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The Molecular Genetics of Congenital Heart Disease: A Review of Recent Developments

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Abstract

Purpose of review—Our understanding of the interactions of genes and pathways during heart development continues to expand with our knowledge of the genetic basis of congenital heart disease (CHD). Along with the discovery of specific genes that cause lesions, recent research has focused on the interactions of some previously identified genes. This review focuses on the progress made during the last year.

Recent findings—T-box, NK, and GATA transcription factors have known associations with a variety of syndromic and isolated congenital heart defects. Discovery of novel interactions of GATA and T-box transcription factors highlights the direction of recent research. In addition, the critical yet somewhat redundant roles of *nkx2.5* and *nkx2.7*, along with the interaction of *nkx2.7* with *tbx20*, have been elucidated. The contributions of still other transcription factor classes are being elucidated. Further understanding of 22q11.2 deletion and microduplication syndromes and their genetic interactions has also been studied. Recent work also highlights *PTPN11* and *NOTCH1* in Noonan syndrome.

Summary—The recent developments in the genetics of CHD are reviewed. In many cases, it is the novel interactions of previously known genes that highlight this year's developments. These interactions will ultimately lead to better understanding of downstream transcriptional or signaling pathways.

Keywords

cardiac septation; congenital heart disease; conotruncal anomalies; somatic mutations

Introduction

Congenital heart disease (CHD) is the most common type of birth defect, with an estimated prevalence of 4 to 50 per 1000 live births.¹ CHD affects approximately 36,000 children each year in the United States.² The prognosis of children with complicated and uncomplicated CHD continues to improve with advancing surgical techniques, but the reported incidence remains unchanged.³ Although cardiac defects can occur in the setting of multiple birth defects as part of a syndrome, most are found as isolated defects with no syndromic association.⁴ For both syndromic and isolated defects, familial cases have been described. Also consistent with genetic etiologies for CHD, epidemiologic studies have shown increased CHD recurrence risk in future pregnancies.⁵ In this review, we present advances

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from the past year in the genetics of CHD. These include studies highlighting interaction of certain genes previously known to be involved with specific lesions and key transcriptional or signaling pathways. Abnormalities in these genes may interrupt or interfere with embryonic cardiogenesis at various stages and produce different CHD phenotypes.⁶

Cardiac septation defects

Defects of cardiac septation are the most common form of CHD and account for approximately 50% of all CHD.¹ Cardiac septation defects include atrial septal defects (ASD), ventricular septal defects (VSD), and atrioventricular septal defects (AVSD), and they have strong associations with some Mendelian syndromes. Nonetheless, septal defects comprise the largest portion of isolated CHD that does not occur in the setting of other birth defects or genetic syndromes.⁴ Genes that encode transcription factors have been previously associated with cardiac septation defects and include *TBX5*, *NKX2.5*, and *GATA4*. Individuals with Holt-Oram Syndrome, which is characterized by secundum ASD and VSD along with upper limb deformity, have mutations in *TBX5*.⁷ Mutations in *NKX2.5*, which interacts with *TBX5*, cause *ostium secundum* ASD and heart block.⁷ *NKX2.5* mutations are also seen in other forms of CHD including tetralogy of Fallot and Ebstein anomaly.^{8,9} Mutations in *GATA4* have also been identified in patients with ASD and VSD.¹⁰

Experimental analyses of *GATA4* mutations have revealed interactions between *Gata4* and *Tbx5*.¹¹ Maitra et al. used mouse genetics to investigate interaction between *Gata4* and *Tbx5*.¹¹ They demonstrated that doubly heterozygous (*Gata4*^{+/-}, *Tbx5*^{+/-}) knockout mice display growth retardation and early neonatal lethality, likely from heart failure. Interestingly, cardiogenesis was normal early in embryogenesis, but all double heterozygotes ultimately developed AVSDs and biventricular myocardial thinning. The authors propose that the myocardial abnormalities may be secondary to downregulated *MYH6* (α Myosin Heavy Chain), mutations in which have also been associated with ASD.¹² Davis et al.¹³ showed that knockout mice that were *Gata6*^{+/-}, *Tbx5*^{+/-} double heterozygotes also exhibited early neonatal lethality. Unlike *Gata4*^{+/-}, *Tbx5*^{+/-} mice, *Gata6*^{+/-}, *Tbx5*^{+/-} mice had no growth retardation and normal cardiac septation. *Myh6* expression in these mice was normal, but the mice did exhibit defects in myocardial proliferation. Thus, future mutational analyses in relevant forms of human CHD may focus on *GATA6/TBX5* interaction domains.

