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Genetic and Developmental Basis of Cardiovascular Malformations

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Abstract

Cardiovascular malformations (CVMs) are the most common birth defect, occurring in 1–5% of all live births. Genetic, epigenetic, and environmental factors all influence the development of CVMs, and an improved understanding of causation of CVMs is a prerequisite for prevention. Cardiac development is a complex, multi-step process of morphogenesis that is under genetic regulation. Multiple developmental pathways act independently or in combination to effect proper cardiac lineage specification, differentiation, and structure. Because of this complexity, there are numerous potential mechanisms by which genetic variation can impact both fetal cardiac development and latent cardiac disease. Although the genetic contribution to CVMs is well recognized, the genetic causes of human CVMs are still identified relatively infrequently. Mouse models are important tools to investigate the molecular mechanisms underpinning cardiac development as well as the complex genetics that characterize human CVMs. In this review we provide an overview of the key genetic concepts characterizing human CVMs, review their developmental basis, and provide examples to illustrate the critical developmental and genetic concepts underlying the pathogenesis of CVMs.

Keywords

Congenital heart defects; congenital heart disease; development; gene dosage

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INTRODUCTION

The underlying causes of CVMs can include cytogenetic abnormalities, single gene disorders, environmental etiologies, or most commonly, multifactorial etiologies (Table 1). Chromosomal abnormalities account for 12–14% of all live born cases and 20–33% of fetal cases of congenital cardiovascular malformations (CVMs), indicating that the proper genetic control of cardiac development is essential [1–4]. CVMs can occur as isolated findings, as part of a well-defined syndrome, or in conjunction with additional extracardiac anomalies not formally recognized as a syndrome [5]. The designation of CVMs as isolated can be problematic since many important distinguishing features of syndromic conditions, such as developmental delay or dysmorphic features, may not be apparent at initial evaluation. As a result, syndromic cases of CVM may be underestimated. In addition, the traditionally cited incidence for CVMs of ~1% of live births likely also underestimates the scope and impact of disease. Taking into account very high rates of CVMs in spontaneous abortuses, common malformations such as BAV (present in 1.2% of the population) and latent cardiac diseases such as aortic dilation which are not included in the birth incidence of CVMs, genetically mediated CVMs are likely much more common than previously thought. When considering the etiology of CVMs, as opposed to the proportion of CVM cases that manifest as disease at birth, the incidence increases to approximately 5%.

Recently, we summarized the overall progress in the molecular genetic analyses of CVMs and current recommendations for clinical application of genetic testing. In particular, we reviewed the utility and limitations of chromosomal microarray analyses (CMAs) and the emerging clinical roles for whole exome sequencing (WES) and other next-generation sequencing (NGS) technologies [6]. Readers with an interest in the current clinical testing approaches for CVMs are referred there. Here, we focus on common genetic and developmental themes across the wide variety of CVMs and the ability of animal models and knowledge of cardiac developmental biology to impact our understanding and approach to CVMs.

The genetic basis of CVMs

Epidemiologic studies suggest that a syndromic form of CVM is identifiable in approximately 20% to 30% of cases [4]. Known genetic causes are extremely heterogeneous, encompassing not only mutations in cardiac relevant genes but also more complex chromosomal abnormalities, submicroscopic duplications/deletions, and whole-chromosome aneuploidies (Table 1). As noted above, CVMs can be isolated or can occur as part of a well-recognized genetic syndrome, and this distinction may be subtle.

Inheritance patterns for many CVM-associated genetic conditions are well characterized (reviewed in [6]) (Table 2). Genetic syndromic conditions associated with CVMs are most commonly de novo or autosomal dominant. For dominantly inherited conditions, such as Noonan or Holt-Oram syndromes, individual recurrence risks for offspring with the syndrome is 50%. Importantly, not all patients with a particular syndrome have associated heart defects and the proportion can vary by syndrome. Furthermore, the presence or severity of a CVM in the parent does not predict the severity in the child.

Isolated CVMs may be inherited as autosomal dominant, autosomal recessive, or X-linked conditions, but are most commonly sporadic with multifactorial etiology (Table 3). Like other conditions inherited as a complex trait, isolated CVMs may show familial clustering with reduced penetrance[7]. For these reasons, recurrence risks for isolated CVMs can be difficult to assign. Consistent evidence of high heritability of isolated CVMs indicate that a strong genetic component exists, even for defects occurring without an obvious mode of inheritance [8].

Gene dosage as a mechanism for CVM

Gene dosage is an important concept underlying genetic disease, including birth defects. For many genes, a missing (deletion) or extra (duplication) copy of that gene results in no phenotypic consequences. In contrast, dosage sensitive genes produce abnormal phenotypes in the absence of two functional genes. Aneuploidies such as Trisomy 21 and Turner syndrome demonstrate that proper chromosome number is required for normal development. CVMs, including AVSD, are seen in approximately 50% of individuals with Down syndrome. Likewise, up to 50% of patients with Turner syndrome will have a CVM, most commonly a defect in the left ventricular outflow tract. Because of the large number of genes with abnormal dosage in these conditions, identifying the causal genes for the cardiac features has proven difficult. Furthermore, the decreased penetrance of the CVMs suggests that genetic modifiers interact with dosage-sensitive gene(s) on the same chromosome (in the case of Trisomy 21) or other chromosomes to cause CVM. Thus, a threshold exists in both aneuploid and euploid populations for the number of genetic perturbations that can be tolerated before CVM results. For example, *Creld1* and *Hey2* were recently identified as potential modifier genes in Trisomy 21[9]. Mice with mutant forms of these potential modifiers were intercrossed to the Ts65Dn mouse model of Down syndrome. Breeding loss-of-function alleles of either *Creld1* or *Hey2* onto the trisomic background causes a significant increase in the frequency of CVM. This supports a threshold hypothesis for additive effects of genetic modifiers in the sensitized trisomic population.

