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Cardiovascular diseases

Transcriptional mechanisms of congenital heart disease

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Over the past decade, clinical studies have identified a number of congenital heart diseases associated with mutations in cardiac transcription factors. Recent reports have shown that several of these transcription factors physically interact with one another, implying they might act in similar molecular pathways. In this review, we outline familial heart diseases linked to cardiac transcription factors and describe some of the emerging technologies being developed as potential therapeutics for these diseases.

Introduction

Congenital heart defects (CHDs) threaten nearly 1% of all newborns and pose a significant threat of infant death; however, the underlying genetic mechanisms of many CHDs remain elusive. Most probably, the majority of these defects have a basis in the complex process of cardiogenesis. Heart development involves a series of highly coordinated events including cell proliferation, differentiation, migration and morphogenesis, and a number of genes involved in these processes have been identified as potential causes of specific cardiac anomalies. Transcription factors are major regulators of developmental processes and play essential roles in cardiogenesis (Fig. 1). Here, we review six CHDs associated with

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deletions or mutations of transcription factors and the current understanding of their molecular bases.

DiGeorge syndrome: *Tbx1*

The presentation of DiGeorge syndrome (OMIM #188400) is one consequence of the most common human genetic deletion, MONOALLELIC MICRODELETION (see Glossary) of chromosome 22q11.2. In most cases, the heterozygous deletion eliminates approximately 3 Mbp of the long arm of chromosome 22, resulting in the loss of an estimated 30 genes [1]. Recent studies have described highly variable clinical indications of patients with chromosome 22q11.2 deletions, even within the same pedigree. However, CHDs are the most common feature of DiGeorge syndrome, or *del22q11*, and may include TETRALOGY OF FALLOT, INTERRUPTION OF THE AORTIC ARCH TYPE B, VENTRICULAR SEPTAL DEFECTS, PULMONARY ATRESIA, OR PERSISTENT TRUNCUS ATERIOSUS (see Glossary for terms; Fig. 2) [1,2].

The use of mouse genetics has recently given clarification to which of the numerous genes deleted in *del22q11* might be responsible for the DiGeorge syndrome phenotype. Targeted mutations in the mouse genome have allowed the majority of the DiGeorge syndrome clinical manifestations to be attributed to haploinsufficiency of *Tbx1* (GenBank accession no. AF012130), one of the genes deleted in *del22q11* patients [1,3]. *Tbx1* is a member of the T-box family of transcription factors and is involved in the patterning of the pharyngeal

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Glossary

Duane anomaly: a congenital eye movement disorder characterized by an absence of abduction, restricted adduction and retraction of the globe on attempted adduction.

Hypoplastic left heart syndrome: a congenital heart defect in which the left side of the heart is underdeveloped resulting in insufficient blood flow.

Patent ductus arteriosus: a cardiovascular defect in which the ductus arteriosus fails to close after birth.

Persistent truncus arteriosus: a congenital cardiovascular defect resulting from the failure of the septum between the aorta and pulmonary trunk to develop and characterized by a common arterial trunk opening out of both ventricles with the pulmonary arteries branching from the ascending common trunk.

Pulmonary atresia: a congenital heart defect characterized by the absence of the normal valve into the pulmonary artery.

Interruption of the aortic arch type B: a congenital heart defect characterized by an interruption between the left carotid artery and the left subclavian artery.

Monoallelic microdeletion: loss of a small fragment of one allele of a gene.

Radial ray defects: congenital malformations of the forelimbs.

Tetralogy of Fallot: a congenital heart defect characterized by a malformation of the ventricular septum, enlargement of the right ventricle, misplacement of the aortic origin, and a narrowing of the pulmonary artery.

