A Decade of Advances in the Molecular Embryology and Genetics Underlying Congenital Heart Defects

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Congenital heart defects (CHD) are the most common type of human birth defect and result in significant mortality worldwide. Despite numerous epidemiologic studies in the past decades, few genetic causes have been identified until recently. CHD result from abnormal morphogenesis of the systematic cardiovascular construction during development. Recent advances in molecular embryology, including the discovery of a new source of cardiac progenitor cells termed the second heart field (SHF), have revealed that the heart arises from multiple distinct embryonic origins. Cells derived from the SHF contribute to the development of the cardiac outflow tract, together with the other progenitor cell lineage called cardiac neural crest cells. Numerous cardiac transcription factors regulate these progenitor cells during heart development. Elucidation of the transcriptional network for these cardiac progenitor cells is essential for further understanding cardiac development and providing new insights into the morphogenesis of CHD. This review outlines the recent discoveries of the molecular embryology of the normal heart and the genetic basis of CHD. (*Circ J* 2011; **75**: 2296–2304)

Key Words: Congenital heart disease; Genes; Genetics; Genotype; Pediatrics

Congenital heart defects (CHD) occur in nearly 1% of all live births and are the major cause of infant mortality and morbidity.¹ Additionally, approximately 3 per 1,000 live births will require some intervention during the first year of life. The multiple surgeries necessary to correct complex anatomical defects can be debilitating, and the quality of life of patients with CHD is often greatly compromised. Despite its clinical importance, the underlying genetic etiology of most CHD remains unknown, and they are so-called “multifactorial” diseases. The formation of the heart proceeds by sequential gene regulatory steps that dictate cell fates and organize specialized cell types into complex 3-dimensional units of structure and function. In order to explore the etiology of CHD, an approach focusing on the individual modular steps in cardiovascular morphogenesis is important, because most CHD result from abnormal morphogenesis in specific structural components of the developing heart and vessels. In this review, we discuss the recent 10-year advances in our understanding of the heart development within the framework of CHD.

Developmental Origins of the Heart

Decades of descriptive embryology, including cell lineage tracings, have improved our understanding of developmental origins of the heart (Figure 1).^{2,3} Cells derived from the anterior lateral plate mesoderm form a crescent shape at approximately embryonic (E) day 7.5 in the mouse embryo, corresponding roughly to week 2 of human gestation. By E8.0 in mice, or 3 weeks in humans, these cells coalesce along the ventral mid-

line to form a primitive heart tube, consisting of an interior layer of endocardial cells and an exterior layer of myocardial cells, separated by extracellular matrix, called “cardiac jelly”, for reciprocal signaling between the 2 layers. The crescent-shaped pool of cardiogenic progenitor cells is now termed as the “first heart field” (FHF).^{2,3} The FHF that forms the heart tube, eventually contributes to specific chambers of the future heart, exclusively to the left ventricle and all other parts of the heart, except the cardiac outflow tract (OFT).

In addition to the FHF, it was reported 10 years ago that there was a second source of myocardial cells.⁴ In the mouse embryo, this source lies medially to the cardiac crescent and then behind the forming heart tube, extending into the mesodermal layer of the pharyngeal arches. This second source is termed the “second heart field” (SHF).^{2,3} The heart tube derived from the FHF may predominantly provide a scaffold upon which cells from the SHF migrate into both the arterial and venous poles of the heart tube, where they subsequently construct the requisite cardiac components. The contribution of the SHF to both the myocardium and smooth muscle of the arterial pole has been well studied (Figure 1). When the heart tube forms, SHF cells migrate into the midline and position themselves dorsal to the heart tube in the pharyngeal mesoderm. Upon rightward looping of the heart tube, SHF cells cross the pharyngeal mesoderm into the anterior and posterior portions, populating a large portion of the OFT, the future right ventricle, and the atria. The addition of SHF-derived myocardium to the OFT results in its elongation, which is necessary to allow the OFT to rotate sufficiently for the correct align-

