23
<i>Madh6</i> (<i>Smad6</i> /mothers against decapentaplegic homologue 6)	CT septation defects	102
<i>Nf1</i> (neurofibromatosis 1)	EC cushion defect, DORV	32
<i>Nfatc</i> (nuclear factor of activated T cells, cytoplasmic 1)	VSD, valve defects	103, 104
<i>Nkx2-5</i> (<i>Csx</i> /NK2 transcription factor related, locus 5)*	ASD,* VSD, TOF, EP	10, 105
<i>Nr2f2</i> (COUP-TFII/nuclear receptor subfamily 2, group F, member 2)	Atrial dysgenesis, ASD, venous malformation (cardinal vein obstruction)	106
<i>Nrg1</i> (neuregulin 1)	Noncompaction, dysmorphic trabeculae	107
<i>Nrp</i> (neuropilin-1)	TGA, PTA	86
<i>Ntf3</i> (neurotrophin 3)	PTA, IAA, CT defects	108
<i>Pax3</i> (paired box gene 3)	PTA, CT defects	109
<i>Pcaf</i> (p300/CBP-associated factor)	PTA, CT defects	110
<i>Pdgfrα</i> (platelet-derived growth factor receptor, α polypeptide)	PTA, DORV, VSD, noncompaction	70
<i>Pitx2</i> (paired-like homeodomain transcription factor 2)	Laterality, AVSD, PTA, TGA, DORV	72, 111
<i>Rarα</i> (retinoic acid receptor, α ; RAR α_1/β_2 , α/β_2 , $\alpha\gamma$ compound mutants similar)	IAA, VSD, PTA, DORV	74

Currently, there is no unified molecular pathway that dictates atrial septal morphogenesis and one cannot assign growth modulating properties to specific molecules within

the septum primum, septum secundum, or other contributing tissues. Additionally, caution must be exercised in the assignment of the ASD phenotype, especially compared with a

TABLE 2. Gene Mutations Demonstrating Cardiac Phenotypes (continued)

Gene	Null Phenotype	Reference
<i>Rar</i> β (retinoic acid receptor, β)	CT defects, VSD	74
<i>Rar</i> γ (retinoic acid receptor, γ)	CT defects, VSD	112
<i>Rxr</i> α (retinoid X receptor, α)	ASD, VSD, AVSD, noncompaction, PTA, AP window	28, 36
<i>Sema3c</i> (semaphoring 3C/sema domain, immunoglobulin domain, short basic domain, secreted)	IAA, PTA	54
<i>Sox4</i> ([sex determining region Y]-box 4)	AVSD, TGA, semilunar valve defects, PTA	113
<i>Tbx1</i> (T-box 1)*	DGS*	44–47
<i>Tbx5</i> (T-box 5)*	HOS*, ASD, VSD, TOF, EP	6
<i>Tead1</i> (Tef-1/TEA domain family member 1)	Noncompaction, trabecular abnormality	114
<i>Tek</i> (Tie2/endothelial-specific receptor tyrosine kinase)*	Venous malformations*	115, 116
<i>Tgf</i> β 2 (transforming growth factor, β ₂)	VSD, DORV	73
<i>Tgf</i> β 3 (transforming growth factor, β receptor III/ β glycan)	Decreased cushion mesenchyme transformation	117
<i>Vcam1</i> (vascular cell adhesion molecule 1)	Noncompaction, VSD	17, 18
<i>Zfp</i> 2 (FOG2 [friend of GATA] 2/zinc finger protein, multitype 2)	TA, ASD, VSD, PS, TOF, AVSD	14, 15

*Genes in which human mutations have been identified.

AP window indicates aorticopulmonary window; ASD, atrial septal defect; AV, atrioventricular; AVSD, atrioventricular septal defect; CT, conotruncal; DGS, DiGeorge syndrome; DORV, double-outlet right ventricle; EC, endocardial cushion; HOS, Holt-Oram syndrome; IAA, interrupted aortic arch; IVS, intact ventricular septum; PS, pulmonary stenosis; PTA, persistent truncus arteriosus; RVOT, right ventricular outflow tract; TA, tricuspid atresia; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; and VSD, ventricular septal defect.

patent foramen ovale, a very difficult diagnosis to make with certainty in a histological section of a mouse. Further studies should build on the work that has demonstrated interactions between different molecules and identification of common pathways along with the identification of downstream genes that play a direct role in tissue morphogenesis.

