Congenital heart disease (CHD) is the most common birth defect in humans, occurring as often as 1 in 125 live births. Because prenatal diagnosis and both medical and surgical care of affected neonatal infants have improved dramatically in the past decades, the number of adult survivors of CHD is increasing. Thus, it has become increasingly important for all cardiologists to be familiar with the basic and clinical characteristics of CHD. At the same time, there has been an explosion in our understanding of the molecular and genetic programs regulating cardiovascular development (for reviews, see Olson, Srivastava and Olson, and Gruber and genetic programs regulating cardiovascular development.

CHD most commonly arises from structural defects caused by errors in the morphogenetic programs regulating heart and outflow tract development. Less commonly, functional defects of cardiac muscle account for congenital cardiac disease such as occur in muscular dystrophies or storage diseases. These disorders are not discussed further here but have been reviewed by Cox and Kunkel, Emery, and Lapidos et al. Distinct and diverse structural abnormalities of the heart and cardiac outflow tract have been classically categorized according to phenotypic characteristics, with less attention to developmental or genetic mechanisms. This is likely to change in the near future. This review is not intended to be an exhaustive survey of the genetic basis of CHD. Rather, a few illustrative examples are discussed with attention to those that highlight recent advances and that may be informative for the development of new approaches to the treatment of adult cardiac disease (see the Table).

Neural Crest and the Secondary Heart Field

Congenital Defects of the Cardiac Outflow Tract

Approximately one third of all congenital heart defects involve abnormalities of the outflow tract and great vessels. Examples include transposition of the great arteries, double-outlet right ventricle, interrupted aortic arch, and persistent truncus arteriosus. During embryonic development, the heart forms as a linear tube that undergoes looping and subsequent septation to form the 4 cardiac chambers and parallel systemic and pulmonary circulations. In particular, the single great vessel emerging from the embryonic heart and several sets of symmetric aortic arch arteries undergo septation and remodeling. The truncus arteriosus is septated to form the aorta and pulmonary artery, and the symmetric aortic arch arteries are remodeled to form the asymmetric adult vasculature with a single left-sided aortic arch.

Septation of the outflow tract and remodeling of the aortic arch arteries are dramatically affected by a population of migratory cells that originate outside the heart field (see the Figure). Cardiac neural crest cells migrate from the dorsal neural tube to the developing outflow tract where they differentiate into vascular smooth muscle and connective tissue. In the absence of normally functioning neural crest cells, the outflow tract fails to septate, and aortic arch artery repatterning is defective. Hence, genes and environmental factors that affect neural crest cells can also cause CHD.

DiGeorge syndrome is a relatively common cause of CHD affecting the outflow tract of the heart. It is also associated with hypocalcemia, thymus defects, learning disorders, and other abnormalities. Most patients with DiGeorge syndrome have a chromosomal deletion affecting 1 copy of chromosome 22. Thus, they are haploinsufficient for a critical gene or genes within the deleted region. Over the past several years, a large number of animal models have been created that recapitulate many of the characteristics of DiGeorge syndrome. In the mouse, a region of chromosome 16 is homologous to the commonly deleted region of human 22q11. Deletion of 1.5 Mb of chromosome 16 in the mouse recapitulates thymus, parathyroid, and cardiac defects of the human disease. A series of complementation experiments in which deleted genes were reintroduced by transgenic approaches into deleted mice implicated a transcription factor gene, Thbx1, as a likely cause of the DiGeorge-like defects. Inactivation of Thbx1 itself leads to CHD in heterozygous mice and causes severe pharyngeal and cardiac defects in homozygous deficient animals.

Approximately 17% of patients with DiGeorge syndrome have no demonstrable chromosomal abnormality. Presum-
ably, these patients harbor mutations in a specific gene or genes that result in the disease phenotype. Recently, specific mutations in \(\text{TBX1}\) were identified in 3 nondeleted DiGeorge families, supporting the conclusion that DiGeorge syndrome can be caused by loss of Tbx1 function.\(^{18}\) However, several other genes in the commonly deleted region have also been implicated as potential causes or modifiers of DiGeorge syndrome, and many nondeleted DiGeorge patients do not have mutations in the coding region of \(\text{TBX1}.\)\(^{18–22}\) Perhaps mutations in noncoding regulatory regions affect \(\text{TBX1}\) expression in these patients, or perhaps mutations in other genes can cause similar phenotypes.