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Figure 1. Developmental origins of the heart. Frontal views of embryos at the cardiac crescent and primitive heart tube stage, a lateral view of an embryo at the looping heart stage, and the 4-chambered heart are shown. In the cardiac crescent, the first heart field (FHF) cells form a crescent shape in the anterior region of the embryo, with the second heart field (SHF) cells medial to it. In the primitive heart tube, the SHF cells lie dorsal to the heart tube in the splanchnic mesoderm of the pharyngeal region. In the looping heart tube, the SHF cells migrate into the anterior and posterior ends of the tube (blue arrows). Subsequently, cardiac neural crest cells (cNCC) migrate into the outflow tract from the dorsal neural tube (yellow arrows) to remodel the outflow tract and the aortic arch system. In the 4-chambered heart, the FHF cells form the left ventricle and atria (red), while the SHF cells form the outflow tract, the right ventricle, and part of the atria (blue). cNCC contribute to the outflow tract cushions (yellow) that eventually form the aortopulmonary septum. Epicardium is derived from the proepicardial organ (green).

ment of the pulmonary and aortic trunks with their respective ventricles.

Cardiac neural crest cells (cNCC) originate from the dorsal neural tube between the mid-otic placode and the caudal boundary of the third somite.⁵ After they delaminate from the dorsal neural tube, cNCC migrate into the caudal pharyngeal arches, and the OFT where they form the septum (Figure 1). Migration of the cNCC is also targeted to pharyngeal arches 3, 4 and 6, which give rise to the future great vessels (Figure 2). Many signaling pathways are involved in the migration and the condensation of cNCC, including reciprocal signaling between cNCC and the SHF that are essential for the development of the OFT and the aortic arch system.⁶

The fourth lineage of cardiac precursor cells is derived from the proepicardium (PE).⁷ The PE develops from the coelomic mesothelium that overlays the liver bud, and the expression of PE-specific genes is induced in naïve mesothelial cells in response to a localized liver-derived signal. Almost all of the cells of the epicardium and the coronary vessels arise from the PE, which develops as multiple epithelial villi protruding from the pericardial mesothelium immediately posterior to the sinoatrium of the looping stage embryonic heart. The PE extends towards the primitive heart, attaches, and spreads over the myocardial surface, forming the epicardium (Figure 1). During PE growth and epicardial formation, some PE/epicardial cells undergo an epithelial–mesenchymal transformation and are moved into the subepicardial space, giving rise to the precursors of the coronary vessels and connective tissue cells.⁸

Transcription Factors Essential for the Heart Development

The molecular mechanisms underlying heart development involving CHD have been elucidated in a decade by numerous

investigations of cardiac specific transcription factors.⁹ After the signal stimulations for cardiac induction, such as Wnt and BMP, heart development is governed by transcription factors that are evolutionally conserved and cross-regulate each other to control cardiac cell fates, the expression of genes encoding contractile proteins, and the morphogenesis of cardiac structures. The homeobox gene, *Nkx2.5*, also known as *Csx*, a homolog of *Drosophila tinman*, is expressed in cardiac progenitor cells, and serves as a target of inductive signals that initiate cardiogenesis. In mice lacking *Nkx2.5*, the initial events of heart formation occur normally, but embryos die from abnormal morphogenesis of the heart tube and failure of left ventricular development, which can be interpreted as a selective loss of derivatives of the FHF.

The *Mef2* gene is required for the differentiation of all types of muscles. Of the 4 vertebrate *Mef2* genes (*Mef2a*, *-b*, *-c* and *-d*), *Mef2c* is required for activation of a subset of cardiac contractile protein genes, as well as for the development of cardiac structures derived from the SHF.¹⁰

Members of the GATA family of zinc finger transcription factors directly regulate numerous cardiac contractile protein genes as well as upstream regulatory genes such as *Nkx2.5*, *Mef2*, and *Hand*.¹¹ Of the 6 GATA genes in vertebrates, three (*Gata4*, *-5* and *-6*) are expressed in the heart and have been implicated in heart development through loss-of-function mutations. Forced expression of cardiac GATA factors in *Xenopus* and zebrafish embryos induces premature activation of cardiac gene expression.¹² Mutation of the GATA gene, *pannier*, in *Drosophila* results in an absence of cardioblasts and a decrease in the number of pericardial cells.¹¹