Ventricular Septal Defects

Ventricular septal defects (VSDs) are a common form of CHD, occurring in more than 1 in 300 live births, as well as a frequent component of more complex lesions.^{1,2} VSDs may occur anywhere in the ventricular septum and are usually classified by their location. There are at least three anatomic classification schemes for ventricular septal defects, each with its own advocates. One system divides VSDs into four types: conoseptal, conoventricular, muscular, and atrioventricular canal-type.¹⁶ Only a small fraction of patients with VSDs ever become symptomatic, thus the diagnosis of VSD is usually initially suspected based on the physical examination alone: a murmur appears as PVR falls and a pressure gradient develops across the defect. Left-to-right shunts through moderate to large VSDs become hemodynamically significant in the first 2 to 6 weeks of life. The vast majority of significant VSDs are closed surgically by sewing a prosthetic patch to the edge of the defect, although some are now being closed by catheter delivered devices.⁵

Similar to atrial septal defects, the heterogeneous composition of the ventricular septum suggests a variety of possible mechanisms leading to developmental defects. The most complex region of the ventricular septum is anterior and superior where, simplistically, the muscular ventricular crest joins with at least two other distinct tissues: the atrioventric-

ular cushions and the conotruncal cushions. Transitory structures, important in cardiac septation, although not present in the final structure (eg, proximal portions of the conal cushions), are also critical to its development. In fact, the location of conal structures appears to be a significant factor in development of a number of complex diseases, from tetralogy of Fallot to interrupted aortic arch. Additionally, cell populations extrinsic to the developing heart, including the neural crest, may influence the process of ventricular septation through inductive interactions with neighboring tissues. The molecular mechanisms responsible for various stages of ventricular septation remain largely unknown, in particular, the processes that mediate morphogenetic movements and fusion between opposing structures. Rigorous phenotypic analysis of VSDs, specifically the location and the adjacent structures is important because the mechanisms by which each of the four anatomic types develop are likely to be different.

Some insights have emerged from animal models in which α_4 integrin or vascular cell adhesion molecule (VCAM-1) are disrupted. These molecules mediate adhesion and are expressed in complementary patterns in critical regions of the closing ventricular septum, with mutant mice exhibiting multiple cardiac and noncardiac defects attributable to failure of tissue-tissue fusion.^{17,18} Further information regarding these critical processes is central to the understanding of a large fraction of complex congenital heart disease because the junction of the ventricular septum and the two cushion tissues appears morphologically to underlie connections between atria and ventricles, connections between ventricles and great vessels, and septation of the heart.

Mutations in a large number of genes in animal models have been associated with VSDs, usually in association with

other complex heart defects. Unfortunately, few of these shed significant light on the development of the ventricular septum. Scattered examples of animal models of isolated VSDs have been identified, while some human syndromic and sporadic cases of VSD have been associated with *NKX2-5*, *TBX5*, and *GATA4* mutations.^{7,19,20} Recent reports have suggested that notch signaling may be critical for ventricular septation because mutations in *JAG1*, encoding a notch ligand, can result in ventricular septation defects in humans associated with Alagille syndrome or tetralogy of Fallot.²¹⁻²³ In addition, mice in which the transcription factor gene *Chf1/Hey2* is inactivated display VSDs.^{24,25} *Chf1/Hey2* encodes a basic helix-loop-helix (bHLH) transcription factor homologous to *Drosophila* Hairy/Enhancer of split proteins that act as nuclear effectors of notch signaling²⁶; the zebrafish orthologue of which is *gridlock*.²⁷ Unfortunately, there is no cross-species confirmation of phenotype with respect to *gridlock* because the Zebrafish cardiovascular phenotype appears limited to aortic coarctation, not seen in murine models. This nonconvergence of phenotypes between animal models is unpredictable and will continue to present a biological challenge for the interpretation of animal studies.