The types of cardiovascular defects seen in DiGeorge syndrome are similar to those produced by neural crest ablation in chick embryos.\(^{10}\) However, Tbx1 is not expressed by neural crest cells. Rather, it is expressed by pharyngeal endoderm, a tissue that may provide secreted signals such as fibroblast growth factor 8 that affect migrating neural crest.\(^{23–26}\) Tbx1 is also expressed by cells that contribute to the myocardium of the outflow tract; defects in these cells may contribute to the cardiac phenotype.\(^{27}\)

Until recently, it was thought that all myocardial cells originated in the cardiac crescent and developed by expansion of the original masses of precardiac mesoderm that form the bilateral cardiac tubes that fuse in the midline of early embryos to form the linear heart tube. However, a series of recent studies have provided strong evidence that a second population of cardiac progenitor cells exists that can be identified in anterior pharyngeal regions and provides a significant contribution of relatively late-arriving myocardial cells that are incorporated into the cardiac outflow tract, the right ventricle, and even portions of the left ventricle and atria.\(^{27–29}\) (Figure). This “secondary” or “anterior” heart field is still being defined, but it is likely that Tbx1 is expressed in at least some of these cells. The secondary heart field is clearly distinct from neural crest, although both populations represent migratory cell types that contribute to the heart. Secondary heart field cells become myocardium; neural crest contributes to vascular smooth muscle of the aortic arch, ductus arteriosus, and the great vessels. Many forms of CHD that involve the outflow tract of the heart may turn out to be defects of the secondary heart field, perhaps especially those involving malrotation or outflow tract dysplasia. Hence, it will be important to define the genetic programs regulating growth and development of this population of myocardial cells and to search for mutations in these genes in appropriate patient populations. As in DiGeorge syndrome, elucidation of the molecular and genetic bases of CHD has revealed that the primary mutation responsible for the observed pathology may not reside within the cells of the outflow tract per se (endothelial cells and/or vascular smooth muscle cells) but rather cells that reside in close physical proximity to the heart and outflow tract, including cells in the pharyngeal endoderm and/or secondary heart field.

### Implications for Adult Disease: Stem Cell Therapies for Damaged Hearts

The rapidly evolving understanding of normal cardiac development, including the elucidation of the secondary heart field

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**Examples of Genes That Function During Cardiac Development and Have Been Implicated in Adult Cardiac Pathophysiology by Human and Animal Studies**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Embryonic Cardiac Function/Associated Congenital Disorder</th>
<th>Adult Cardiac Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{NKX2–5})</td>
<td>Looping morphogenesis/ASD(^{46})</td>
<td>Conduction system(^{57})</td>
</tr>
<tr>
<td>(\text{GATA4})</td>
<td>Midline fusion of cardiac primordial/ASD(^{42–44})</td>
<td>Cardiac hypertrophy(^{63,14})</td>
</tr>
<tr>
<td>(\text{TBX5})</td>
<td>Hypoplasia/ASD(^{44,55})</td>
<td>Conduction system(^{46})</td>
</tr>
<tr>
<td>(\text{Islet1})</td>
<td>Secondary heart field(^{27})</td>
<td>Cardiac progenitors(^{31})</td>
</tr>
<tr>
<td>(\text{MEF2})</td>
<td>Looping morphogenesis, myofibrillogenesis(^{48})</td>
<td>Coronary artery disease, cardiac hypertrophy(^{49,50})</td>
</tr>
<tr>
<td>(\text{HOP})</td>
<td>Cardiac myocyte proliferation, maturation(^{6,36})</td>
<td>Cardiac hypertrophy, failure, conduction system(^{51,57})</td>
</tr>
</tbody>
</table>
field, has contributed to exciting advances in the development of regenerative approaches for the treatment of adult cardiac disease. Several independent groups have reported that the heart may possess a resident population of cardiac stem cells or myocyte progenitors that could be used to regrow heart muscle after ischemic damage.\textsuperscript{30–33} From the emerging understanding of the secondary heart field, Chien and colleagues\textsuperscript{31} identified a subpopulation of cells in the embryonic heart and neonatal mouse, rat, and human heart possessing the properties of determined cardioblasts. These cells are characterized by expression of Isl1 (Isl1\textsuperscript{+}), an LIM-homeodomain transcription factor expressed primarily in the secondary or anterior heart field.\textsuperscript{27} Isl1\textsuperscript{+} is expressed in other cell lineages, including pancreatic islet cells from which its name is derived. Isl1\textsuperscript{+} cells give rise to cardiac myocytes populating the cardiac outflow tract and right ventricle, a subset of left ventricular cells, and a large number of atrial cells.\textsuperscript{27} Consistent with this finding, Isl1-deficient mouse embryos lack an outflow tract and right ventricle.\textsuperscript{27}