Multiple T-box (Tbx) genes function to control cardiac fate, differentiation, and patterning of the dorsal vessel.¹³ At least 7 Tbx genes display overlapping expression in the FHF, SHF and other cardiac progenitors of amniotes. Mice lacking *Tbx5*

display defects in the posterior region of the heart tube, which gives rise to the atria.¹⁴ Tbx5 and Tbx20 have also been implicated in the formation of the cardiac conduction system and ventricular chambers.¹⁵ Tbx2 and Tbx3 function as repressors of chamber myocardium and are associated with the development of the conduction system.¹⁶

The *Drosophila* genome encodes a single member of the Hand family of bHLH transcription factors that is required for normal development of the dorsal vessel.¹⁷ The correlation between gene duplication and cardiac complexity is especially intriguing with respect to the *Hand* genes, which regulate ventricular growth. Although in zebrafish there is only 1 *Hand* gene that is essential for the development of the single ventricle of this organism,¹⁸ there are 2 *Hand* genes (*Hand1* and 2) in mice.¹⁹ *Hand1* and *Hand2* are preferentially expressed in derivatives of the FHF and SHF, respectively. Mice lacking *Hand2* do not form a right ventricle, whereas *Hand1* mutant embryonic stem cells are unable to contribute to the outer curvature of the heart that gives rise to the left ventricle.²⁰ Evolutionary duplication of the ventricular chambers, thus, correlates with duplication of the *Hand* genes.

Molecular Regulation of the SHF

Since the discovery of the SHF in 2001, molecular pathways regulating the SHF have been extensively investigated (Figure 3). The SHF is marked by the LIM-homeodomain transcription factor, Islet1 (Isl1), and comprises the dorsal-medial aspect of the cardiogenic plate, whereas the FHF comprises the ventral aspect of the cardiogenic plate (Figure 1).²¹ Expression of Isl1 is extinguished as progenitor cells express markers of cardiac differentiation, although Isl1 is necessary for the SHF-derived cells to populate the heart. After the heart tube has formed, canonical Wnt signaling is maintained in

the SHF.²² β -catenin is required in the Isl1-expressing SHF progenitors. Genetic ablation of β -catenin results in pharyngeal arch remodeling defects, a single outflow vessel, and embryonic lethality, indicating disruption of the myocardial contribution from the SHF. Loss of Wnt signaling reduces the number of Isl1-positive cells, leading to OFT and right ventricular defects, whereas excess Wnt signaling expands the Isl1-positive population.²³ Canonical Wnt signaling is responsible for promoting proliferation and maintaining cells in a progenitor state. Meanwhile, β -catenin induces both BMP4 and non-canonical Wnt11 expression in the SHF, suggesting that the early canonical Wnt signaling also sets the stage for differentiation. Non-canonical Wnt signaling, on the other hand, promotes cardiac differentiation and is specifically required for the OFT development.²⁴

Hand2 is a good candidate for controlling the development of the SHF because it is expressed predominantly in the right ventricle and the OFT. *Hand2*-null mice show severe hypoplasia of the right ventricle and growth retardation from E9.5, with death by E10.5.²⁰ However, the early embryonic lethality of *Hand2*-null mice has precluded lineage-specific study of its function in myocardial progenitors. Recently, we used a floxed allele of *Hand2* to ablate its expression in specific cardiac cell populations at defined developmental points. We found that *Hand2* expression within the mesoderm-derived SHF progenitors was required for their survival and deletion in this domain recapitulated the complete *Hand2*-null phenotype.²⁵

Similarly, right ventricular hypoplasia in mice lacking *Mef2c* may also be a defect of the SHF. *Mef2c* is a target of Isl1, Gata4, Foxh1, and Tbx20 during the development of the SHF.^{26,27} As a matter of fact, many of transcriptional regulators of the FHF, including Nkx2.5 and Gata4, are expressed in a population of cardiogenic progenitor cells derived from the SHF and may target its development.