In many cases, animal models of complex cardiac disease include VSDs. For instance, mutation of the *retinoic acid X receptor α* gene (*RXR α*),²⁸⁻³⁰ the *Type 1 Neurofibromatosis* gene (*Nf1*),^{31,32} and *Pax3*³³ all result in VSDs, although the etiology is likely to be indirect and unrelated in each case. The number of murine congenital heart abnormalities in which a cell autonomous myocyte defect has been identified in molecules other than structural proteins is small. *RXR α* defects may primarily relate to an epicardial abnormality.³⁴ *Nf1* cardiac defects are due to a primary role for neurofibromin in endocardial cells, and *Pax3* functions in neural crest. Hence, diverse mechanisms in multiple cell types can converge to result in a phenotype that includes VSD.

Atrioventricular Canal

Atrioventricular canal affects over 1 in 2800 live births.^{1,2} Although there are many anatomic variations, the most common form consists of a combination of defects in the (1) atrial septum primum, (2) the inlet portion of the ventricular septum, and (3) a common atrioventricular canal (CAVC).³⁵ Anatomically, CAVC lesions are divided into three general groups: partial, transitional, and complete. All defects result from deficiencies of the anterior portion of the atrial septum and posterior portion of the ventricular septum as well as a common AV valve. Partial or incomplete common AV canal consists of a common AV valve with two orifices (one entering each ventricle) and dense attachment of the valve tissue to the crest of the ventricular septum. Thus, despite ventricular septal deficiency, there is no space beneath the valve, hence no VSD. This type is sometimes referred to as ostium primum ASD with cleft mitral valve; although in fact there is no separate mitral valve. The complete type may have one or two orifices and varying degrees of attachment to the ventricular septal crest, but they all have a large space between leaflet and septum, hence a large VSD. The transitional type generally has two orifices but a small space beneath the valve (a restrictive VSD component). In the

immediate neonatal period, these infants may present with mild cyanosis due to large intracardiac communications and relatively high pulmonary vascular resistance. If not detected in the neonatal period, these infants typically present early in infancy with congestive heart failure (CHF) because of the large left-to-right shunt, which increases as the pulmonary vascular resistance falls. Complete surgical repair is undertaken electively at 3 to 6 months, with earlier repair in symptomatic patients. Repair of CAVC involves closure of atrial and ventricular defects—either with one or two separate patches—and the AV valve is reconstructed and resuspended by various techniques.

Few animal models clearly recapitulate the clinical disorder. In mice, mutation of the *Friend of Gata 2* (*Fog2*) transcription factor gene results in a phenotype with features of CAVC including a common AV valve, but epicardial abnormalities resulting in coronary artery defects are also present.^{14,15} The *RXR α* mouse²⁸ is a good example of rodent-human phenotype convergence with heterozygotes demonstrating a spectrum of defects from subtle (cleft anterior leaflet of the mitral valve) to severe (complete AV canal).³⁶ Recently, conditional inactivation of *Bmp4* in the heart resulted in defects of AV septation that more closely resemble the range of phenotype seen in human common AV canal (sometimes referred to as AVSD).³⁷ Difficulties with unified nomenclature and description of early embryonic lethal phenotypes in mouse embryos can lead to differing anatomic descriptions of similar phenotypes (eg, *Fog2*^{14,15} and *Chf1/Hey2* knockouts).^{24,25,38} It is likely that additional mouse models of CAVC remain unrecognized.

Trisomy 21/Down's syndrome is a common human chromosomal disorder, and 20% of Down's patients have AV canal abnormality.³⁹ This is usually a balanced single common AV valve with the aorta connected exclusively to the left ventricle. This is genetically mirrored in mice with an extra copy of the syngeneic mouse region, resulting in Trisomy 16 (Ts16).⁴⁰ However, phenotypically, Ts16 mice are markedly different from human patients and usually demonstrate an unbalanced AV connection with separate superior leaflets. These phenotypic details are critically important to both human disease and basic biology, because molecular correlates are either difficult to draw or misleading in the absence of accurate phenotype. Missense mutations of the cell adhesion molecule gene *CRELD1* are associated with nontrisomy 21-related partial atrioventricular canal.⁴¹ *CRELD1* was identified as a candidate gene from the AVSD2 locus (3p25-pter) and analysis of 50 patients associated mutations with partial AVSD. However, there is no animal model to support this association.

