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Review article

Genetic regulation of heart valve development: Clinical implications

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ABSTRACT

Cardiac malformations, most commonly valve defects, are some of the predominant causes of cardiovascular morbidity and mortality worldwide. Up to a third of all patients with complex congenital heart defects and numerous syndromic conditions, as well as a significant amount of the general population, exhibit valve defects. These observations have not only major implications in infancy; they also have a major impact on the adult population and the growing number of adults with congenital malformations. Over recent years, a large number of Mendelian inheritance patterns and syndromic causes have been identified, shedding light on the importance of genes encoding components of the extracelluar matrix in valve disease. Nevertheless, we still know little about the genetic origin of sporadic and more complex family traits. It is unclear to what extent genetic variations play a role in disease pathogenesis and influences phenotypes rooted in early development. Such knowledge would be greatly beneficial for counseling and treatment of patients. Therefore, this review summarizes the findings in human non-syndromic and syndromic valve disease with a special focus on extracellular matrix proteins, and discusses them in the context of vertebrate valve development.

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INTRODUCTION

With a worldwide incidence of 1–2%, valve disease is one of the predominant causes of cardiovascular morbidity and mortality in all age groups. Valve defects account for a quarter of all congenital heart diseases (CHD) [1–4]; 30% of complex CHD, such as tetralogy of Fallot (TOF) and hypoplastic left heart syndrome (HLHS), as well as a high number of known syndromes, involve valve defects [5]. The predominant valve phenotypes are bicuspid aortic valve (BAV), with a prevalence of up to 2% [6,7], and mitral valve prolapse (MVP), found in 2.4% of the general population [8]. Valve disease has not only major implications with respect to infant mortality and morbidity; it is also a major health concern in the adult population and among the rapidly growing number of adults with congenital malformations [9–12].

Attempts to understand cardiac circulation and its underlying anatomy date back to Aristotle [13], the 12th century [14], and Leonardo da Vinci, who was the first to describe valve physiology including BAV [15]. Over the last decade, a much better understanding of the interrelatedness of genetic factors and developmental processes in heart and valve formation has emerged, and the genetic underpinnings of cardiac morphogenesis are increasingly known. Cardiac progenitor cells are traceable to the cranial-anterior two thirds of the primitive streak, which are among the earliest embryonic cells to gastrulate [16,17]. A key factor for early cardiovascular specification is *Mesp1*, a basic helix-loop-helix transcription factor [18–20] promoting the expression of a number of cardiac transcription factors such as *Hand2, Gata4, Tbx20,* and *Nkx2.5* [21]. The expression domains of these transcription factors demarcate the heart fields in the anterior lateral plate mesoderm and act in combinatorial fashion to specify cardiac progenitors in the cardiac crescent. Expression of these factors is controlled by endoderm-derived signals such as fibroblast growth factors (FGFs) and bone morphogenetic proteins (BMPs) [22–25]. The first human heartbeats are observed after fusion of the two bilateral fields at day 21 of embryonic development. The linear heart tube consists of two cell layers (myocardium and endocardium) separated by an extracellular matrix produced by myocardial precursor cells, the cardiac jelly [26]. Although the tubular heart lacks valves, it still is able to generate unidirectional blood flow, through a peristaltic-like pattern of lumen occlusion. During further stages of development, the cardiac chambers balloon out at the outer curvature and the cardiac jelly diminishes resulting in alterations of the pumping pattern [27]. This process is still poorly understood; hemodynamic shear stress has been found to be an important contributor to cardiac morphogenesis at this point [28]. Endocardial cushions first appear during rightward looping of the linear heart tube at the level of the atrioventricular (AV) canal and outflow tract (Fig. 1). At the molecular level, these regions are marked by the expression of specific markers, e.g. *Tbx2* [29]. A second wave of myocardial cells, which are derived from the second heart field, enter the heart at both poles of the tubular heart leading in particular to the formation of the right ventricle and the outflow tract. On the molecular level this process is characterized by the expression of *Fgf10* and *Isl1, although Isl1* is also expressed transiently in first heart field cells [30–33]. As depicted in Fig. 1, several sources contribute cells to the mature heart, such as the cardiac neural crest [34,35], and the proepicardial cells [36–40]. Altogether, these migratory cell population processes play an important role in valvulogenesis, including endothelial-to-mesenchymal transition (EMT), remodeling, and maturation of the semilunar (SL) and atrioventricular (AV) valves in the outflow tract (OFT) and AV canal (AVC).