Submicroscopic chromosome deletions and duplications also underlie many genetic syndromes, and the term genomic disorder is used to refer to these conditions. Two classic genomic disorders, 22q11.2 deletion syndrome and Williams-Beuren syndrome, are discussed in further detail below.

Understanding the genetic basis of syndromic CVM can identify important genes for isolated CVM

Williams-Beuren syndrome (WBS) is a relatively common genetic syndrome associated with CVM caused by deletion at 7q11.23, resulting in haploinsufficiency of multiple genes, including elastin, *ELN*. Supravalvar aortic stenosis (SVAS) is the most classic cardiac finding in WBS, although other defects, including peripheral pulmonic stenosis, occur. Subsequent to the description of WBS as a deletion at 7q11.23 in 1993[10], Ewart et al. showed close linkage of *ELN* and supravalvular aortic stenosis (SVAS) in two families[11]. Deletions or point mutations limited to the *ELN* gene appear to result in nonsyndromic SVAS, whereas larger deletions spanning multiple genes lead to the WBS. Studies in a

mouse model with elastin deficiency have successfully corroborated the genetic findings with regard to SVAS and latent aortic disease [12, 13].

22q11.2 deletion syndrome provides a second example of a genomic disorder that led to the identification of a single gene causing CVM. CVMs occur in approximately 75% of patients with 22q11.2 deletion syndrome, with conotruncal defects predominating. After the identification of 22q11.2 deletion syndrome, significant effort was put forth to delineate the dosage sensitive gene(s) responsible for the CVMs using mouse development and genetics, ultimately identifying *Tbx1*[14, 15]. Yagi et al. investigated *TBX1* mutations in families who had 22q11.2 deletion syndrome phenotype but no detectable deletion and found that *TBX1* mutations are responsible for many major phenotypes of the syndrome, including CVMs. In much the same way that modifiers for the Ts65Dn mouse model of Down syndrome were identified, sonic hedgehog and retinoic acid developmental signaling pathways modify the phenotypes of a 22q11.2 deletion syndrome mouse model, suggesting that mice with reduced gene dosage are sensitized to these morphogens[16]. These disorders illustrate the concept that genes which cause CVMs may be associated with syndromic or isolated presentations. Comprehensive identification of dosage sensitive candidate CVM genes and integration into an understanding about the genetic and developmental origins of CVM would facilitate the development of therapies to rescue the CVMs associated with both syndromic and isolated CVM.

Blurring the boundaries: single gene defects that can cause both syndromic and isolated CVMs

Genetic testing technologies have identified several genes that cause both syndromic and nonsyndromic CVMs (Table 2–3) [6]. For example, CVMs, including aortic aneurysm, are reported in syndromic patients (i.e., Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS)) with mutations affecting the TGFβ pathway (*TGFB2, TGFBR1, TGFBR2, SMAD3, FBN1*) (Table 2)[17, 18]. Non-syndromic aortic disease is a frequently asymptomatic but potentially lethal disease characterized by familial cases of thoracic aortic aneurysm and dissection (FTAAD). This monogenic but genetically heterogeneous condition is primarily inherited as an autosomal dominant disorder with variable penetrance and expressivity. Mutations in *TGFB* genes have also been described in nonsyndromic patients with isolated CVM or aortic aneurysm (Table 3) [18–21]. These facts complicate the clinical approach to patients with CVMs and the choice of genetic testing. Furthermore, patients with mutations in *ACTA2*, a gene known to cause isolated FTAAD, can have a syndromic presentation [22] and pediatric patients with FTAAD frequently have subtle signs of a connective tissue disorder [23]. As the ability to identify genetic etiology improves, boundaries between syndromic and nonsyndromic disease often become less distinct. Careful phenotyping and improved interpretation of genetic variation are important to better refine our understanding of the spectrum of clinical effects of specific genetic variation.

The developmental basis for CVMs: genes and pathways required for critical stages of heart formation

Genetically engineered mice are extensively used in CVM research and have contributed greatly to an understanding of the genetic control and mechanistic basis of CVMs. Multiple