Truncus Arteriosus

Truncus arteriosus, or persistent truncus arteriosus (PTA), is an uncommon form of CHD, affecting 1 in 10 000 live births. PTA consists of a single great artery arising from the heart. The truncal valve is often anatomically abnormal and a coexisting VSD is present in nearly all cases. Both extracardiac and cardiac abnormalities such as right aortic arch, arch hypoplasia, aortic coarctation, and interruption are frequent.³⁹

The majority of infants with truncus arteriosus present with symptoms of congestive heart failure in the first weeks of life. These infants may be cyanotic, but congestive heart failure symptoms and signs predominate. In contrast to VSD and CAVC discussed above, CHF occurs earlier and may be more severe with less than 25% surviving 1 year. The specter of irreversible pulmonary vascular disease and hemodynamic instability make prompt surgical repair the treatment of choice. Repair is performed by removing the pulmonary arteries from the common trunk and placement of a right ventricular to pulmonary artery conduit. Unfortunately, nearly all children require reoperations for conduit revision.⁵

Recently, microdeletion of chromosome 22 has been noted in as many as 1/3 of patients with truncus arteriosus. Chromosome 22 deletions are commonly associated with DiGeorge syndrome, which includes parathyroid and thymus defects, craniofacial abnormalities, and cardiovascular defects.⁴² However, PTA can occur as a result of this chromosomal abnormality in the absence of other signs of DiGeorge syndrome. Animal studies have recapitulated certain features of PTA and DiGeorge syndrome with engineered deletions of regions of mouse chromosome 16, which are homologous to human chromosome 22q11.⁴³ The critical gene for cardiac development on chromosome 22 centers around *TBX1*.^{44–46} Although mutations in human patients have been difficult to identify, a recent study reports *TBX1* mutations in three unrelated patients without the 22q11 deletion.⁴⁷ Additional neighboring genes may modify *Tbx1* function or may independently cause PTA.^{48,49}

The developmental mechanisms of truncal septation have been extensively studied and are well reviewed elsewhere.⁵⁰ This structure is key to the proper formation of the heart and likely related to many forms of congenital heart disease. Neural crest cells play a critical role in truncal septation. This multipotent cell population is specified in the dorsal neural tube and a subpopulation migrate through the pharyngeal arches to the developing outflow tract of the heart. Mutation of the murine *Pax3* gene, which is expressed by premigratory neural crest, results in PTA. *Pax3*-expressing neural crest derivatives populate the conotruncus in a rostral to caudal direction, although the precise role for these cells is still being determined.^{46,51} Neural crest cells differentiate into the smooth muscle of the aortic arch and ductus arteriosus. Interestingly, *Tbx1* is not expressed by migrating neural crest. It is thought to mediate an effect on conotruncal septation by influencing the expression of secreted growth factors by pharyngeal endoderm, which subsequently signal to migrating neural crest.⁵² Recent experiments of the *van gogh* locus (*tbx1*) in zebrafish confirm a key role for *Tbx1* and suggest interactions with *Hand2* and *Edn1*.⁵³ The migratory behavior of neural crest cells may be influenced by members of the semaphorin guidance molecule family and mice with mutations in *Sema3C* exhibit PTA.⁵⁴ The Wnt, bone morphogenetic protein (BMP), and vascular endothelial growth factor (VEGF) signaling pathways have also been implicated in outflow tract septation.^{55–57}

Uncovering the molecular controls that regulate conotruncal development and septation will require the precise assignment of individual molecular functions in time and space.

Lineage tracing through Cre-recombinase–based methods of reporter mice and their crosses into mice defective in other molecules has provided key insights into the contributions of various tissue subtypes.^{58–60} However, the overlay of temporal interactions will be critical in order to unravel these complex interactions. This is another area in which clinical expertise can inform basic science and vice versa. The anatomy of the conotruncus, with different cushion components variously contributing to truncal septation, valvulogenesis, and intracardiac septation, is complex. The heterogeneity of the conotruncal cushions is poorly emphasized in murine cardiac developmental biology, although the interesting PTA phenotype demonstrated by *Bmpr2* knockout mice are informative.⁵⁷ Likewise, portions of aorticopulmonary septum, the superior aspects of the conus, and the semilunar valves are infrequently discussed in terms of their common embryological origin.