VALVE DEVELOPMENT AND ORIGIN OF VALVULAR PRECURSOR CELLS

Cushion formation in the OFT and AVC in vertebrates is initiated during early looping when signals from the myocardium induce endothelial-to-mesenchymal transformation (EMT) in adjacent endocardial cells. This, in turn, increases the synthesis of extracellular matrix (ECM) and leads to cell invasion of the cardiac jelly between the myocardium and the endocardium, resulting in the formation of endocardial cushions (Fig. 1), which over time effectively prevents blood regurgitation in the developing heart [41]. Further steps in valve formation include the remodelling and maturation process in which heart valve progenitor cells diversify and differentiate into interstitial valve fibroblasts [42,43]. In the course of this remodelling process, the ECM becomes compartmentalized and displays region-specific differences in cellular and matrix composition. It consists of the *fibrosa*, the *spongiosa* and the *ventricularis* of the *semilunar* valves (SL) or the *atrialis* of the atrioventricular (AV) valves [44]. The precise distribution of extracellular matrix components within these layers contributes to valve development through cell guidance and life-long maintenance of proper function.

Histopathological evidence shows that this spatiotemporal coordination of ECM organization is perturbed in diseased valves [45].

The origin of cells within the developing valve is not fully understood. Several studies systematically investigated the contribution of the different cell lineages in the outflow tract, the AV canal as well as valve leaflets and suggest multiple origins [46–50]. The precursor for myocardial and endocardial cells of the OFT arises from the second heart field in the splanchnic mesoderm [51]; early mesenchymal cells for endocardial cushion formation are primarily of endocardial origin [47,52–56]. However, there is conflicting evidence about other subpopulations of cells (e.g. neural crest and proepicardium) in the individual leaflets suggesting they might arise from distinct or different embryonic sources. In addition, little is known about the origin of the valve interstitial cells (VICs) and whether or not they contain different subpopulations, with specific contributions to mature valve structure and function [57].

THE GENETIC NETWORKS UNDERLYING VALVE DEVELOPMENT

Several hallmarks of valve development, such as cell migration and EMT, are morphological characteristics in cancer and inflammation. Not surprisingly, similar molecular signatures are found in valve development, such as TGFβ- and WNT-signalling (Figs. 2 and 3).

The TGFβ superfamily consists of more than 30 members, all of which are secreted proteins. TGFβ-signalling can exert its function through the canonical pathways and the non-canonical/non-SMAD pathways. The latter activates different pathways including MAP kinase,