cell types contribute to the development of a fully septated four-chambered heart, including the first heart field (FHF) and second heart field (SHF), cardiac neural crest (NC), epicardial (Epi), and endocardial (EC) cell lineages (Fig. 1) [24]. Both cardiac NC and endocardium-derived cushion mesenchyme are important precursors of the outflow tract (OFT) septa and semilunar valves[25]. At embryonic day 9.5 (E9.5), endocardium in the proximal OFT region gives rise to cushion mesenchyme via epithelial-mesenchymal transition (EMT) (Fig. 1) [25, 26]. NC cells enter the distal OFT (E10), proliferate, and eventually colonize the endocardial ridges of the proximal cushions[27]. NC cells undergo apoptosis (E11.5 – 13.5), and are necessary for aorticopulmonary septation and OFT alignment[28]. Endocardium-derived OFT cells also undergo proliferation but remain restricted to the endocardial ridges of the proximal OFT cushions (Fig. 2). Remodeling and fusion of the endocardial ridges of the proximal cushions results in the formation of fibrous OFT septum that undergoes differentiation (E13.5–15.5) and eventually becomes a completely muscular structure (E16.5–18.5) through a process called myocardialization[26]. Despite abundant contributions of NC to the OFT mesenchyme, few NC derivatives are present in the mature semilunar valves[27]. Although the precise role of NC cells or their interaction with endocardial EMT-derived OFT cells remains unclear, more recent studies have suggested that NC cells are also important for the remodeling of the semilunar valves [27]. Abnormal OFT cushion remodeling often results in semilunar valve thickening, defective OFT septation (persistent truncus arteriosus, PTA) or alignment defects such as double-outlet right ventricle (DORV) and ventricular septal defect (VSD) [25]. The signals and cellular events that mediate valve remodeling are poorly characterized, although apoptosis and alterations in extracellular matrix production have been described [29, 30]. Similar developmental events are noted in AV cushion formation and remodeling except that the cardiac NC are absent in the AV cushions and both dorsal mesocardium and epicardium provides additional cushion components of the AV cushion mesenchymal complex (Fig. 2) [31].

Developmental pathways acting independently or in combination contribute to heart development and have been reviewed recently [6, 32–34]. For example, TGFβ and BMP family members play different roles during cardiac development (Fig. 3) (reviewed in [26, 35]), and mutations in these genes result in distinct phenotypes [35–38]. In Loeys-Dietz syndrome (LDS), mutations in the TGFβ pathway genes cause thoracic aortic aneurysm (TAA; Table 3) and are also highly associated with BAV[39]. Paradoxically, elevated levels of TGFβ1 are seen in these patients. Similar increases in TGFβ1 activity is also associated with BAV in Turner syndrome [40]. Overall, it remains unclear whether the loss-of-TGFB function and/or gain-of-TGFB function is the primary cause of CVM. On the other hand, BMP signaling is required to induce differentiation of early cardiac progenitors, but BMP signaling is inhibited at later stages by Smad6a to permit chamber development mediated by Tbx2 and Tbx20. Importantly, the role of a particular signaling pathway can vary as development proceeds. For example, Wnt signals are critical for early cardiac precursor induction and proliferation, but later become inhibitory. Combinatorial interactions are the rule. Notch signaling interacts with both BMP and TGFβ pathways [6, 41]. The cardiac transcription factor Nkx2.5 physically and functionally interacts with Gata4, Tbx5, and Mef2c, each of which forms additional unique and shared connections with other molecular, genetic, and signaling

components [6, 33, 34]. Such signaling and transcriptional networks hint at the possibility that some CVMs may result from additive effects of multiple low-effect susceptibility alleles.

Phenotypic heterogeneity and locus heterogeneity

Studies of gene targeted mouse models indicate that loss of a single gene can result in a spectrum of CVMs (Table 4–5, Fig. 4; non-comprehensive examples). For example, the OFT malformations of the TGFβ2-deficient fetuses include DORV, PTA, abnormal morphology and thickening of aortic and/or pulmonary valves, aortic arch artery malformations (i.e. IAA), DILV and/or overriding of tricuspid valves orifice via a perimembranous inlet VSD, and abnormal morphology and thickening of tricuspid and mitral valves. Similarly, a range of CVM phenotype is seen in mice which lack *Tbx1*, *Nkx2-5, Tbx20, Tbx5, and Gata4*.

Different CVM phenotypes are noted in patients with identical mutations, even among members of the same family (Table 4). Null mutation in several genes can cause AVSD in mice, including *Nkx2-5, Gata4, and Tbx1* [31]. Additional examples are presented in Fig. 4. Developmental mechanisms that cause different CVMs in response to a mutation in a single gene remain incompletely understood.

Mutations in developmental pathways may result in latent disease

There are numerous potential mechanisms through which genetic mutations could affect the complex differentiation and morphogenetic processes in heart development. Once developed, the cardiovascular system must undergo homeostasis to maintain function throughout life. This ability to repair and remodel following stress and injury uses many of the same mechanisms involved in the original development and remodeling of those tissues[38]. Failure of these processes can result in late onset disease. Genes associated with CVMs (Fig. 4) [35, 38] are ideal candidates for these homeostatic, stress response and repair processes. Improvement in outcomes requires a better understanding of mechanisms underlying CVMs and dysregulated homeostatic/repair processes.

There are many interesting examples of genes in which homozygous gene deletion in mice results in CVMs in embryos and latent cardiac disease in adult mice, including elastin, emilin 1, periostin, and fibrillin 1. Elastin null ($Eln^{-/-}$) mice die perinatally secondary to severe arterial obstruction reminiscent of SVAS[12], whereas arteriopathy in the $Eln^{+/-}$ mouse manifests as systemic hypertension[42]. Juvenile $Eln^{+/-}$ mice demonstrate normal valve function, but progressive valve disease (predominantly aortic regurgitation) is identified in 17% of adult and 70% of aged adult $Eln^{+/-}$ mice by echocardiography[13]. Thus $Eln^{+/-}$ mice are a model of latent aortic valve disease and reduced elastin leads to dysregulation in valve pathogenesis. Other good examples of mouse models of latent aortic disease include *Emilin1*, *Fibrillin-1*$^{+/C1039G}$, and *Fibrillin1*$^{mgR/mgR}$ (*Fbn1*$^{mgR/mgR}$), the latter two being mouse models of Marfan syndrome (MFS). The *Fbn1*$^{mgR/mgR}$ mice die spontaneously from rupture of the thoracic aorta between 2 to 4 months of age, and are useful in testing therapeutic strategies for aortic aneurysm. On the other hand, *Fibrillin-1*$^{+/C1039G}$ mice, where a point mutation seen in MFS has been made, represent a viable mouse model to study the development and progression of aortic aneurysm. The early manifestation of elastic fiber fragmentation and aberrant TGFβ signaling suggests that these processes are

crucial intermediate factors which provide novel information for diagnosis and treatment of patients with aortic disease [23, 43].