GATA5, although a member of the *GATA* transcription factor family, has not been previously implicated in human CHD. While it is present in developing endoderm and mesoderm, there has been little delineation of direct involvement in mammalian cardiac development. However, Singh et al.¹⁴ have now used transgenic mice to study potential redundant roles of *Gata4* and *Gata5* in heart development. They have demonstrated that haploinsufficiency of both *Gata4* and *Gata5* results in defects characterized by thinning of the ventricular wall. Loss of a second *Gata5* allele leads to profound cardiac malformations including ASD, VSD, and endocardial cushion defects among others. Thus, Singh et al.¹⁴ conclude that *Gata4* and *Gata5* play redundant, yet critical roles in heart development. The exact contribution of *Gata5* to this process will likely be the subject of future studies.

Recent experimental data has also highlighted *NKX2.7* as a candidate CHD gene. Tu et al.¹⁵ investigated *nkx2.7* and *nkx2.5* redundancy during zebrafish cardiogenesis. Both of these factors are known to participate in cardiac looping. Using knockdown transgenic zebrafish lines, the authors demonstrated that both *nkx2.7* and *nkx2.5* are crucial for later stages of normal cardiogenesis. While hearts of *nkx2.5* deficient fish fail to loop appropriately, *nkx2.7* deficient hearts exhibit defects in ventricular myocardial proliferation and differentiation. Interestingly, doubly deficient (*nkx2.7/nkx2.5*) fish also display upregulation of other

cardiogenic genes (e.g. *bmp4*, *versican*, and *tbx20*) at later stages of heart development. Tu et al.¹⁵ suggested that such increased gene expression contributes to defective cardiogenesis since *tbx5* and *tbx20* inhibit cardiac proliferation, and are negatively modulated by *nkx2.7*.¹⁶ Thus, *nkx2.7* and *nkx2.5* both play critical and somewhat redundant roles in cardiac morphogenesis. The interaction of *nkx2.7* with *tbx20* especially influences late cardiac morphogenesis. While pre-looping cardiac development proceeds normally, these transcription factors appear to have a significant influence on late morphogenesis and the eventual phenotype.¹⁵

Much remains to be done to understand the contributions of *NKX* gene mutations to human congenital heart diseases. Although constitutional mutations have been shown to contribute to Mendelian transmission, the role of somatic mutations has been more controversial. *HAND1* is a basic helix-loop-helix (bHLH) transcription factor that plays a key role in heart development.¹⁷⁻¹⁸ Cardiac specific deletion of *Hand1* in the developing mouse heart has been implicated in VSDs, AV valve malformation, hypoplastic ventricles, and outflow tract abnormalities.¹⁹ Reamon-Beuttner et al.²⁰ studied the contribution of *HAND1* mutations to pathogenesis of septation defects in the human heart. Using formalin fixed hearts, they found multiple mutations in the *HAND1* gene, most frequently occurring in the N-terminal region and the bHLH domain. These areas are essential for DNA binding and protein-protein interactions of *HAND1*. Interestingly, *NKX2.5* and *GATA4* mutations were found in the same collection of hearts. Reamon-Beuttner et al.²⁰ proposed that somatic mutations may modify combinational interactions of well known cardiac transcription factors to affect heart development, specifically atrial and ventricular septation. By contrast, Draus et al.²¹ found no evidence of somatic *NKX2.5* mutations in 28 samples of fresh frozen tissue and suggested that others' findings may have resulted from formalin fixation induced DNA damages. In the future, such surveys will continue to tackle the possibility of constitutional and/or somatic mutations in other transcriptional factors involved with CHD.

Recent studies have shown that *Tbx2* plays a key role in the formation of the AV canal, the left ventricular base, and the cardiac conduction system.²² *Tbx3* and *Tbx18* play key roles in sinoatrial (SA) node development.²³ *TBX20* mutations have been reported in familial secundum ASDs.²⁴ Posch et al.²⁵ describe a novel mutation in *TBX20* associated with familial ASD spanning three generations of kindred. This mutation apparently was a gain-of-function one which significantly enhanced transcriptional activity of two target genes, *NKX2.5* and *GATA4/5*. These findings support the idea that the development of the atrial septum is a result of the complex interaction of multiple transcription factors. These and other factors will be ripe substrate for mutational analysis.