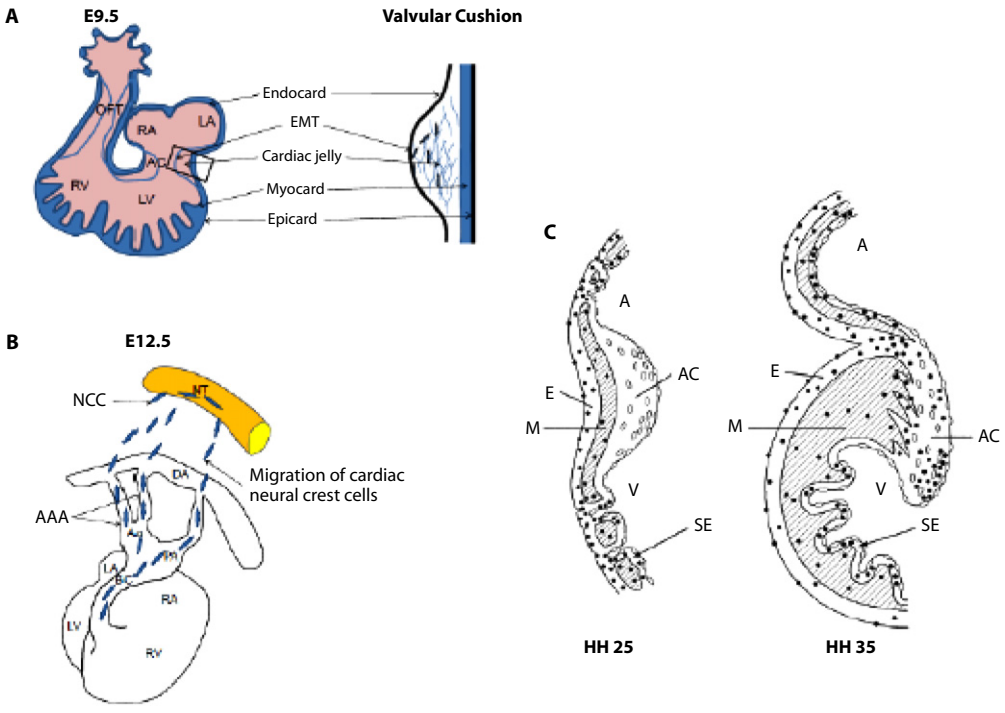


Figure. 1 Important events during valve development. A. Schematic figure of the first signs of valvular cushion formation in mouse embryos at Stage 9.5 dpc. The heart is already looped at this point in the OFT, and the AVC-EMT derived cells migrate in to the cardiac jelly to establish a functioning valve apparatus. Adapted from [58]. **B.** Migration of the neural crest cells into the outflow tract at stage 12.5 dpc. Neural crest cells play a major role in the formation of the outflow tract and its valvular structures. Disturbances in this migration process results in severe outflow tract anomalies including malfunctioning valves. Adapted from http://php.med.unsw.edu.au. **C.** Cell immigration into the mesenchyme of the AVC cushions. The Black dots show epicardial cells in the valvular region at stage HH 25 and HH 35 in the chick. Grey dots demarcate the endocardial cells after EMT. Taken from [47]. Abbreviations: NT — neural tube; DA — dorsal aorta; NCC — neural crest cells; RA — right atrium; LA — left atrium; RV — right ventricle; LV — left ventricle; OFT — outflow tract; EMT — epithelial-mesenchymal transition; AV — atrio-ventricular canal; AAA — aortic arches arteries; Ao — aorta; PA — pulmonary artery; A — atrium; V — ventricle; M — myocard; E — epicard; SE — endocard.

RHO-like GTPase and phosphatidylinositol-3-kinase/AKT pathways. The canonical TGFβ-signalling cascade initiates phosphorylation by type I receptor (ACTR-like kinases ALKs) of type II receptor (BMPRII, ACTRII and ACTRIIB).

The role of the non-canonical TGFβ-signalling pathway in EMT is mostly demonstrated through the activation of ERK and MAPK (Fig. 2). Remarkably, so far it is unclear in mice if the TGFβ pathway is essential for EMT, since mice with a conditional endocardial and myocardial deletion of TGFβ RII only show a reduced growth rate of the cushion [59]. Nevertheless, the importance of TGFβ for valve development in the avian embryo has been shown on several occasions [60–62]. In addition, the bone morphogenetic proteins (BMPs), members of the TGFβ-superfamily, also demonstrate important functions in valve development. Cardiac deletion of *Bmp2* results in a loss of *Tbx2* expression, which is necessary for chamber-specific gene expression, enhanced cardiac jelly formation and activation of *Has2, Twist1 and Notch1, all of which* are involved in valve development [63]. As shown in Fig. 2 these effects occur via the activation of SMAD1/5/8 [64]; whereas, the activation of SMAD2/3 and the resulting induction of the transcription factor SLUG occurs via TGFβ [65,66]. The importance of BMP signaling is not restricted to early events of valvulogenesis. Deletion of *Smad6* in mice leads to hyperplasia of the valve primordia [67] and *Bmp4* mouse mutants show hypocellular OFT and AVC cushions [68].

In a comparative gene expression analysis, active WNT/ β-CATENIN signalling and *Fog1* expression was identified in developing endocardial cushions [69]. Consistent with this observation, increased WNT/ β-CATENIN signaling, such as in the *Apc* knockout mouse, leads to excessive cushion formation, whereas over expression of dickkopf1 and subsequent down regulation of WNT signaling leads to hypo-cellular cushions (Fig. 2) [70,71]. In addition, targeted *Notch1* mutations in mice result in hypo-cellular valve primordia due to EMT defects. Several processes are necessary for this effect (depicted in Fig. 2); *Notch1* induction of *Snail* and the lateral promotion of selective TGFβ-signalling [72]. Functional analysis demonstrated that the *Notch1* target genes *Hey1* and *Hey2* are involved in myocardial restricted expression of *Bmp2* and *Tbx2* to the presumptive valve myocardium [73,74]. Highlighting the importance of the endocardial-myocardial interactions during valve development via NOTCH1/BMP2-induced *Snail1* expression and the nuclear accumulation of