Figure 3. Molecular pathways controlling the second heart field. Direct in vivo regulations are indicated by blue lines, and genetic expression data are indicated by black lines. See text for detail.

The significance of transcriptional regulation in the SHF is highlighted by the CHD in 22q11.2 deletion syndrome (22q11DS), the most common chromosome microdeletion syndrome in humans.^{28,29} A T-box transcription factor, TBX1, on the 22q11.2 region is a major genetic determinant of cardiac and craniofacial disorders. Tbx1 is expressed in the SHF, but not in the FHF or cNCC.³⁰ OFT defects, such as persistent truncus arteriosus (PTA) and tetralogy of Fallot (TOF), and aortic arch anomalies are highly associated with 22q11DS, as well as craniofacial defects, such as cleft palate. *Tbx1*-null mice phenocopy the 22q11DS phenotype. Tissue-specific disruption of *Tbx1* in the expression domain of *Nkx2.5* developed the single OFT with no evidence of an aortopulmonary septum, suggesting that loss of Tbx1 function in the SHF or pharyngeal endoderm may result in OFT defects.³¹ Shh plays a role in maintenance of the expression of Tbx1.^{30,32} The expression of Tbx1 in the SHF is directly regulated by forkhead-containing (Fox) transcription factors, Foxa2, Foxc1 and Foxc2, through at least 2 enhancer regions including the consensus Fox binding sites.^{32,33} The *Tbx1*-hypomorphic mouse demonstrated that the development of the OFT was more sensitive to Tbx1 dosage than craniofacial development. The increased dose-dependency in the OFT is likely related to amplification of Tbx1 via an autoregulatory loop involving Foxa2 and Tbx1 in the SHF, resulting in upregulation of the downstream targets, Fgf8 and 10.³⁴ In experiments using knockout mice, Foxc1 and Foxc2, and Foxh1 were involved in the development of

the OFT, although *Foxa2*-null mice die too early in utero to study cardiac development. *Foxc1*- or *Foxc2*-null mice and compound heterozygous mice for *Foxc1* and *Foxc2* display aortic arch defects reminiscent of *Tbx1* heterozygous mice.³⁵ Interestingly, they also display a reduction in the size of the OFT and right ventricle, suggesting a dose-dependent regulatory mechanism of Foxc1 and Foxc2 in the SHF.³⁶ Moreover, embryos homozygous-null for both *Foxc1* and *Foxc2* display an absence of the OFT and right ventricle, as well as down-regulation of Tbx1, Fgf8, and Fgf10.

In contrast, there is no evidence of genetic interaction between Foxh1 and Tbx1, although Foxh1 plays an important role in the SHF development. Consistent with this observation, the consensus binding sequence for Foxh1 is different from those for Foxa2, Foxc1, and Foxc2. A recent study demonstrated that Foxh1 was able to form a transcriptional complex with Nkx2.5 and to regulate Mef2c expression in the SHF and OFT, and that *Foxh1*-null mice have OFT defects.²⁷ A Foxa2/c1/c2–Tbx1 pathway, therefore, may be parallel to or independent of the Foxh1/Nkx2.5–Mef2c pathway in terms of regulating the SHF (Figure 3).

Intracellular calcium signaling is known to play essential roles in cardiac physiology and hypertrophy, but its function in cardiac development remains to be elucidated. Inositol 1, 4,5-trisphosphate receptor (IP₃R) is an intracellular calcium release channel on the endo/sarcoplasmic reticulum. Three subtypes of IP₃R (1, 2, and 3) have been identified in many organs

during embryogenesis and the postnatal period.³⁷ Recently, we reported a redundant role of IP₃R1 and IP₃R2 during cardiogenesis, implicating the calcineurin/NFAT signaling pathway in the development of the endocardial cushion.³⁸ Furthermore, we showed that IP₃R1 and IP₃R3 are redundantly essential for the development of the SHF.³⁹ Although the molecular mechanisms underlying IP₃R-mediated Ca²⁺ signaling in the SHF are not fully understood, our study demonstrated that redundant roles of IP₃R1 and 3 might be implicated in the regulation of cell survival and the Mef2c-Smyd1 transcriptional cascade during the development of the SHF.