Ventricular septal defect: a congenital heart defect characterized by an abnormal opening between the cardiac ventricles, usually resulting from failure of the spiral septum to close the interventricular foramen and allowing blood to pass directly from the left to the right ventricle.

endoderm and aortic arches as well as cardiac outflow tract development in a gene dosage-dependent manner [2]. Attempts to further connect *Tbx1* to DiGeorge syndrome have led to searches for mutations in this gene in patients lacking the typical chromosomal deletion. To this end, five patients have been identified as carrying only a *Tbx1* gene mutation [4]. Though these individuals do not exhibit all characteristics of DiGeorge syndrome, this demonstrates that mutations in human *Tbx1*, as in mouse, are capable of causing many of the defects associated with *del22q11*. In an effort to understand the molecular mechanisms of TBX1 function, recent observations have resulted in a model in which *Fgf8* in the pharyngeal endoderm is regulated by TBX1 to control the proper patterning of the aortic arch through epithelial–mesenchymal interactions (Table 1) [2]. Additionally, *Tbx1* transcription has been shown to be regulated by the *sonic hedgehog* (*shh*) signaling pathway via the *Foxc1* and *Foxc2* transcription factors which are expressed in the head mesenchyme and the mesenchyme surrounding the aortic arch arteries (Table 1) [2]. Together, studies such as these demonstrate signaling cascades by which *Tbx1* is transcribed and can initiate proper patterning events; however, the complete mechanism of TBX1 action remains unknown.

Familial cardiac septal defects: *Nkx2.5* and *Gata4*

Cardiac septal defects (CSDs) are a common form of CHD and are defined by a hole in the septal wall allowing blood transfer

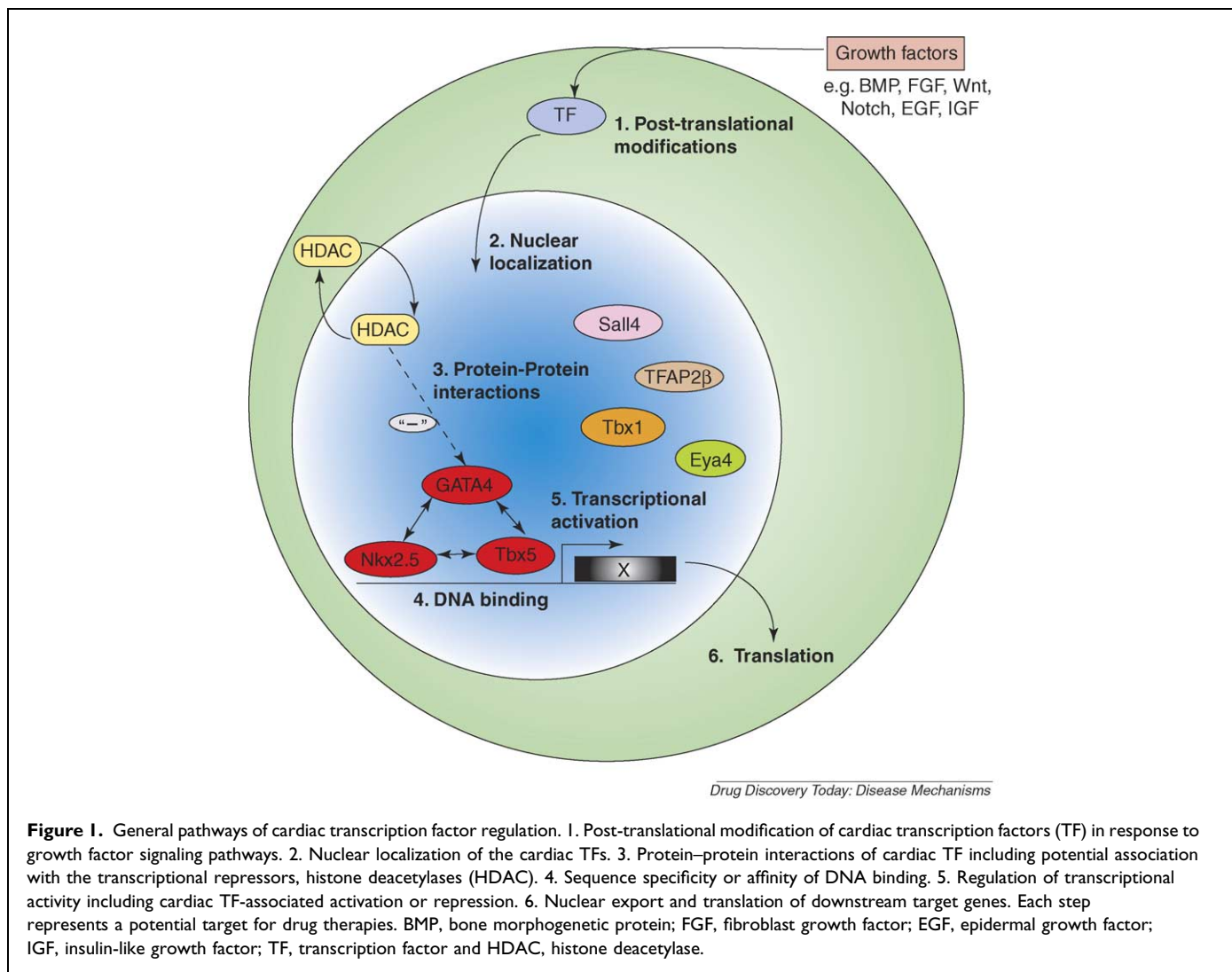
between the atria or ventricles. Atrial septal defects (ASDs) affect over one in 1000 live births, while ventricular septal defects (VSDs) are the most prevalent CHD, occurring in approximately one in 300 live births (Fig. 2). Over time, persistent left-to-right shunting of blood between the atria or ventricles leads to pulmonary hypertension, arrhythmias, and atrial and ventricular dysfunction. Fortunately, severe ASDs and VSDs can be treated by surgical- or catheter-based procedures that employ a prosthetic patch to close the defect. Despite the high incidence of CSDs, the precise molecular mechanisms directing septal morphogenesis remain unclear. However, genetic studies have implicated mutations in the *Nkx2.5* and *Gata4* loci as genetic causes of familial CSDs.