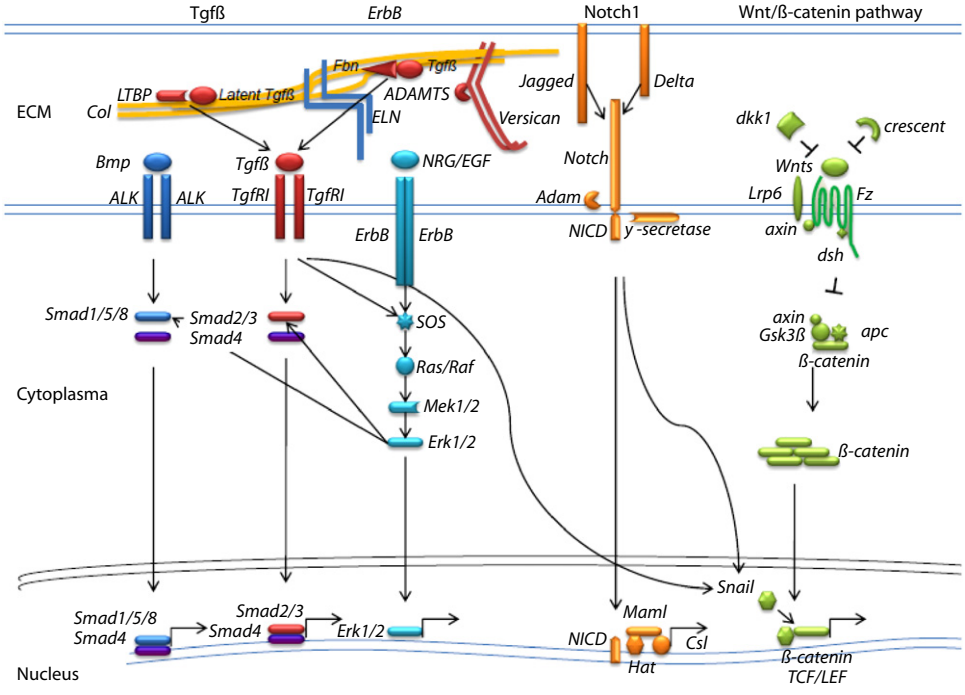


Figure. 2 Signalling networks in valve development. Several transcription factors, extracellular matrix proteins as well as signalling factors have been identified to play a role in valvulogenesis; the TGF β, NOTCH, ERBB and the WNT/ β-CATENIN pathway. Aberrant expressions of individual factors during early and late events result in valvular disease.

BMP2-mediated SNAIL1 to regulate the extent of EMT in AVC and OFT [75–77]. The interplay of these pathways is not restricted to early events; it also has implications in aortic valve disease by later controlling the master regulator of osteogenesis CBFA1/RUNX2 [78]. Several additional factors have been identified in later events of valvulogenesis. The direct NOTCH target *Runx3* is necessary for maintaining the mesenchymal fate after termination of NOTCH signaling, [79] and *Nfatc1* promotes ECM-remodeling [80,81]. *Nfatc1* is activated by *Rankl* and exerts its functions via its downstream target *CathepsinK* [82]. Interestingly, *Vegf* together with *NFatc1* are necessary to maintain the endothelial cell layer during endocardial cushion formation through activation of MEK1-ERK1/2 or JNK1/2 signalling [60,83,84]. Activation of ERK1/2 within the cushion mesenchyme [85,86] has also been observed in Noonan syndrome caused by mutations of *SHP-2*. Araki et al. showed that increased ERK activation, downstream of ERBB family receptor tyrosine kinases results in an extended interval for EMT and causes valve defects [87]. Of note is the observation that the ERBB receptor family knockout mice show heart and/or valve phenotypes resulting in lethal outcome [88–93]. Similar observations have been made for additional essential component of the NRG1/ERBB signaling pathway such as *Adam17* and *19* [90,94,95], which are necessary for the ectodomain shedding of neuregulins.

The interplay of these genetic pathways as shown in Figs. 2 and 3 with hemodynamic factors in the developing valve is at the basis of early and late valve disease. Studies in zebrafish show that valve and cardiac morphogenesis depend on the geometry of the beating heart, suggesting that the physical environment including hemodynamics plays a critical role in its development [96,97]. Remarkably, the full extent to which hemodynamic factors are necessary for normal valve development is not clear, since the *silent heart* mutant develops normally, suggesting that hemodynamics are not important. However, this conclusion is probably not correct, since evidence for an important morphogenetic role of hemodynamics in heart development comes from mouse studies. Yashiro et al. [98] showed that *Pitx2* expression in the outflow tract has an impact on asymmetric remodeling of the great arteries through hemodynamics. In addition, experiments in zebrafish focusing on the atrioventricular (AV) canal development before and during valvulogenesis have shown that the amount of retrograde flow determines valve growth and reduces *klf2a* expression in valve precursors [28,99]. These experiments highlight the important effects of hemodynamics on valve morphogenesis. It remains to be seen how important and to what extent hemodynamics are morphogenetic and if they are equally strong compared to the genetic effects in human valve development. Interestingly, abnormalities in early development uncovered by cumulative hemodynamic stress and environmental factors in adulthood point to a substantial genetic component.

GENETICS OF NON-SYNDROMIC HUMAN VALVE DISEASE

In most cases, valve disease in humans occurs on a sporadic basic and in the absence of an underlying medical syndrome. The causes for these diseases remain largely unknown, with most studies showing that valve disease is a genetically-heterogeneous, complex trait [57,100,101]. As an example, family-based studies have identified loci for BAV and hypoplastic left heart syndrome on chromosomes 5, 13 and 18 [102–104], and for mitral valve prolapse (MVP) on chromosome 13, 11, 16 and X [105–109]. In all clinical and genetic studies on human valve disease, variable expressivity, reduced penetrance and allelism are widely observed.