LacZ-tagged Isl1\textsuperscript{+} cells isolated from postnatal mouse hearts continue to express Isl1 and proliferate in mixed culture with cardiac mesenchymal cells. Phenotypically, proliferating Isl1\textsuperscript{+} cells express Nkx2-5 and GATA4 but do not express genes encoding mature cardiac markers.\textsuperscript{31} Interestingly, Isl1\textsuperscript{+} cardiac cells do not express c-kit of Sca-1, demonstrating that this resident population of cardiac cells is distinct from those identified previously.\textsuperscript{32,33} When cocultured with neonatal cardiac myocytes, Isl1\textsuperscript{+} cells differentiate into cardiac myocytes and express the full repertoire of cardiac-restricted contractile and ion channel genes. Moreover, these cells display spontaneous contractile activity, inotropic responses, and spontaneous periodic calcium oscillations.\textsuperscript{31}

However, Isl1\textsuperscript{+} cells are exceedingly rare in the neonatal heart (500 to 600 per rat heart) and may not exist in the adult heart. As such, these cells may not provide myocyte reserve capacity in response to cardiac injury, a role analogous to that of satellite cells in skeletal muscle. The therapeutic potential of these cells will depend on the capacity to isolate them from the embryonic or neonatal heart (or perhaps from the adult heart) and to expand them ex vivo. However, these cells represent an excellent system in which to examine the molecular basis of cardioblast-to-cardiocyte transition. At least in this cell population, Isl1 serves as a useful marker for the elusive cardiac myoblast. This body of work provides an instructive example of the contribution of developmental insights to the development of new therapeutic possibilities.

**Defects of Atrial Septation and Associated Conduction Abnormalities**

Closure of the atrial septum is a complex process that occurs in multiple stages throughout mid and late gestation and after birth. The septum primum initially divides the left and right atria by actively growing from the dorsal wall of the primitive atrium toward the endocardial cushions in the AV canal. Complete closure is prevented initially by a residual connection just above the endocardial cushions, the ostium primum. Before this intra-atrial connection is closed by active fusion of septal tissue with the endocardial cushions, additional fenestrations appear in the septum primum that form the ostium secundum. The ostium secundum is later closed by the septum secundum, which, like the septum primum, actively grows to divide the atria. However, the septum secundum never fuses with the endocardial cushions and acts as a flap valve across the ostium secundum. This allows for requisite right-to-left blood flow via the residual intra-atrial connection, the foramen ovale, during embryonic life (while there is little pulmonary circulation and most blood is shunted from right to left atrium). This also allows prompt closure of the intra-atrial connection when pulmonary blood flow increases at birth, thus elevating left atrial pressure in association with pulmonary venous return. After birth, the foramen ovale closes completely in most but not all individuals.

Atrial septal defects (ASDs) are common, occurring in \( \approx 1 \) in 1000 individuals.\textsuperscript{1,2} This form of CHD is often not appreciated until the fourth or fifth decade of life, when complications can include paradoxical emboli, pulmonary hypertension, and Eisenmenger’s syndrome. ASDs are also commonly associated with other forms of complex CHD, especially those including cardiac conduction system abnormalities.