Interrupted Aortic Arch

Interrupted aortic arch (IAA) is a rare disease whose clinical presentation—shock or severe CHF in the first 2 weeks of life—is remarkably similar to aortic coarctation, although developmental mechanisms are different. IAA is not a lesion isolated to the great vessels, but is a complex defect that occurs frequently in association with posterior malalignment of the infundibular septum with the muscular septum—almost a “mirror image” of tetralogy of Fallot in which there is anterior malalignment. This posterior positioning results in a narrowed left ventricular outflow tract that is occasionally atretic. This severe obstruction is associated with decreased growth, hypoplasia, and interruption of the aortic arch. Thus, in addition to the ventricular septal defect and narrowed subaortic area, there is complete atresia of a segment of the aortic arch. There are three anatomic subtypes of interrupted aortic arch based on the location of the interruption, likely with differing developmental mechanisms: type A distal to the left subclavian artery; type B between a subclavian artery and a carotid artery; and type C between the two carotid arteries. Type B is the most common variety. Surgical reconstruction is the only therapeutic option and is performed as soon as the patient is hemodynamically stable, typically utilizing a corrective approach with aortic arch reconstruction and VSD closure.

Specific gene mutations in humans have not been described in patients with IAA, although it is associated with chromosome 22 deletions. However, a number of candidate genes have been identified in animal models. In general, there is phenotypic overlap in these models between IAA and PTA. For example, some *Sema3C*-deficient embryos display IAA, whereas others display PTA.^{54,61} Defects in the endothelin signaling pathway also result in isolated IAA.⁶² The region of the aortic arch that is deficient is similar to the segment of the aortic arch in which smooth muscle derives from neural crest,⁶³ and the endothelin A receptor (*Endra*) is expressed by neural crest cells. The ligand for this receptor, endothelin 1 (*Edn1*), is expressed by neighboring tissues as is the enzyme, endothelin-converting enzyme (*Ece1*), that is required for activation of this ligand. Mutation in any component of this signaling system results in IAA.^{62,64–67} Mutations in two

related forkhead transcription factors, *Foxc1* and *Foxc2*, can also result in IAA.⁶⁸ Interestingly, *Foxc1* and *Foxc2* can function downstream of sonic hedgehog in pharyngeal tissues and can directly activate expression of *Tbx1*.

Double-Outlet Right Ventricle

Double-outlet right ventricle (DORV) is an uncommon group of lesions that have as a common feature both great arteries arising predominantly from the morphological right ventricle. Some of the disease entities include DORV with mitral atresia, DORV with malalignment type subpulmonary VSD with or without subaortic stenosis and coarctation (Taussig-Bing type), DORV with malalignment type subaortic VSD and pulmonary stenosis (“tetralogy” type), DORV with malalignment type subaortic VSD without outflow obstruction (“VSD” type), DORV with single RV, DORV with superior-inferior ventricles usually with an inlet type VSD. Children with DORV present in a wide spectrum of ways dependent on the relationship of the great vessels to the VSD, whether or not there is outflow obstruction, and the size of the left ventricle. For example, patients with subaortic VSD and no pulmonary stenosis have increased pulmonary blood flow and congestive heart failure, whereas those with pulmonary stenosis present with cyanosis; patients with a subpulmonary VSD present with cyanosis (transposition physiology) and may also have signs of coarctation of the aorta. Definitive therapy for DORV is surgical, although palliative procedures may allow for temporary improvement in medical condition. There are a large number of procedures that have been devised to treat the complex intracardiac anatomy associated with DORV and allow for a biventricular repair. Each of these follows the principle of baffling blood from the systemic ventricle to the aorta, maintaining or establishing continuity from the right ventricle to the pulmonary artery, and closing the VSD. In some cases, such as the Taussig-Bing heart with DORV and subpulmonary VSD, it may require an arterial switch. Those cases with mitral atresia and DORV present with varying degrees of pulmonary venous obstruction and require a single ventricle pathway approach. Cases with an inlet type VSD may also be best served with a single ventricle approach, because that VSD may not be an optimal pathway for baffling a great artery to the LV.