As an example, mutations in BMP receptors and their downstream targets are involved in a wide variety of vascular and cardiac conditions [64,110,111]. Decreased BMP signaling caused by *ALK2* mutations has been implicated as a cause of atrioventricular septal defects; conversely, gain-of-function mutations in the same gene are the cause of progressive fibrodysplasia ossificans [112,113]. Knockdown of endothelial *Alk3* and neural crest *Alk2* in mice results in severe endocardial cushion defects [112,114,115]. Within this same pathway, mutations of *SMAD4* have recently been identified in the context of aortopathy and mitral valve disease [116]. This gene had previously been identified as a cause of juvenile polyposis syndrome [117–119]. Dominant missense mutations of *GATA4* can result in impaired binding to SMAD4, thus linking the two cascades at the transcriptional level [120]. Interestingly, mutations in *SMAD3* have recently been reported to cause a syndrome presenting with perturbations of the whole arterial tree, including aneurysms and early-onset osteoarthritis [121].

Identified in both familial and sporadic cases, mutations in the *NOTCH1* gene on chromosome 9q34 have been shown to contribute to BAV and aortic calcifications [122–125]. The importance of the NOTCH pathway in human valve disease is further strengthened by the finding that mutations of *JAGGED1*, a NOTCH ligand, cause Alagille Syndrome, a multisystem disorder with pulmonary valve stenosis [126,127]. The identification of specific mutations reducing JAGGED1-induced NOTCH1 signalling provides further evidence of tightly controlled signal transduction during valve development and highlights its role as a modifier or causal gene for human valve disease [123]. Further downstream of NOTCH, several components of cardiac-specific transcription cascades, such as NKX2.5, GATA4, TBX5, HEY2, and CITED2 (Table 1) have been implicated in human valve disease based on candidate gene and linkage studies, respectively [128–136].

In most human studies, variable expressivity and reduced penetrance in obligate mutation carriers have been the rule rather than the exception. For example, patients with aortic aneurysm due to mutations *ACTA2* [137] and *MYH11* [138] show BAV with reduced penetrance (Table 1). These observations are not limited to humans: mice heterozygous for a targeted *Nkx2.5* allele, have an increased incidence of valvuloseptal defects [139], and 25% of mice with a homozygous deletion of *Gata5* have BAV (Fig. 4) [140]. Using a targeted breeding strategy of *Nkx2.5* heterozygous mice, Winston et al. showed that a balance between several modifying loci contributes to both heterogeneous heart defects as well as normal heart development in this mouse model [139]. Knowing that proper heart development is guided through a combinatorial genetic network, it is tempting to speculate that multiple perturbations in the pathways described above account for the non-Mendelian patterns of human valve disease.

GENETICS OF SYNDROMIC HUMAN VALVE DISEASE

Several syndromic conditions shed additional light on the genetics of valve disease in humans. For the purpose of this review, we will focus on mutations in extracellular matrix (ECM) proteins. Such