Decreased penetrance, variable expressivity and complex inheritance: lessons from mouse models

Genetically engineered mice can serve as a useful example of modifying genetic influences that affect phenotype. A good example is the different phenotypes seen in *Tgfb2* null mice on mixed (129/Bl-Swiss) and inbred (C57BL/6) genetic backgrounds. The OFT malformations of the inbred null fetuses included DORV (100% cases), PTA (27.2% cases), and semilunar valve defects (100% cases). In addition, the null fetuses developed DILV and/or overriding of tricuspid valves orifice via a perimembranous inlet VSD (100% cases), and tricuspid/mitral valve defects (100%). Notably, the overall penetrance of the observed cardiac valve and septal defects was significantly higher in C57BL/6 inbred null fetuses compared to *Tgfb2* null fetuses on the mixed genetic background. [44] This difference is attributed to the differences in genetic modifiers between the strains.

Epigenetic factors in CVM

An increasing recognition of epigenetic factors has revealed an unanticipated breadth to the causes of CVMs[45]. Epigenetics refers to functionally relevant changes to the genome that do not involve change in the DNA sequence. DNA methylation and histone modification are major epigenetic mechanisms which alter chromatin remodeling and gene expression without altering the underlying genetic information [5]. A recent study by Pediatric Cardiac Genomics Consortium of the NHLBI identified de novo point mutations in several histone modifying genes that collectively contribute to approximately 10% of severe CVM[5, 21]. Refinements in technologies such as ChIP-seq and systems biology approaches will aid the understanding of global regulation and functional redundancies in cardiac transcription factors in CVMs[33, 34]. MicroRNAs (miRNAs), a class of "small" non-coding RNAs, negatively regulate the expression of their target genes through post-transcriptional processes and also interact with epigenetic machinery[46]. Regulation of gene expression via mechanisms that affect epigenetic machinery will identify novel etiologies for CVMs.

Future developments

The effect of gene variation on the assembly of distinct cardiac and extracardiac cell lineages during heart development is an important area that warrants future investigation. The relative importance and role of different cell types in cardiac morphogenesis and remodeling remains to be fully understood. Defining gene function in specific cell types in mouse models at high resolution will enable predictions to be made about the phenotypic consequences of variants in humans that currently lack functional interpretation. Experiments that delineate fundamental differences/similarities in loss-of-function and gain-of-function genetic backgrounds in mice will provide insight into the consequences of gene dosage perturbation in humans and mechanisms of genetic disease. Finally, incorporation of new technologies such as next generation sequencing, gene expression profiling (i.e., RNA seq), and CRISPR/Cas9 -based methodologies to discover and validate novel genes involved in CVMs will significantly enhance the understanding of cardiac genetics and development.

SUMMARY

Cardiac development is a complex, multi-step process under genetic regulation. A detailed understanding of the molecular basis of cardiac development is necessary to understand disease causation. The field of cardiovascular genetics is progressing at a rapid pace, leading to novel diagnostic genetic testing for CVMs. Recent efforts to integrate developmental studies from animal models with systems biology approaches offers significant promise for future CVM research. Understanding how genetic mutations affect the integration of multiple signal transduction pathways to cause CVM is an active area of research. The information gained from these developmental and genetic investigations should generate novel hypotheses for future experimentation and to provide diagnostic and therapeutic avenues for CVM patients.

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KEY POINTS

- There is a strong genetic contribution to cardiovascular malformations (CVMs).
- Genes important for syndromic CVM may also cause nonsyndromic CVM.
- An understanding of the genes and pathways required for critical stages of heart formation informs the approach to genetic testing and diagnosis.
- The same gene or genetic locus may cause different types of CVMs (phenotypic heterogeneity)
- The same CVM may result from mutations in different genes (locus heterogeneity)
- Mouse models are important tools to investigate the complex genetics of CVMs.

Best Practices Box

What is the current practice?

Genetic Testing in Cardiovascular Malformations

- Genetic testing practices for congenital heart defects have yet to be standardized in many centers and testing is frequently underutilized.
- Guidelines for cardiac imaging and genetic testing for thoracic aortic aneurysm and cardiomyopathy
- A genetic diagnosis has important implications for patient management, screening recommendations for family members, and recurrence risk counseling

Genetic Testing Options

- Chromosome analysis is the gold standard for diagnosis of aneuploidies and other large chromosomal abnormalities.
- Chromosomal microarray (CMA) and fluorescence in situ hybridization (FISH) permit identification of microdeletion and duplication syndromes resulting from abnormalities too small to be detected by conventional chromosome analyses.
- Next generation sequencing (NGS) panels are the test of choice for some syndromic congenital heart defects, thoracic aortic aneurysm and cardiomyopathy.
- CMA and targeted NGS are non-redundant tests. Consulting a geneticist is important for establishing a differential, ordering the appropriate test(s), and interpreting results.

Implications for family members

- First degree relatives of patients with specific cardiovascular malformations (i.e. left ventricular outflow tract obstructive defects, thoracic aortic aneurysm) should undergo cardiac screening
- Family based risk assessment and recurrence risk information differs by type of cardiovascular malformation and should be provided to the family be a knowledgeable genetics professional.