A large number of mouse mutations result in a DORV phenotype. Mutations in the *type IIB activin A receptor* result in complex defects, which includes DORV.⁶⁹ *Cited2*,¹² multiple members of the endothelin signaling pathway [*Ece1*^{64,65} and *Endra*⁶⁶], *Pdgfra*,^{70,71} *Pitx2*,^{56,72} *Tgfb2*,⁷³ and compound mutants of the retinoic acid receptor family [*RARα*, *RARβ*, and *RARγ*⁷⁴] all result in DORV.

Why does great vessel alignment appear especially susceptible to mutations? Some of the high frequency is likely due to difficulties in histological phenotyping. In some instances, the appearance of two great vessels and one ventricular chamber is interpreted as DORV. However, the diagnosis of DORV is impossible to make without reference to the ventricular septum which is exceedingly difficult to accurately assess before E13.5. In addition, a sensitivity to this defect is probably due to the confluence of tissues responsible for appropriate great vessel alignment. There is an opportu-

nity for basic science to inform clinical practice through careful phenotyping. Because DORV is a spectrum of defects that results from spatial malalignment of separate structures, there may exist a similar spectrum across an allelic series of genes that also produce DORV.

Hypoplastic Left Heart Syndrome

Hypoplastic left heart syndrome (HLHS) is a fairly common (1 in 4000), heterogeneous group of anatomic abnormalities in which there is a small or absent left ventricle with hypoplastic or atretic mitral and aortic valves.^{1,2} Although medical stabilization is important in the treatment of these children, surgical therapy is necessary for survival. Surgical palliation of HLHS involves either staged reconstruction (with a neonatal Norwood procedure followed by a staged Fontan operation later in childhood) or, less commonly, neonatal cardiac transplantation. The first stage reconstruction for hypoplastic left heart syndrome depends on three principles: the creation of unrestricted ventricular outflow via an anastomosis between the aorta and pulmonary artery, an atrial septectomy to assure adequate pulmonary venous outflow, and a shunt to provide a reliable source of pulmonary blood flow.

Although a genetic etiology has not yet been elucidated, there are increasing reports of HLHS occurring in families with associated left-sided cardiac disease such as a bicuspid aortic valve. This association suggests a genetic etiology. HLHS is rare in trisomy 21, but has been reported in other trisomies such as 13 and 18, as well as in Turner’s syndrome. However, recent evidence in zebrafish⁷⁵ supports earlier work⁷⁶ suggesting a critical role for hemodynamics in the etiology of congenital heart disease. Experimental disruption of cardiac blood flow, by constriction of inflow to or outflow from the left ventricle can affect left ventricular development, suggesting that HLHS may occur secondary to hemodynamic perturbations rather than exclusively as a primary genetic or developmental defect intrinsic to the left ventricle. Interplay between both epigenetic and genetic factors that influence development of HLHS is likely to exist.

Cardiac chamber-specific regulation of gene expression has been documented for a large number of myocardial structural and regulatory genes. For instance, *Nkx2-5* is expressed throughout the developing heart, but chamber-specific enhancer elements mediate expression in right ventricle and left ventricle separately.⁷⁷ The same is true for a series of other cardiac-specific genes. This implies that chamber-specific transcriptional pathways exist that mediate right and left ventricular gene expression. The bHLH transcription factor gene *Hand1* is predominantly restricted to the left ventricular and outflow tract myocardium, whereas the related gene *Hand2* is restricted to the right ventricle.⁷⁸ Nevertheless, genetic animal models of HLHS remain to be described.

Pulmonary Atresia With Ventricular Septal Defect (PA/VSD)

PA/VSD is sometimes also known as tetralogy of Fallot with pulmonary atresia (TOF/PA), as the ventricular septal defect is almost always of the malalignment type with severe

obstruction of the right ventricular outflow tract. It is a rare disease in which the ventricles are of normal size and there is usually a severely hypoplastic infundibulum with complete atresia of the pulmonary valve. The pulmonary architecture and the source of pulmonary blood flow is variable, ranging from normal-sized pulmonary arteries supplied by the ductus arteriosus, to diminutive pulmonary arteries and a small ductus, to discontinuous pulmonary arteries supplied by bilateral ductus, to virtually absent central pulmonary arteries with the pulmonary blood flow supplied by multiple aortopulmonary collateral arteries.