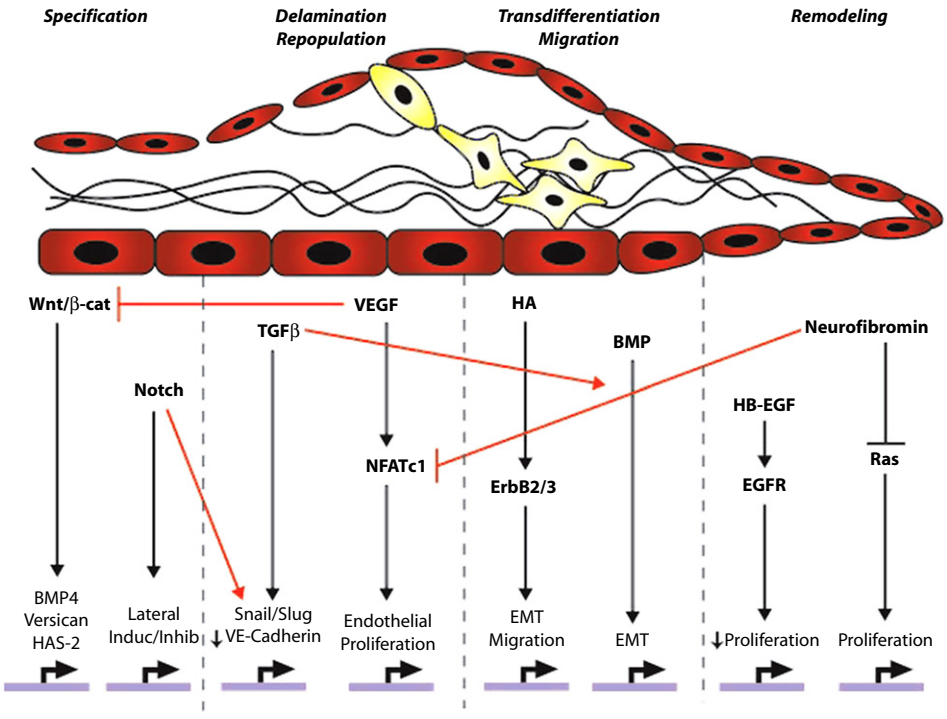


Figure. 3 Anatomic and signaling events during early valve development. Numerous pathways either synergistically (black arrows) or through inhibitory interactions (blunt red arrows) coordinate the early events of endocardial cushion formation and EMT (for detailed overview see Fig. 2). The repopulation, differentiation and migration of endothelial cells into the ECM/ cardiac jelly are the first signs of the developing valves. Picture taken from Armstrong and Bischoff Circ. Res. 2004; 95; 459–470 [60].

mutations have been described to disturb valve formation and are associated with specific histopathological phenotypes.

William's syndrome is caused by mutations in ELASTIN *(ELN)* and goes along with supravalvular aortic stenosis [141]. This phenotype is partly recapitulated in heterozygous *Eln* knockout mice [142–144]. ELN is the earliest structural matrix protein expressed by smooth muscle cells (SMC) and closely linked to the evolution of the closed vertebrate circulatory system [145]. Transcripts are detected initially in the *Truncus arteriosus* [146], and its expression is initiated during cusp development [147]. ELN and Collagen (COL) molecules are linked by Lysyl oxidases (LOX) and microfibrils consisting of Fibrillin (FBN) to form a macromolecular complex in the ECM. Mutations in *COL* have been found in a variety of different syndromes, such as the Osteogenesis imperfecta and Stickler syndrome [148–151]. Valve anomalies are also observed in patients with Ehlers–Danlos syndrome, characterized by mutations in different COL proteins [152]. Cardiac and valve phenotypes have been observed for *ColVa1* and *ColXIa1* mutants [153]; and homozygous mutant *Col3A* mice partially replicate the human phenotype [154]. Also, loss of function in LOX proteins necessary for linking of ELN and COL molecules in the ECM [155] shows a cardiac phenotype. Indeed, the phenotype of targeted *Lox*- mice ($Lox^{-/-}$) is lethal soon after birth, most likely due to severe defects in the cardiovascular system (valvular regurgitation, aortic aneurysms and cardiac dysfunction) and diaphragmatic rupture [156,157]. Furthermore, their function is not restricted to scaffolding. Studies have shown that they also modulate the SMC [158], interact with TGFβ [159] and have a direct effect on the promoter of *Eln* [160] and *Col* [161]. Notably, LOXL1, a member of the LOX-like proteins interacts with FIBULIN (FBLN) 5 [162]. Mice deficient for *Fbln5* and *Fbln4* show disorganized elastic fibers leading to defects in the lung, blood vessels and skin resulting in perinatal death. The vascular phenotype in *Fbln4* mice is much more severe than that of *Fbln5* knockout mice, owing to a more pronounced perturbation of the vascular tree, including a narrowed and tortuous aorta characterized by irregular ELN aggregates [163,164]. Valvular phenotypes in *Fbln4* knockout mice include

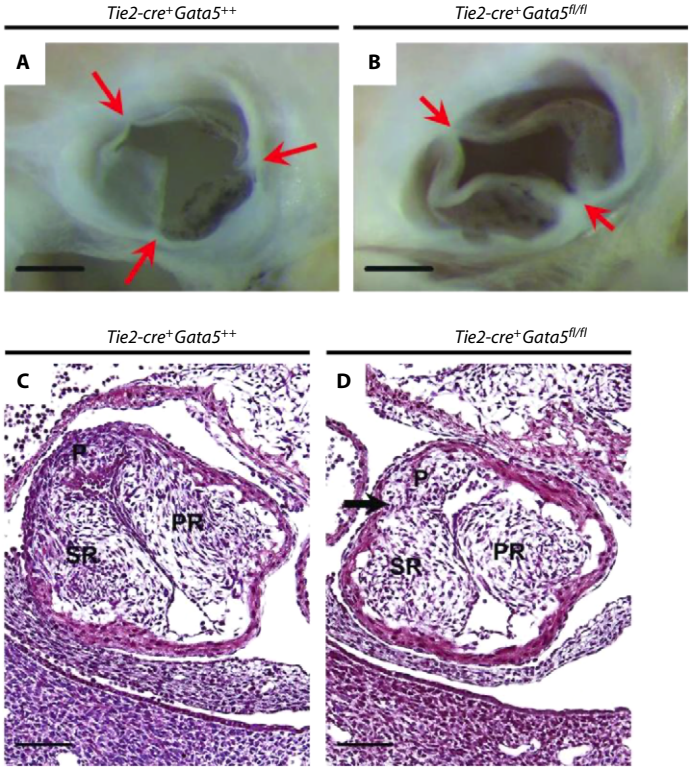


Figure. 4 ***Gata5*** **is required for aortic valve formation. (A and B) Anatomic analysis reveals the presence of BAV in Tie2-cre^{+}Gata5$^{fl/fl}$ mice. Arrows point to an attachment of the valve cups to the aortic wall. (C and D) Histological analysis of the outflow tract of (C) wild type, and (D) Tie2-cre^{+}Gata5$^{fl/fl}$mutant at E11.5. Arrow shows abnormal fusion resulting in an R-N BAV. Figure reproduced with permission from [140].**