Molecular Regulation of cNCC

The specification and induction of cNCC involves a number of secreted signals, including Wnt, BMP, FGF and retinoic acid, that are critical for the induction of the neural plate border.⁴⁰ Importantly, the canonical Wnt β -catenin pathway is specifically required for cNCC induction.⁴¹ The combined action of these secreted signals triggers the expression of neural crest specifier genes, such as transcription factors, Snail2, Sox9 and FoxD3.⁴²

The cNCC have definitive migration patterns and stereotypic destinations.⁴³ Throughout their journey, cNCC interact with a multitude of signaling factors, many of which may help guide them and reciprocally, the cNCC may pattern and contribute to many of the structures in proximity to these secreted

factors. The role for FGF signaling in migrating cNCC development remains somewhat elusive. *FGF8*-hypomorphic mice show apoptosis of cNCC as they emigrate from the neural tube and in the pharyngeal arches.⁴⁴ *FGF2* expression is promoted by FGF8 and is a prerequisite for the differential localization of FGF2, which is essential for chemotaxis of mesencephalic NCC migration.⁴⁵ Decades of ablation studies have revealed much of what is known about the roles of cNCC.^{5,46}

The final migration of cNCC into the OFT is guided by Semaphorin 3C (SEMA3C). The myocardium underlying the pulmonary side of the undivided OFT expresses SEMA3C while cNCC express its receptor, Plexin A2 (PLXNA2), and are attracted by the SEMA3C ligand.⁴⁷ Semaphorins are secreted, transmembrane and GPI-linked proteins that were initially found to be important in axonal guidance in the developing nervous system.⁴⁸ Semaphorin ligands bind to plexin and neuropilin receptors, which lead to alterations in the cytoskeleton and microtubule network. This signaling is required for the correct migratory patterning of cNCC into the OFT (Figure 4). In 75% of newborn *Sema3c* mutant mice, there is incomplete septation of the OFT and these animals have a single semilunar valve with 4 cusps and a ventricular septal defect just below the valve.^{49,50} Interestingly, the PLXNA2 receptor is expressed in not only migrating, but also postmigratory cNCC, suggesting that the final step of migration of cNCC into the OFT may be orchestrated by SEMA3C-PLXNA2 signaling.

The transforming growth factor β (TGF β) superfamily,

Table. Genes Encoding Transcription Factors Associated With CHD		
Gene	CHD	References
NKX2.5	TOF, ASD, DORV, VSD, HLHS, CoA, IAA, TGA, Ebstein	Schott JJ et al; Hirayama-Yamada K et al; Gutierrez-Roelens I et al; Hosoda T et al; Watanabe Y et al; Gutierrez-Roelens I et al; Elliott DA et al; Rifai L et al; Benson DW et al; Goldmuntz E et al; McElhinney DB et al; De Luca A et al; Akcaboy MI et al; Konig K et al; Ikeda Y et al; Bjornstad PG et al; Pabst S et al; Gioli-Pereira L et al; Akcaboy MI et al; Rauch R et al
GATA4	ASD, AVSD, VSD, TOF, HRHS, PAPVC	Garg V et al; Hirayama-Yamada K et al; Okubo A et al; Sarkozy A et al; Tomita-Mitchell A et al; Zhang W et al; Rajagopal SK et al; Nemer G et al; Schluterman MK et al; Posch MG et al
ZIC3	TGA, ASD, PS	Chhin B et al; Megarbane A et al; Ware SM et al; De Luca A; Gebbia M et al
FOG2	TOF, DORV	Pizzuti A et al; De Luca A et al
FOXH1	TGA, TOF	Roessler E et al
GATA6	PTA, PS, AVSD	Kodo K et al; Lin X et al; Maitra M et al
NKX2.6	PTA	Heathcote K et al
TBX20	ASD, CoA, VSD, PDA, MS, HLHS	Kirk EP et al; Posch MG et al
CITED2	VSD, ASD	Sperling S et al
TBX5	ASD, VSD, AVSD	Basson CT et al. *Associated with Holt-Oram syndrome.
TBX1	VSD, IAA, PAVSD	Yagi H et al; Rauch R et al; Gong W et al; Rauch A et al. *Associated with DiGeorge/Conotruncal anomaly face syndrome.
ANKRD1	TAPVC	Cinquetti R et al
HAND1	TOF, Atrial isomerism	Hatemi AC et al; Wang J et al
HAND2	TOF	Shen L et al