What changes in current practice are likely to improve outcomes?

- Continued integration of genetic testing services into cardiovascular practice will improve diagnostic and prognostic accuracy and will support risk assessment and family planning initiatives.
- NGS technologies promise to greatly benefit patient diagnosis and gene discovery efforts.
- Appropriate cardiac screening and surveillance in at risk relatives will identify latent disease.

Is there a Clinical Algorithm?

- An algorithm for congenital heart defects has been proposed recently. [6]
- Guidelines summarize genetic testing for syndromic and non-syndromic thoracic aortic aneurysm as well as cardiac screening in first degree relatives.[47]

Major Recommendations

Genetic testing and referral decisions should be determined based on the nature of the cardiac defect.

A detailed pedigree should be obtained in all cases of CVM.

Chromosome analysis is recommended for patients with suspected aneuploidy.

Patients with multiple congenital anomalies, neurological findings, developmental delay, and/or dysmorphic features should be referred for genetic evaluation.

CMA and/or FISH should be used in patients with conotruncal defects.

Patients with apparently non-syndromic LVOTO, RVOTO, AVSD, heterotaxy, or other complex defects should have CMA.

Patients with TAA should have a genetics evaluation and appropriate genetic testing.

Specific CVMs should trigger cardiac screening of first degree relatives.

Rating for the Strength of the Evidence

C Recommendation based on consensus, usual practice, expert opinion, disease-oriented evidence, and case series for studies of diagnosis, treatment, prevention, or screening

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Summary

1. Genetic evaluation and testing are increasingly important for cardiovascular malformations. Improvements in genetic testing

technologies have assisted gene discovery and helped to reshape standards of patient care. Risk assessment of family members and recurrence risk counseling are important components of management and care.

Key Developmental Stages	EMT	Neural Crest Entry	Cushion Remodel-ing	Leaflet Formation	Valve Maturation		Mature Leaflet	
E	8.5	9.5	10.5	11.5	12.5	13.5	14.5	16.5	18.5

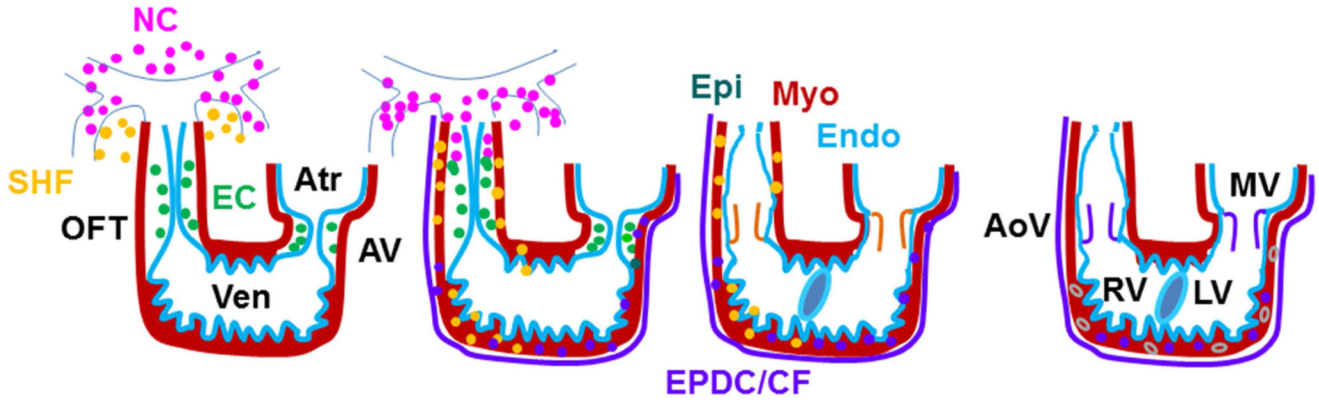

Figure 1. Diagrammatic representation of heart development

Endocardium (EC) (aqua blue line) forms the endocardial cushions (green circle) via cushion EMT (E9.5). Neural crest (NC) (pink circle) cells migrate into the OFT during E10.5-12.5. Valve leaflets undergoing differentiation (orange color) and maturation (purple color) (E13.5-18.5) are clearly indicated. Only one semilunar valve is shown. SV, semilunar valves; AV, atrioventricular canal; RV, right ventricle; LV, left ventricle