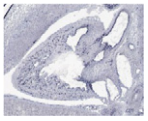
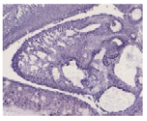
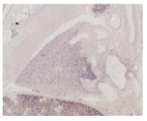
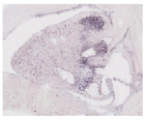
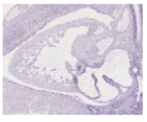
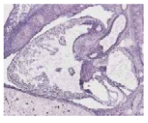
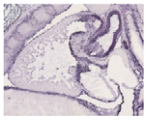
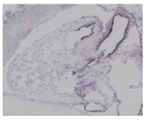
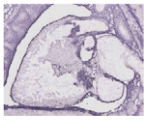
thickening of the aortic valvular leaflets and are associated with aortic valve insufficiency/stenosis [165]. Observations in patients with cutis laxa syndromes caused by mutations in *FBLN5* or *FBLN4* corroborate these findings from animal models [166,167].

The best-studied model for defects in the extracellular matrix and changes in the TGFβ signaling cascade is the Marfan syndrome caused by inherited or *de novo* mutations in *FBN1*. Diagnostic criteria for this syndrome include skeletal, ocular and cardiovascular manifestations [168,169]. Valvular phenotypes are well described including thickening of the mitral and aortic valves [170]. Similar changes are observed in null mutant mice, since *FBN1*-deficient mice develop MVP [171]. In addition *FBN2* mutations have been observed in patients with the Beal syndrome, an autosomal dominant disease with vascular anomalies including MVP [172,173]. The phenotype observed in mice lacking *Fbn2* only recapitulates the skeletal, but not cardiac, anomalies most possibly through alterations in BMP signaling [174]. Remarkably, mutated *FBN2* in Zebrafish results in notochord and vascular defects [175]. Mutations of *FBN1* are not limited to Marfan syndrome; they also cause the allelic conditions Shprintzen–Goldberg syndrome, MASS syndrome, stiff skin syndrome, isolated ectopia lentis, and autosomal dominant Weill–Marchesani syndrome.

Two type of the Loeys–Dietz syndrome (LDS) inherited in an autosomal dominant pattern are caused by mutations in *TGFBR1* also *TGFBR2*. Type 1 shows arterial turtosity aneurysms, hypertelorism and bifid uvula or cleft palate as well as a widespread systemic involvement. Type 2 lacks the craniofacial involvement and only occasionally shows a bifid uvula [176]. Cardiac manifestations include the predisposition for aortic dissection and subsequent rupture; MVP is also observed but to a much lesser extent than in Marfan syndrome [177]. Additional cardiac findings include a patent ductus arteriosus, atrial septal defects, and bicuspid aortic valve. Interestingly, mutations of the *TGFBR1* gene, which is predominately mutated in Loeys–Dietz syndrome, are also found in the Shprintzen–Goldberg syndrome [178,179]. Commonly, mitral valve prolapse without aortic dissection is seen in a number of other type 1 fibrillinopathies either caused by or associated with mutations in the *FBN1* gene such as several MASS (**m**itral valve prolapse, upper limits of **a**ortic root diameter, **s**tretch marks of the skin and **s**keletal conditions) phenotypes. The Weil–Marchesani syndrome (WMS) includes a subluxation of microspherophakic lenses, short stature, brachydactyly and congenital heart disease—mainly valvular anomalies—and is caused by either mutations in *FBN-1* or one of the two metalloproteinases *ADAMTS10* and *17* [180,181]. No animal model has been established in WMS so far, but heterozygous *Adamts9* mice show anomalies in the aortic wall, valvulosinus, valve leaflets and spongy myocardium, consistent with non-compaction of the left ventricle [182,183]. In addition, metalloproteinases are tightly linked to cleavage of VERSICAN (VCAN), which may directly affect a key regulatory network in the vascular wall centered around FBN1 and regulates TGFβ signaling [184,185]. VCAN, a member of the *Aggrecan/Versican Proteoglycan* family, is a major component of the ECM and has been shown to be important for EMT in the production of cardiac mesenchyme [186,187]. Mice with an altered *Vcan* gene product die of heart defects. Secreted VCAN is regulated by proteolysis, a process that enables subsequent deposition of ECM molecules throughout cardiac development, especially during atrioventricular remodeling, cardiac outlet formation as well as growth and compaction of the trabeculae in the ventricular myocardium [188–190]. Increased VCAN cleavage is correlated with regression of neointimal thickening and the loss of proteoglycans [191]. It has also been shown that proteoglycans play a significant role in the adaptation to the high shear stress generated by blood flow in the aorta [192] pointing to a possible mechanism of lifelong hemodynamic stress in valve disease.