CHD, congenital heart defects; TOF, tetralogy of Fallot; ASD, atrial septal defect; DORV, double outlet right ventricle; VSD, ventricular septal defect; HLHS, hypoplastic left heart syndrome; CoA, coarctation of aorta; IAA, interrupted aortic arch; TGA, transposition of great arteries; AVSD, atrioventricular septal defect; HRHS, hypoplastic right heart syndrome; PAPVC, partial anomalous pulmonary venous connection; PS, pulmonary valve stenosis; PTA, persistent truncus arteriosus; PDA, patent ductus arteriosus; MS, mitral valve stenosis; PAVSD, pulmonary atresia with ventricular septal defect; TAPVC, total anomalous pulmonary venous connection.

Figure 5. Novel mutations in *GATA6* associated with persistent truncus arteriosus result in failure of transactivation of downstream target genes, *SEMA3C* and *PLXNA2*. (Top) The construction of *GATA6* with positions of novel mutations (N466H and E486del) and conservation of alignment between species is shown. *GATA6* mutant proteins show a significant decrease in transcriptional activity on *SEMA3C* (Lower left) and *PLXNA2* (Lower right) promoters compared with the wild-type (WT). Zf, zinc finger domain; NLS, nuclear localization signal; Ter, terminal codon; luc, luciferase reporter.

including BMP and TGF β ligands, is one of the most widely studied signaling families that affect cNCC development.⁵¹ The TGF β subfamily ligands signal via a receptor complex composed of 2 type II receptors and 2 type I receptors. Upon ligand binding, type II receptors, which are constitutively active kinases, phosphorylate and activate type I receptors (ALKs). Type I receptors phosphorylate and activate a specific set of downstream signaling molecules called Smads. In general terms, TGF β s bind to the TGF β type II receptor (TGF β RII) and TGF β type I receptor (ALK5) activating Smad2 and 3, while BMPs bind to the BMP type II receptor and type I receptors ALK2, 3, or 6, activating Smad1, 5 and 8.⁵² Alk2 is required either for normal migration and/or maintenance of cNCC in the pharyngeal arches and OFT.⁵³ Canonical TGF β or BMP signaling results in phosphorylation of different Smads, but these phosphorylated Smads all bind the universally used co-Smad4.⁵⁴ The Smad4 complex is then translocated to the nucleus where it alters transcription. Conditional deletion of Smad4 in cNCC using Wnt1-cre leads to failure of the OFT septation.⁵⁵

Molecular Genetics of CHD

The association between mutations in NKX2.5 and atrial septal defect (ASD) with atrioventricular block was first published in 1998.⁵⁶ Since then, some cardiac transcription factor genes have been demonstrated in a decade to be associated with various CHD (Table).

Recently, we screened mutations of cardiac transcription factors in patients with selected non-syndromic CHD, namely PTA, representing the most severe cardiac OFT defect, and identified 2 different GATA6 mutations in 2 probands, but not in 182 unrelated controls with no CHD.⁵⁷ Our subsequent biological analyses revealed that SEMA3C and PLXNA2 were directly regulated by GATA6, and that both GATA6 mutant proteins failed to transactivate these genes (Figure 5). Transgenic analysis further suggested that the expression of SEMA3C and PLXNA2 in the OFT was dependent on GATA transcription factors during heart development. Together, our data implicate mutations in GATA6 as novel genetic causes of CHD involving the OFT, as a result of disruption of the direct regulation of semaphorin-plexin signaling (Figure 4).