Management is based on the source of pulmonary blood flow and palliation may consist of either a systemic-pulmonary artery shunt followed by definitive correction. Alternatively, the complete repair may be done in the neonatal period. Outcomes are dependent on the status of the pulmonary vascular bed at birth. If the pulmonary arteries are essentially normal in size, the long term outcomes are similar to those of other forms of tetralogy of Fallot. If there are diminutive pulmonary arteries, the prognosis is worse.

Specific gene mutations in humans and animal models have not been identified that result in PA/VSD. However, transgenic overexpression of *Tbx1* can result in a phenotype remarkably similar to PA/VSD.⁴⁴ Thus, it is possible that genetic pathways involved in DiGeorge syndrome and cardiac outflow tract development are similar or identical to pathways disrupted in PA/VSD.

Transposition of the Great Arteries (TGA)

Transposition of the great arteries (TGA) (S,D,D), meaning situs solitus of viscera and atria, ventricular D-loop, and D or right-sided position of the transposed aorta, is the most common cyanotic lesion presenting in the first week of life, affecting approximately 1 in 3100 live births. The term transposition was first applied by Farre in 1814 and since then numerous anatomic classification systems have attempted to describe this complex lesion of ventriculoarterial discordance.⁷⁹ In TGA, the aorta arises from the right ventricle and the pulmonary artery from the left ventricle resulting in nearly separate (parallel) systemic and pulmonary circulations. Existence of a communication between the left and right sides via a PDA, PFO/ASD, or VSD is required for survival. Atrioventricular alignment is usually concordant with fibrous continuity between the pulmonary artery and mitral valve. One way of interpreting conal positioning during development and the resulting anatomic outcome is that TGA is one extreme of the DORV spectrum that begins with TOF, tetralogy type, progresses through DORV, and ends in TGA.^{80,81} However, all malalignment defects do not appear to arise from identical mechanisms. This is supported by the fact that animals harboring mutations that result in TOF, DORV, or TGA, even when only partially penetrant, do not frequently display a full range of defects. Coronary artery malpositioning is the most frequently associated anomaly occurring in a third of patients whereas VSD (25%), true ASDs (10%), aortic arch abnormalities (5%), and tricuspid valve abnormalities (4%) are less common.⁸² Children present in the first few days of life with cyanosis and tachypnea requiring surgical correction by an arterial switch procedure

in which the great arteries are divided and proper ventriculoarterial concordance restored. The coronary arteries are removed from the aorta and sewn to the neo-aorta and any intracardiac shunt repaired. Untreated, half of children die in 1 month and 90% in 1 year.

The *Hspg2* (Perlecan) knockout is the closest genetic animal model to common TGA (S,D,D),^{83,84} although humans with mutations of *HSPG2* do not demonstrate congenital heart disease.⁸⁵ Inactivation of the *type II activin receptor*⁶⁹ in mice results in TGA, although these animals also harbor multiple other abnormalities that are more reflective of other forms of more complex TGA. Mutations in *Dvl2*,⁵⁵ *Pitx2*,⁵⁶ *Endra*,⁶⁶ and *Neuropilin-1*⁸⁶ all demonstrate a series of defects that include TGA, suggesting a role for neural crest-derived tissues in the pathogenesis of TGA. The modulatory effect of retinoic acid on these basic factors, the interplay of molecules in conotruncal maturation, and the movements of tissues resulting in the various forms of TGA, PTA, and DORV remain to be determined.

Conclusion

We have discussed specific examples of congenital heart disorders that are associated with single gene defects or which have been replicated in mouse models. Many of these murine models fall outside traditional classification schemes and therefore demand careful phenotyping. However, cross fertilization of basic biological information from informative animal models with insight from common clinical pathologies will lead to mutual advancement and should provide an increasingly powerful strategy to unravel the molecular basis of this devastating complex of diseases.

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