Mutant alleles of the *Nkx2.5* locus (GenBank accession no. BC025711) correlate with ASDs in rare families in which the defect is inherited (Fig. 2) [5]. Genetic studies in a wide variety of organisms demonstrate that NKX2.5 functions at many stages of cardiac development and in a variety of cardiac tissues [6,7]. Complete loss of NKX2.5 in mice results in early embryonic lethality with severe cardiac defects [8], whereas mice heterozygous for the *Nkx2.5* allele suffer from ASDs only occasionally [9]. This suggests that genetic modifiers are important for ASD penetrance. NKX2.5 interacts with other transcription factors associated with CHDs such as GATA4 and TBX5, and many cardiac-specific genes contain NKX2.5-binding sites in their promoters, highlighting the importance of *Nkx2.5* in the cardiac transcriptional program (Table 1) [10]. Chien *et al.* reported that mice harboring a ventricular muscle-cell restricted knockout of *Nkx2.5* mimic CHD and implicated persistent bone morphogenetic protein-10 (BMP-10) expression as playing an important role in the onset and progression of observed cardiac defects [11]. This study suggests that antagonizing BMP-10 signals could represent a new therapeutic approach to prevent the progression of *Nkx2.5*-associated CHDs.

ASDs as well as VSDs and atrioventricular septal defects, are also associated with *Gata4* (GenBank accession no. AY740706) haploinsufficiency (Fig. 2) [12–14]. A study of a large pedigree revealed a missense mutation in *Gata4* linked to an autosomal dominant disorder where ASD was fully penetrant. *Gata4* encodes a zinc-finger transcription factor essential for cardiogenesis and directly interacts with the cardiac transcription factors NKX2.5 and TBX5 to synergistically activate cardiac gene expression [10,15]. Inherited mutations in *Gata4* result in reduced DNA binding and transactivation of target genes as well as loss of TBX5 interaction [12]. In addition to NKX2.5 and TBX5, GATA4 associates with a variety of binding partners thought to create specific transcriptional complexes that confer tissue-specific gene expression during heart development (Table 1) [6,10].

Holt-Oram syndrome: *Tbx5*

Holt-Oram syndrome (HOS, OMIM #142900) is an autosomal dominant condition that occurs in approximately one of



every 100,000 live births. HOS generally presents highly variable phenotypes including both upper limb and cardiac defects. Though rare, there is much to learn from its presentation of CHDs, which range from single or multiple ASDs and VSDs to more complex malformations such as tetralogy of Fallot and HYPOPLASTIC LEFT HEART SYNDROME (see Glossary; Fig. 2) [16]. Mild to severe cardiac arrhythmias are also common [17].