Several transcription factor genes that are important during cardiac development are mutated in some patients with ASDs. NKX2-5 encodes a homeodomain-type DNA binding protein that has highly conserved homologues in species as divergent as flies and man. In *Drosophila melanogaster* (fruit fly), the homologous gene is called *tinman*, because flies with mutations in this gene have no heart.\textsuperscript{34,35} In humans, NKX2-5 mutations have been identified in families with secundum-type ASDs and cardiac conduction defects, including AV conduction block.\textsuperscript{36} Interestingly, animal models have indicated that *Nkx2-5* functions in the embryo during development of the cardiac chambers and in the adult by maintaining integrity of the conduction system.\textsuperscript{37} Hence, the well-known association of ASDs with conduction defects may be related both to the structural proximity of the atrial septum and the specialized conduction tissue and to the common molecular programs used during development of the atrial septum and maintenance of the conduction system. This recently appreciated function of Nkx2-5 in the adult conduction system raises the possibility that polymorphisms or mutations might predispose to adult arrhythmia such as heart block, sick sinus syndrome, or atrial fibrillation. *Nkx2-5* mutations have also been identified in patients with more complex forms of CHD, including tetralogy of Fallot.\textsuperscript{38,39}

ASDs, in association with limb malformations, are prominent characteristics of Holt-Oram syndrome, which is caused by mutations in another transcription factor gene, *TBX5*.\textsuperscript{40} Like *TBX1, TBX5* is a member of the T-box family of transcription factors that are defined by the presence of a conserved DNA binding domain. Interestingly, Tbx5 is able to interact both physically and functionally with Nkx2-5,\textsuperscript{41} suggesting that deficiency of either member of this transcriptional complex can cause ASDs. More recently, another factor that can interact with both Nkx2-5 and Tbx5 has been found to be mutated in some patients with ASDs. *GATA4* encodes a transcription factor that is required for midline migration and fusion of the bilateral embryonic cardiac
Implications for Disorders of Cardiac Conduction in the Adult

The specialized conduction system of the adult heart is derived from embryonic myocytes that transdifferentiate to form Perkinje fibers–associated conduction tissues. The genetic programs that regulate this transdifferentiation process are poorly understood, and pathways that maintain conduction activity and function into adulthood are not well characterized. However, emerging data suggest that molecular cascades that are functional in embryonic myocardium are redeployed in the adult conduction system and that defects in these cascades can lead to common forms of adult cardiac dysrhythmias.

In the embryo, Nkx2-5 functions to regulate multiple cardiac-specific genes, including the homeodomain factor HOP (homeodomain only protein). Recent data suggest that both of these factors are expressed at relatively high levels in adult conduction tissues and that loss of expression is associated with defects of conduction especially involving the AV node and the proximal His conduction region. Tbx5 expression is also functional and robust in cardiac conducting tissues. Genetic deletion of Nkx2-5 in the adult mouse heart leads to progressive degeneration of AV nodal tissue and AV block. This is associated with decreased expression of HOP. Independent experiments show that loss of HOP expression in mouse adult hearts is associated with conduction delay and loss of connexin-40 expression. These data suggest that maintenance of adult conduction integrity is an active process, although validation in human studies is still generally required. Loss or impairment of gene function might therefore be the cause of common forms of AV conduction disease in adults, which represent frequent indications for pacemakers in elderly patients. Likewise, predisposition to stress-induced atrial and ventricular arrhythmias such as those frequently complicating cardiac and noncardiac surgeries may be influenced by polymorphisms in developmental genes that function in adult conduction tissues. A better understanding of regulation of Nkx2-5, Tbx5, HOP, and related factors may allow interventions that halt decay of the conduction system or minimize risk of arrhythmia.

Conclusions

Significant and rapidly advancing discoveries in genetics and cardiovascular development are deciphering the molecular causes of CHD. New insights from developmental biology such as the identification of the secondary heart field may provide the foundation for partial reclassification of some forms of CHD. Further understanding of molecular cascades that are active during cardiac formation are proving useful for the identification and manipulation of embryonic and adult cardiac stem cells that offer dramatic opportunities for the
treatment of adult and congenital disease. Elucidation of fetal programs is also proving useful for the development of new therapeutic options for treatment of congestive heart failure and cardiac hypertrophy, conditions characterized by the reactivation of fetal gene programs.

**Disclosure**

Dr Parmacek has served on the Boston Scientific Science Advisory Board and is a consultant for Abbott Laboratories.

**References**


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