Conotruncal and Outflow Tract Defects

Outflow tract and aortic arch anomalies account for 20–30% of CHD.¹ The 22q11 deletion CHD syndrome encompassing DiGeorge, velo-cardio-facial, and conotruncal-face syndrome has been well studied. Deletions affecting the 22q11.2 chromosomal locus and the *TBX1* gene likely account for much of the human phenotype, but the contribution of other genes in the deleted region remain a focus of research. Lammer et al.²⁶ investigated chromosomal abnormalities in patients with conotruncal cardiac defects to identify whether 22q11.2 deletions were more strongly associated with one specific lesion. They concluded that children with TOF, compared with those with transposition of the great arteries (TGA) or double outlet right ventricle (DORV) have a significantly higher incidence of 22q11.2 deletions. Goldmuntz and colleagues²⁷ previously found that 15.9% of TOF patients had 22q11.2 deletions. Lammer et al.²⁶ continue to highlight the potential role of other 22q11.2 genes such *JARID2*, an ortholog of the murine gene *jumoni*, which encodes a protein

required for normal heart development. They also observed that four infants in their cohort had a sex chromosome abnormality, the significance of which warrants further investigation.

Rauch et al.²⁸ investigated genotype-phenotype correlation in a cohort of 230 patients with TOF. Their findings support previous studies that the common 22q11.2 deletion represents the most frequent genetic diagnosis in TOF. A small number of their cohort had atypical proximal deletions in 22q11.2 as well as mutations in *TBX1*, *NKX2.5*, and *JAG1*. Rauch et al.²⁸ also showed intragenic *TBX1* mutations may cause non-syndromic TOF, postulating that loss of *TBX1* transcriptional activity due to aggregation of the mutant protein is responsible. Interestingly, they found that AVSD associated with TOF is highly suggestive of trisomy 21 and excludes 22q11.2 deletion.

Considerable attention is now devoted not only to deletion but also to microduplication of 22q11.2.²⁹ 22q11.2 microduplication may occur with a frequency approximately half of that of deletions.³⁰ The phenotype of patients with microduplication 22q11.2 is extremely variable, but significantly overlaps with the features of DiGeorge and Velo-Cardio-Facial syndromes.³¹ The spectrum of CHD associated with microduplication 22q11.2 is vast, including conotruncal and non-conotruncal defects, VSD, hypoplastic left heart syndrome (HLHS), valvular anomalies, and total anomalous pulmonary venous return (TAPVR) among others.³² Interestingly, there is highly variable inter- and intrafamilial expression even among family members with the same size duplication.³⁰ Consistent with the effects of these chromosomal duplications, Zweier et al. reported that *TBX1* gain of function mutations produce human conotruncal malformations.³³

In addition to microduplications, increasing attention is now being given to copy number variants (CNV). These represent a significant source of inter-individual genetic variation that may explain the variable expression of inherited disorders, variable phenotypical presentation of disorders, and potentially non-syndromic CHD.³⁴ Greenway and colleagues³⁵ identified seven new loci that substantially increase the risk for non-syndromic TOF. A CNV at 1q21.2 was present in 1% of sporadic TOF in their cohort. Of the seven CNVs, three encode genes previously associated with CHD (*TBX1*, *NOTCH1*, *JAG1*). They conclude that as much as 10% of sporadic TOF is the result of *de novo* CNVs. The mutations at the loci they identified provide further candidate genes for CHD.

Fifty-six percent of 11q- (Jacobsen syndrome) patients have clinically significant conotruncal defects, most of which are characterized as “flow” defects (HLHS, DORV, d-TGA). A cardiac “critical region” has been identified in distal 11q that is thought to contain putative genes for CHD.³⁶ Multiple candidate gene mutations in this region may provide novel insight into the genetic basis of non-syndromic lesions. *Jam-C*, the murine analogue of *JAM-3*, has previously been implicated in causing CHD in 11q-.³⁷ By contrast, however, Ye et al.³⁸ found that *Jam-C* knockout mice did not exhibit structural heart defects. There was early neonatal lethality, though the cause was unclear, and Ye et al. postulated that it was unlikely to result from CHD.