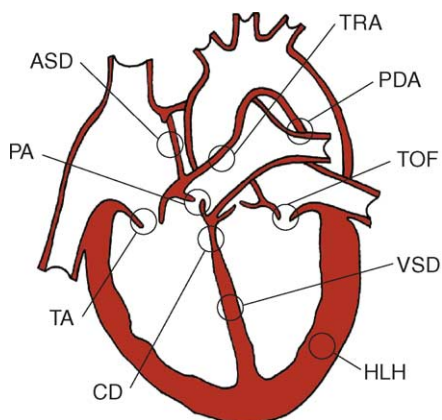
The genomic locus responsible for the HOS phenotypes was previously mapped to chromosome 12q24.1. Since then, HOS has been linked to more than 30 mutations distributed throughout *Tbx5* (GenBank accession no. U80987), generally thought to result in *Tbx5* haploinsufficiency [16,18]. *Tbx5*, like *Tbx1*, is a T-box containing transcription factor that is essential for proper vertebrate tissue patterning and differentiation [19]. Though familial studies and studies in mouse have attempted to correlate the location of the many *Tbx5* mutations along the locus with the wide variation in severity of limb and cardiac defects, there is currently insufficient evidence to support such a hypothesis [16]. Currently, it is

thought that loss of transactivation, reduced interaction with other cardiac transcription factors such as NKX2.5, GATA4, and TBX20, or mis-sorting of mutant forms of TBX5 are the main causes for HOS pathogenesis (Table 1) [6,15,20].

Okhiro syndrome: *Sall4*

Okhiro syndrome (OMIM #126800) is an autosomal dominant condition consisting of DUANE ANOMALY, RADIAL RAY DEFECTS and deafness (see Glossary). The phenotype might include cardiac defects, anal stenosis, pigmentary disturbance, renal abnormalities, or facial asymmetries. The specific cardiac defects are most often ASDs, VSDs, or tetralogy of Fallot (Fig. 2) [21,22].

Familial studies of individuals affected by Okhiro syndrome have identified mutations in the *Sall4* gene (GenBank accession no. NM_020436) and suggest that haploinsufficiency of this gene is responsible for the clinical phenotype [23–25]. *Sall4* (*spalt-like 4*) is a member of the *Sal* gene family, which encodes a group of four probable zinc-finger transcription factors [26]. Thus far, a total of 11 different mutations



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Figure 2. Congenital heart defects associated with mutations in cardiac transcription factors. Mutations in cardiac transcription factors such as *Tbx1*, *Nkx2.5*, *Gata4*, *Tbx5*, *Sall4*, and *TFAP2B* have been associated with multiple human congenital heart defects including atrial septal defects (ASD), ventricular septal defects (VSD), tetralogy of Fallot (TOF), conduction system defects (CD), hypoplastic left heart (HLH), pulmonary atresia (PA), patent ductus arteriosus (PDA), tricuspid atresia (TA), and truncus arteriosus (TRA). Schematic shows the relative position of each defect.

over the entire *Sall4* gene have been described in relation to Okihiro syndrome [21,23,24]. In addition, Borozdin *et al.* demonstrated that Okihiro syndrome can also be caused by deletions of either the entire *Sall4* gene or of single coding exons [25]. Based on work with the closely related *Sall1*, it is evident that these mutations probably result in truncated proteins, possibly having the dominant effect of an upregu-

lated repressor [27]. However, at this point there are no known upstream effectors or downstream targets of *SALL4*.

Char syndrome: *TFAP2B*

Char syndrome (OMIM #169100) is an autosomal dominant trait characterized by facial dysmorphism, hand anomalies, and PATENT DUCTUS ARTERIOSUS (see Glossary; Fig. 2). Char syndrome has been mapped to chromosome 6p12-p21 and further analyses point to inherited mutations within the *TFAP2B* (transcription factor AP-2 beta) (GenBank accession no. NM_003221) locus as the genetic cause of Char syndrome [28–30]. *TFAP2B* encodes a neural crest-related transcription factor belonging to the TFAP family, whose members play an important role in retinoic acid-induced differentiation [31]. Char syndrome probably results from abnormal neural crest development, as neural crest cells are important for the development of several affected tissues [32]. *TFAP2B* mutations associated with Char syndrome inhibit target gene activation through a dominant-negative mechanism or cause abnormal mRNA splicing resulting in *TFAP2B* haploinsufficiency [29,30]. However, the precise molecular mechanisms underlying the effects of aberrant *TFAP2B* activity resulting in Char syndrome remain to be elucidated.