After our study, there have been 2 reports on mutations in GATA6 associated with CHD. Maitra et al showed 2 novel sequence variations in GATA6 (A178V and L198V) from the screening of 310 individuals with non-syndromic CHD.⁵⁸ These variants were identified in 2 individuals, 1 with TOF and the other with atrioventricular septal defect, but not in 288 ethnically-matched healthy controls. Biochemical analysis demonstrated that the GATA6 A178V mutant protein resulted in increased transactivation ability for cardiac genes compared with the wild-type. Lin et al screened 270 individuals with non-syndromic CHD and identified a missense mutation (S184N) of GATA6 in 2 individuals, 1 with TOF and the others with ASD.⁵⁹ Although incomplete penetrance of the phenotype by this mutation was observed, the mutation was

not found in 500 ethnically-matched healthy controls. Their subsequent biological analysis revealed the decreased transcriptional activity of GATA6 with the S184N mutation for regulation of several important cardiac genes, suggesting that the GATA6 S184N mutation may play an important role in the pathogenesis of CHD.

To date, the implication of GATA6 and GATA4 in CHD, as well as their genetic interaction, has been suggested. Genetic disruption of Gata6 in mice results in early embryonic lethality from defects of endodermal differentiation. Conditional inactivation of Gata6 specifically in cNCC causes PTA, suggesting an essential role of Gata6 during OFT development.⁶⁰ Homozygous Gata4 knockout mice die in utero and develop 2 symmetric promyocardial primordia that fail to migrate ventrally and form the heart tube. Mice with heterozygous Gata4 mutations exhibit septal defects and endocardial cushion defects.⁶¹ Mice that are compound heterozygous for Gata4 and Gata6 null alleles die in utero and exhibit a spectrum of CHD, including septal and OFT defects.⁶² It has also been shown that GATA6 and GATA4 regulate the expression of each other during development and that GATA6 may function in concert with GATA4 to direct tissue-specific gene expression essential for formation of the mammalian heart. It is of note that GATA6 mutations identified in humans are predominantly associated with OFT defects, whereas GATA4 mutations are commonly associated with septal defects, although there is some phenotypic overlap (Kodo K, Yamagishi H, unpublished data), probably resulting from a redundant role of these genes. Although the clinical phenotype of individuals with GATA6 mutations commonly involves the OFT, it is distinct from that of 22q11DS and, rather, manifests as non-syndromic CHD. To date, no molecular link has been demonstrated between GATA6 and TBX1; however, our recent study suggests that the expression of Sema3c in the OFT is altered in mouse embryos deficient for *Tbx1* (Kodo K, Yamagishi H, unpublished data), suggesting that GATA6 may share, at least in part, a common molecular pathway with TBX1 during OFT development.

Concluding Remarks

Through the use of animal models combined with human genetic investigations, a molecular framework that controls cardiovascular morphogenesis and a molecular basis of CHD have emerged in this decade (Figure 6).^{2,3,63} Such advances in understanding lead to an approach to the etiology of “multifactorial” CHD in the post-genomic era, although the underlying genetic etiology of the majority of CHD that manifest as non-syndromic and non-familial phenotypes still remains to be elucidated. Environmental factors, epigenetic factors and/or other genetic modifiers may also be responsible for the final phenotype of CHD. Further studies are necessary for more delineated genotype–phenotype correlations between gene mutations and CHD phenotype, and the next challenge is identifying factors that modify or influence the phenotype. An improved understanding of possible genetic causes gives an insight into the pathogenesis, and, eventually, the regenerative medicine for CHD. Our next hope is to target prevention or genetic intervention of CHD, utilizing such progress in basic science.

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