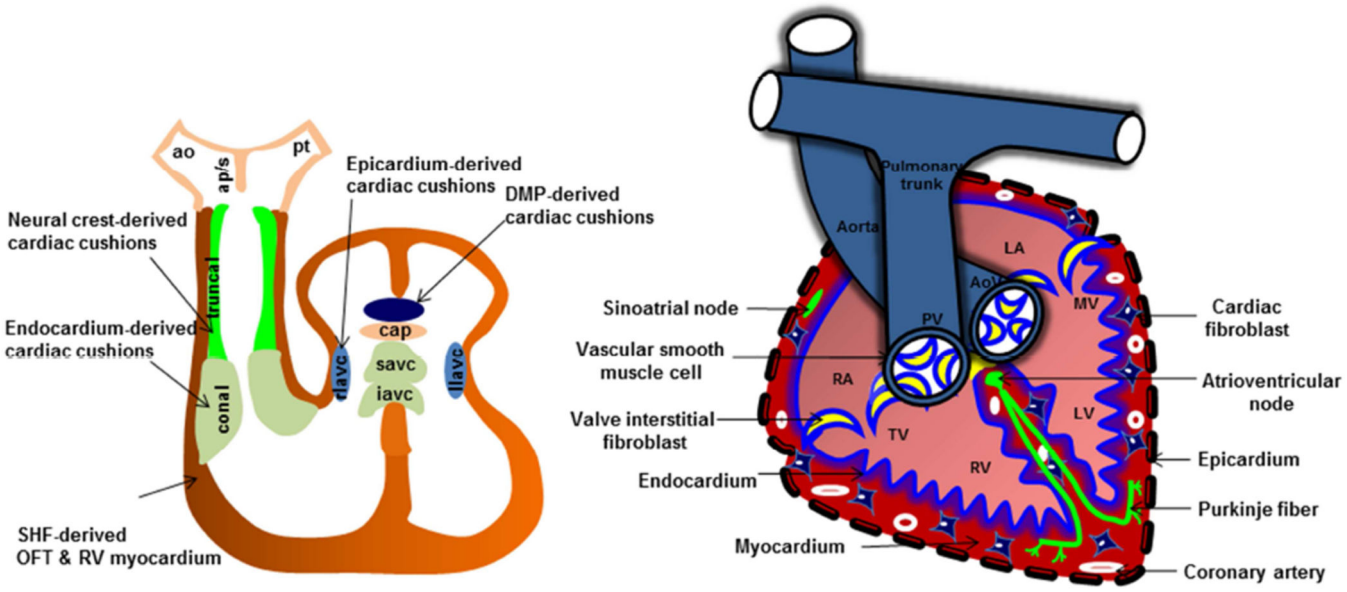

Figure 2. Cardiac remodeling and septation

Myocardium, endocardium, cardiac fibroblasts, and epicardium are major cell types in the heart. *Left side*, Components of OFT and AV cushions are indicated. OFT contains well demarcated conal (endocardium-derived) and truncal (NC-derived) cushions. AV cushion mesenchymal complex contains superior and inferior AV cushions (predominantly EC-derived), right and left lateral AV cushions (rlavc, llavc) (with contributions from both epicardium and EC), mesenchymal cap (cap) and dorsal mesenchymal protrusion (dmp) (dorsal mesocardium-derived). *Right side*, fully septated 4-chambered heart. The valve annulus and vascular wall of the aorta and pulmonary trunk are predominantly comprised of smooth muscle cells. Heart valves predominantly contain valve interstitial cells. Endocardium/endothelium is the innermost layer, whereas epicardium is the outermost layer. Both ventricular and atrial regions contain myocardium and cardiac fibroblasts along with coronary vasculature. Purkinje fiber and atrioventricular and sinoatrial nodes constitute the cardiac conduction system. AoV, aortic valve; PV, pulmonary valve, RA, right atrium; TV, tricuspid valve; RV, right ventricle; LV, left ventricle; MV, mitral valve; LA, left atrium.