Investigating another Jacobsen syndrome candidate gene in 11q-, Ye et al.³⁹ demonstrate that loss of the murine analogue of the *ETS-1* gene, located in the cardiac critical region of 11q, causes VSD and abnormal ventricular morphology in mice. *ETS-1* is a transcription factor with functions that include the regulation of cell growth, lymphocyte development, and angiogenesis.⁴⁰ *Ets-1* knockout mice display large membranous VSDs as well as abnormal ventricular morphology. Of interest, *Ets-1* was not detected in myocardium, evoking the possibility that it is its expression in endocardium which is required for normal myocardial development. The role of *ETS-1* in CHD will likely be the subject of future investigation in patients with 11q- as well as non-syndromic patients.

Defects in either the right ventricular outflow tract (RVOT) or the left ventricular outflow tract (LVOT) vary in location and severity, and in the most extreme circumstances may lead to hypoplasia of the corresponding ventricle. Mutation of *ELN* is responsible for the supravalvular stenosis in Williams syndrome⁴¹. Mouse models showed that a genetic defect mutation in *Ptpn11* (which encodes the Ras signaling cofactor *Shp2*), result in dysplastic semilunar valves.⁴² In humans, *PTPN11* mutations cause Noonan syndrome, which often presents with pulmonic stenosis.⁴³ Araki et al.^{44,45} studied genetically engineered mice to demonstrate that expression of a human *Ptpn11* mutation results in endocardial expression of mutant *Shp2* and Noonan syndrome. They suggest that Noonan syndrome valvular defects relate to defective endocardial-mesenchymal transformation in the endocardial cushions, which is, at least in part, modulated by Ras/Erk signaling.

The Ras-mitogen activated protein kinase (MAPK) signaling pathway is also involved with cardio-facio-cutaneous syndrome (CFCS). This syndrome is characterized by mental retardation, facial dysmorphisms, ectodermal abnormalities, and heart defects.⁴⁶ Mutations in *BRAF* as well as *MEK1/MEK2* have been implicated in CFCS.⁴⁷ Dentici et al.⁴⁸ analyzed a cohort of CFCS patients for *MEK1/MEK2* mutations compared with *BRAF* mutant individuals. While most clinical features were similar, CHD incidence was found to be lower in the *MEK1/MEK2* cohort. Specifically, mitral valve defects and septal defects were less frequent in the *MEK1/MEK2* patients. One patient with an *MEK1* mutation had TOF and abnormal pulmonary venous return, a phenotype not previously described in the Ras/MAPK pathway.

Ongoing investigation continues to highlight the role of NOTCH signaling in CHD. Alagille syndrome, which includes biliary atresia and right sided heart defects, is caused by mutations in *JAGGED-1*, a membrane bound ligand for the Notch 1–4 family, members of which are involved in embryonic patterning and cellular differentiation.⁴⁹ *JAGGED-1* mutations have also been identified in non-syndromic patients with CHD.⁵⁰ In addition, *NOTCH1* mutations have been implicated in familial bicuspid aortic valve (BAV), the most common form of CHD.⁵¹ Interestingly, the nuclear-localized, cleaved and active form of *NOTCH*, the *NOTCH1* intracellular domain, has been found to play a role in epicardial cell transition and early coronary artery development.⁵² High et al.⁵³ demonstrated an essential role for *Notch1* and *Jagged1* in the secondary heart field of transgenic mice during cardiac outflow tract and aortic arch development. Inhibition of *Notch1* or deletion of *Jagged1* resulted in downregulated *Fgf8* in secondary heart field derived myocardium, hypocellular endocardial cushions, and neural crest defects. *Fgf8* is critical to endocardial cushion development and outflow tract septation.⁵⁴ While the exact mechanism of *Notch1/Jagged1* – *Fgf8* interaction remains to be elucidated, this interaction provides a candidate pathway for pathogenesis of Alagille syndrome as well as non-syndromic outflow tract abnormalities.

Conclusion

Advances in our understanding of CHD genetics continue to accrue at an exciting pace. The importance of these developments has never been more significant as individuals with CHD are surviving into adulthood and having children of their own. Recent discoveries in CHD research have continued the trend of demonstrating that mutations in genes that encode proteins with transcriptional functions and/or downstream regulatory factors are central to the genetic basis for CHD. Future work will likely focus on better delineation of genetic causes for non-syndromic CHD, as well as the continued identification of novel interactions between previously and newly discovered genes and proteins. Ultimately, this information will prove invaluable in our treatment and management of CHD patients and their offspring.

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