Dilated cardiomyopathy with sensorineural hearing loss: *Eya4*

Cardiomyopathy is the leading cause of heart failure and is most commonly associated with a dilated cardiomyopathy (DCM) phenotype, defined by increased diastolic and systolic ventricular volumes and contractile dysfunction [33]. Often, DCM is presented in conjunction with defects of the inner ear

Table 1. Human CHDs, associated transcription factors and molecular interactions^a

Human clinical manifestation	Associated transcription factor	Co-factors/upstream molecules	Potential Downstream cardiac genes	Refs
DiGeorge syndrome	<i>Tbx1</i>	VEGF, FOXc1, FOXc2	<i>Fgf8</i>	[1–4,6,7,10]
Familial ASD	<i>Nkx2.5</i>	GATA4, TBX5	<i>Nppal/ANF</i> , <i>Bnp</i> , <i>eHand</i> , <i>Mef2C</i> , <i>Mlc2V</i> , <i>Msx2</i> , <i>N-Myc</i>	[5–10,15]
Familial ASD/VSD	<i>Gata4</i>	FOG2, GATA6, MEF2C, NFATc4, NKX2.5, SRF, TBX5	<i>Nppal/ANF</i> , α / <i>b</i> -MHC, Cardiac α -actin, Cardiac TnC, Cardiac Tnl, <i>Gata6</i> , <i>Nkx2.5</i>	[6,7,10,12–15]
Holt-Oram syndrome	<i>Tbx5</i>	GATA4, NKX2.5, TBX20	<i>Nppal/ANF</i> , <i>Cx40</i> , <i>Gata4</i> , <i>Hey2</i> , <i>Irx4</i> , <i>Mlc2v</i> , <i>Nkx2.5</i>	[6,7,10,15–20]
Okihiro syndrome	<i>Sall4</i>	Unknown	Unknown	[6,21–27]
Char syndrome	<i>TFAP2B</i>	Unknown	Unknown	[15,28–30]
Dilated cardiomyopathy with sensorineural hearing loss	<i>Eya4</i>	SIX, DACH	Unknown	[6,34–38]

^a Abbreviations: VEGF, vascular endothelial growth factor; FOXc1/2, human forkhead-box subfamily c1/2; GATA, GATA-binding protein; TBX, T-box; FOG2, friend of GATA; MEF2C, myocyte enhancer factor 2C isoform; NFATc4, nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent-4; NKX2.5, NK2-related homeobox; SRF, serum response factor; *Fgf8*, fibroblast growth factor 8; *Nppal/ANF*, natriuretic peptide precursor a/atrial natriuretic factor; *Bnp*, brain natriuretic peptide; *eHAND*, heart- and neural crest derivatives-expressed 1; *mlc2v*, myosin light chain-2 ventricular isoform; *Msx2*, muscle segment homeobox 2; *N-Myc*, neuroblastoma-myelocytomatosis viral-related oncogene; α / β -MHC, alpha-, beta-myosin heavy chain; TnC, troponin C; Tnl, troponin I; *Cx40*, connexin 40; *Hey2*, hairy/enhancer of split-related with YRPW motif-2; *Irx4*, iroquois 4.

resulting in sensorineural hearing loss (SNHL). Although not typically characterized as a classical CHD, cardiomyopathy is discussed here because the origins of its pathophysiology can also result from mutations in cardiac transcription factors.

Several studies have demonstrated that approximately 25–30% of DCM cases could be familial [33]. Until recently, the significant mortality and the late onset of this disease hindered the work to identify the genomic location of the responsible disease loci. Schönberger and co-workers have identified a human mutation that causes dilated cardiomyopathy and associated heart failure in addition to previously described sensorineural hearing loss [34,35]. The identified mutation is a 4846 bp deletion of the human gene *Eya4* (GenBank accession no. Y17114), one of the four vertebrate orthologs of the *Drosophila melanogaster* gene *eyes absent* (*eya*) [36]. EYA4 is a transcriptional coactivator that interacts with members of the *sine oculis* family (*Six1-Six6*) and *Dach* transcription factors leading to gene activation (Table 1) [36–38]. The characterization of the human mutation is supported by work in zebrafish, as attenuated *eya4* levels produce the morphological and hemodynamic features of heart failure. In addition, Schönberger *et al.* demonstrate critical roles for EYA4-SIX regulation of transcription in normal heart function [34].