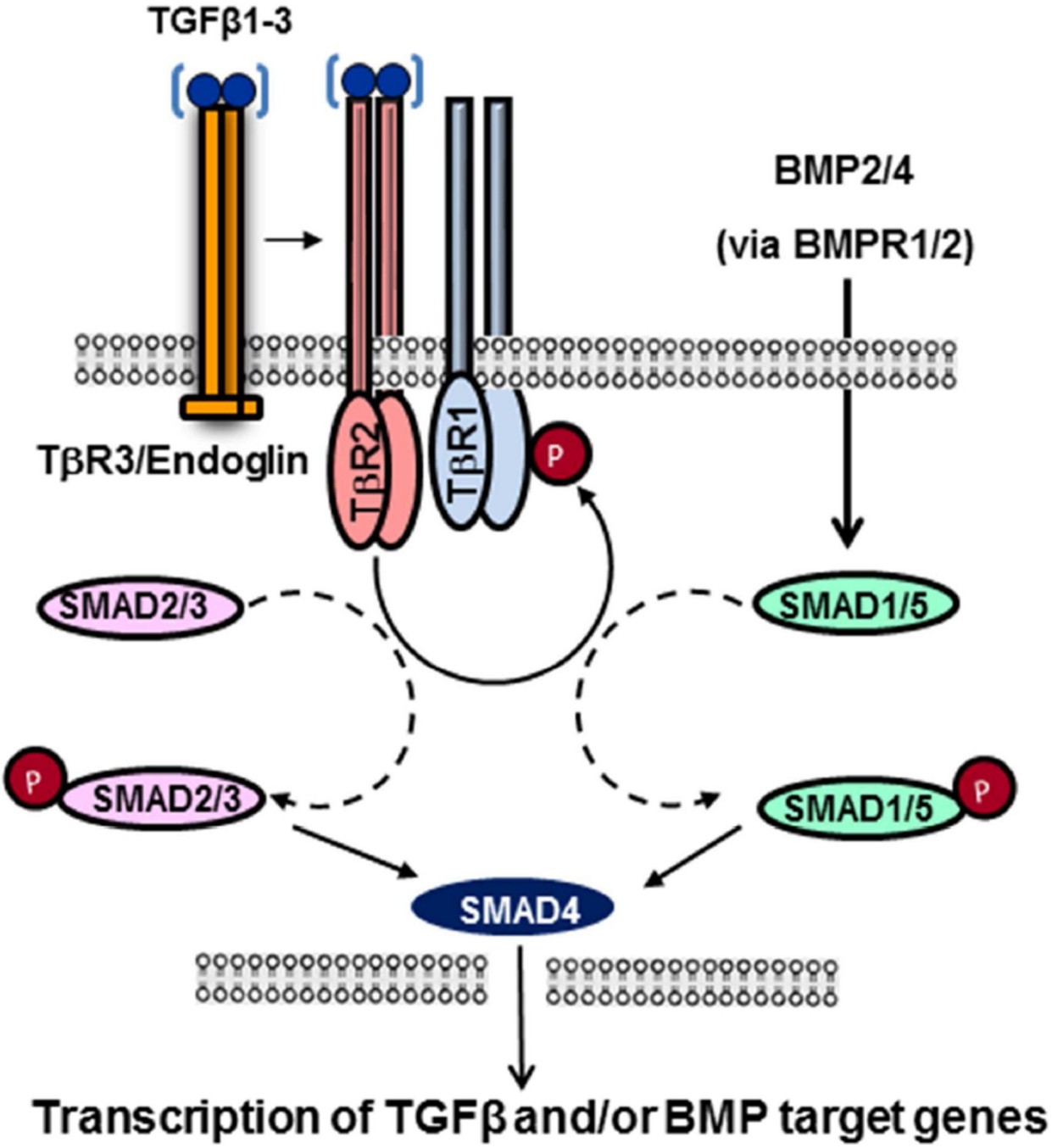

Figure 3. Schematic diagram illustrating the TGFβ signaling pathway

TGFβs binds to a common TGFβ receptor complex, and signals through phosphorylation of the canonical TGFβ-specific SMADs (i.e., pSMAD2/3). The pSMAD2/3 forms a complex with SMAD4, which accumulates in the nucleus and can regulate target gene expression. SMAD4 also binds to BMP-specific SMADs (SMAD1/5/8), and therefore regulates BMP-target gene expression in heart development.

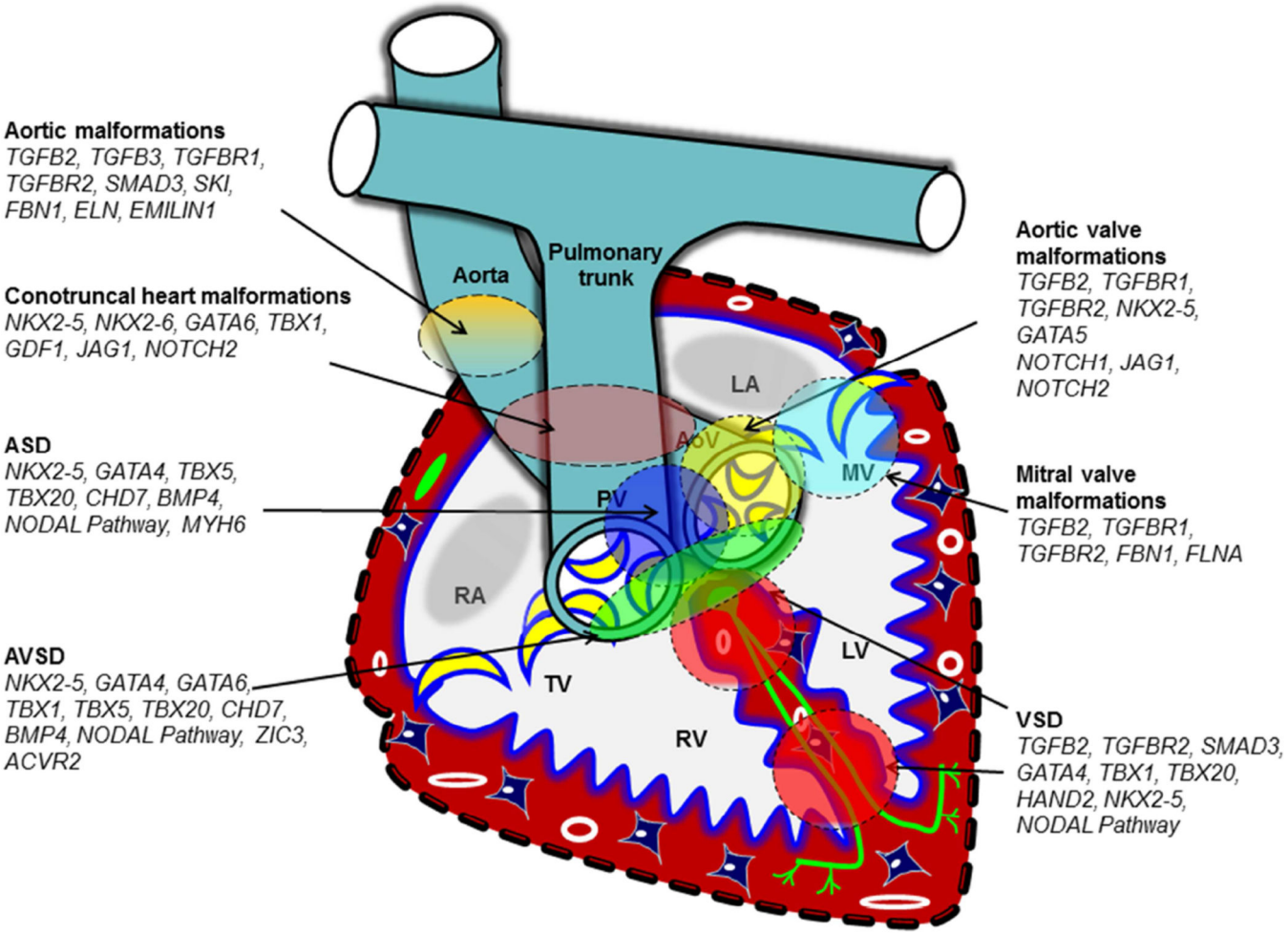

Figure 4. Phenotypic and genetic heterogeneity in CVMs

A single genetic abnormality can cause multiply types of CVMs. In addition, the same genetic abnormality can result in different CVMs. CVMs are indicated with colored circles.

Table 1

Causes of Cardiovascular Malformations

Cause	Example	Characteristic CVMs
Environmental/teratogenic	Lithium Chloride	Ebstein's anomaly
Genetic		
Chromosomal	Trisomy 21	Atrioventricular canal defect
Contiguous gene/CNV	22q11.2 deletion syndrome	Conotruncal malformations
Single gene	Noonan syndrome	Pulmonary valve stenosis
Epigenetic		
DNA methylation	De novo SMAD2 mutations	Conotruncal malformations LV obstructions Heterotaxy

Table 2

Examples of common syndromes with CVMs caused by single gene mutations

Gene	Syndromes	Common cardiac anomalies
CHD7, SEMA3E	CHARGE syndrome	ASD, VSD, TOF
FBN1	Marfan syndrome	Aortic dilation
JAG1, NOTCH2	Alagille syndrome	PS, peripheral PS, TOF
KMT2D	Kabuki syndrome	ASD, VSD, TOF, CoA
PTPN11, KRAS, NRAS, HRAS, RAF1, SOS1, NF1, CBL, BRAF, SHOC2, MAP2K1, MAP2K2	Rasopathies: Noonan, Cardiofaciocutaneous, Costello syndromes	PS, HCM
SKI	Shprintzen-Goldberg syndrome	Aortic dilation
TBX5	Holt-Oram syndrome	ASD, VSD, AVSD, conduction system disease
TFAP2b	Char syndrome	PDA
TGFB2, TGFBR1, TGFBR2, SMAD3	Loeys-Dietz syndrome types 1–4	Aortic dilation
TGFB3	Rheinhoff syndrome	Aortic dilation
ZIC3	X-linked heterotaxy syndrome	heterotaxy

ASD, atrial septal defect; AVSD, atrioventricular septal defect; CoA, coarctation of the aorta; PDA, patent ductus arteriousus; PS, pulmonic stenosis; TOF, tetralogy of Fallot; VSD, ventricular septal defect

Table 3

Genes causing isolated heart defects

Gene	Protein
ETS1	V-Ets avian erythroblastosis virus E26 oncogene homolog 1
TGFB2, TGFB3	Transforming growth factor ligand 2, 3
TGFBR1, TGFBR2	Transforming growth factor receptor 1, 2
SMAD2, SMAD3	Mothers against decapentaplegic, drosophila, homologs 2, 3
HAND1, HAND2	Heart and neural crest derivatives expressed 1, 2
GATA4, GATA5, GATA6	GATA binding protein 4–6
TBX1	T-box 1
TBX20	T-box 20
CITED2	Cbp/P300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2
MESP1	Mesoderm posterior 1 homolog
IRX4	Iroquois homeobox 4
MYOCD	Myocardin
Nkx2-5, Nkx2-6	NK2 homeobox 5, 6
NFATC1	Nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 1
NOTCH1	Notch1
ELN	Elastin

Table 4

Phenotypic heterogeneity: the same genetic abnormality causes different heart defects

Gene	CVMs in genetic mouse models
TGFB2	VSD, TAA, BAV
NKX2-5	VSD, AVSD, ASD
GATA4	ASD, VSD, AVSD
TBX1	AVSD, VSD,
TBX20	VSD, AVSD, ASD
BMP4	VSD, AVSD, ASD
GATA6	VSD, AVSD, ASD
ZIC3	Heterotaxy, d-TGA, DORV, AVSD, other heterotaxy spectrum heart defects
JAG1	PS, ASD, TOF
GDF1	DORV, TOF, d-TGA
TBX5	ASD, VSD
Trisomy 21	ASD, VSD, PDA
45, X	BAV, HLHS, CoA
22q11.2 deletion	TOF, VSD, IAA type B

ASD, atrial septal defect; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; CoA, coarctation of the aorta; DORV, double outlet right ventricle; d-TGA, d-transposition of the great arteries; HLHS, hypoplastic left heart syndrome; IAA, interrupted aortic arch type A; PDA, patent ductus arteriousus; PS, pulmonic stenosis; TAA, thoracic aortic aneurysm; TOF, tetralogy of Fallot; VSD, ventricular septal defect

Table 5

Locus heterogeneity: the same CVM results from distinct genetic loci

CVM Type	Examples of genetic etiologies
BAV	TGFB signaling pathway single gene mutations, aneuploidy (45,X)
VSD	TGFB and BMP signaling pathway single gene mutations, aneuploidy (45,X; Trisomy 21), 22q11.2 deletion
DORV	*GDF1, TBX1*, 22q11.2 deletion
AVSD	*ACVR2, NKX2-5, GATA4*, 22q11.2 deletion, aneuploidy (Trisomy 21)
PS	*JAG1, NOTCH2*
TOF	*JAG1, NOTCH2*, 22q11.2 deletion
MVP	*TGFB2, FBN1, FLNA*
TAA	TGFB signaling pathway single gene mutations, *MYH11, ACTA2, MYLK1, FBN1*,
HCM	Rasopathy gene mutations Sarcomeric gene mutation