Potential therapies

In general, transcription factors have historically been poor targets of drug therapy due to their nuclear localization, lack of enzymatic activity and the difficulty associated with reprogramming transcriptional networks (Fig. 1). Presently, the most effective therapy for cardiac diseases is heart transplantation. However, due to the shortage of organs, cost and inaccessibility of treatment for most affected individuals, this remains a limited therapeutic option. Alternative treatment is the administration of drugs that improve myocardial contractility, though this treatment is only effective as a short-term therapy, with the 5-year survival rate using current agents being less than 60% [39]. More recently, new strategies have focused on two main approaches for treatment of transcription factor-associated heart disease, cardiac stem cell transplants and chemical modulators of transcriptional activity.

The ability to isolate and propagate cell populations that can differentiate into cardiomyocytes *in vivo* offers the opportunity to treat a wide range of cardiac diseases. The existence of cardiac precursor or stem cells in adults remains a contentious issue. However, recent reports suggest that cardiac precursor or stem cells are present, albeit, in a very low number. In addition to endogenous cardiac stem cells, other studies have shown that multipotential cells, most notably embryonic stem (ES) cells and bone marrow-derived stem cells, have under defined conditions differentiated into cardiomyocytes. Although these studies offer the promise of

growing cells for use in repairing the damaged cardiac tissue, three major hurdles must be overcome before stem cells can be considered as a therapy for cardiac disease. First, the molecular, biochemical and cellular properties of these different cell populations must be established. Second, studies must demonstrate that precursor cell populations can be maintained and expanded to suitable numbers to be used as a cardiac therapy while maintaining their multipotentiality. Finally, results must show multipotential cells, once transplanted to the heart, can give rise to functioning cardiomyocytes while not undergoing uncontrolled differentiation leading to cardiac teratomas or fibrosis [40].

An alternative therapeutic strategy for transcription factor-associated cardiac disease is to screen small chemical libraries to identify agents that either exacerbate or ameliorate transcription factor activities (Fig. 1). These agents could either act in an intercellular signaling cascade that turns on, off, or modifies transcription factor activities, most notably agents that act in the calcium or phosphate signaling pathways, or act directly on transcription co-factors such as histone acetyltransferases (HATs) or histone deacetylases (HDACs) [39]. The major obstacle is the availability of an inexpensive, quick screen for these molecules. However, recent observations have shown the sequence, expression and function of many cardiac disease-associated transcription factors are evolutionarily conserved, opening the possibility of using fish or frog model systems as bioassays to test for agents that modulate these pathways.

Conclusions

Given the number of transcription factors demonstrated to play essential roles in vertebrate cardiogenesis, the current pool of CHD-associated transcription factors is probably underrepresented. We speculate that more correlations between mutations in cardiac transcription factors and CHD will be made. To this end, disruptions in the function of at least six cardiac transcription factors have been associated with human CHD. Haploinsufficiency of the genes *Tbx1*, *Tbx5*, *GATA4* and *Sall4* have been correlated with CHDs such as DiGeorge syndrome, Holt-Oram syndrome, familial ASDs and VSDs and Okhiro syndrome whereas similar disruptions in *Nkx2.5*, *TFAP2B*, and *Eya4* are associated with familial ASDs, Char syndrome and cardiomyopathies. Although the identification of genes associated with CHD is an important first step towards the goal of curing cardiovascular disease, it has become clear that understanding the genetic pathways and the molecular mechanisms of transcription factors will be the key to our ability to identify therapeutic agents for CHD. Based on our current understanding of these mechanisms and of heart development in general, possible treatment options can eventually grow to include cardiac stem cell transplants and chemical agents. However, these possibilities lie in the future, and their devel-

opment will rely upon studies using a variety of animal models and our growing knowledge of CHD.

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