Interestingly, another protein belonging to the large superfamily of ADAMTS proteases and ADAMTS-like proteins ADAMTSL2 causes autosomal recessive geleophysic dysplasia (GD) going along with valvular thickening and atrial septal defects. The fact that ADAMTSL2 enhances TGFβ signaling most likely through the binding of LTBP1 [193–195] shows again the importance of this signaling pathway in valve disease and points to possible curative strategies that are being successfully implemented in Marfan Syndrome [196–200]. Given the structural and functional similarities between many ADAMs and ADAMTSs, it is tempting to speculate that they contribute to human valve disease in an allelic fashion.

Table 1. Mutated human genes with valvular defects and its cardiac expression pattern at E14.5 in mouse embryos. All of the depicted genes show a localized expression in the endothelium and the valves, highlighting the importance of these genes in valve development. Pictures are taken from www.eurexpress.org [213]. Abbreviations: Mitral valve prolaps (MVP), Pulmonal stenosis (PS), Bicuspid aortic valve (BAV), Aortic stenosis (AS), Mitral valve anomaly (MVA).

Gene	Locus	Valve phenotype	Syndrome/associated phenotypes	Reference	Expression pattern in the mouse heart at E14.5
NOTCH1	9q34.3	BAV	non-syndromic	[122–125]	
ACTA2	10q23.31	BAV	non-syndromic	[137]	
NKX2.5	5q35.1	MVA, AS	AV block	[128–130,201]	
GATA4	8p23.1	PS	Atrial septal defect	[133,201–204]	
TBX5	12q24.1	AS, MVP	HOLT-ORAM	[134,205,206]	
FLNA	Xq28	MVP	OTOPALATODIGITAL SPECTRUM DISORDER	[207,208]	
ELN	7q11.2	BAV, MVP	WILLIAMS-BEUREN	[141]	
FBN1	15q21.1	MVP, BAV	MARFAN, MASS, WEILL-MARCHESANI, SHPRINTZEN-GOLDBERG	[179,209,210]	
COL1A1	17q21.31-q22	MVP	OSTEOGENESIS IMPERFECTA TYPE	[151]	

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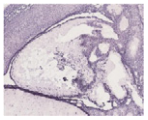
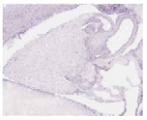
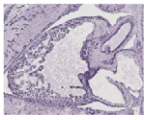
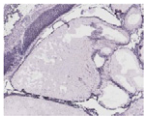
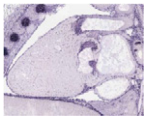
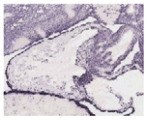
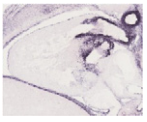
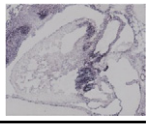
CONCLUSIONS

Taken together, the presented studies highlight the importance of several interacting gene networks acting at the growth factor/receptor, extracellular matrix protein, and transcriptional control levels, which are essential to the morphogenesis and structural integrity of the valves throughout life. Although a number of Mendelian traits and many syndromic causes have been identified, we still know little about the genetic origin of sporadic and more complex family traits. Insight into these processes will be certainly coming from the steadily growing number of NextGeneration sequencing data and further studies in animal models. For instance, it has to be elucidated if the proteins mutated

Table 1 (*continued*)

Gene	**Locus**	**Valve phenotype**	**Syndrome/associated phenotypes**	**Reference**	**Expression pattern in the mouse heart at E14.5**
COL1A2	7q22.1	MVP	OSTEOGENESIS IMPERFECTA TYPE	[151]	
TGFBR1	3p22	MVP	LOEYS-DIETZ	[176]	
COL4A1	13q34	MVP	PORENCEPHALY, FAMILIAL	[211]	
COL2A1	12q13.11	MVP	STICKLER	[150,212]	
COL9A1	6p21.3	MVP	STICKLER	[149]	
COL3A1	2q31	BAV, MVP	EHLERS-DANLOS	[152]	
FBLN5	14q32.1	PS	CUTIS LAXA	[167]	
FBN2	5 q23-q31	MVP	BEALS-HECHT	[172,173]	

are acting as modifiers or directly causing the disease phenotype. This is especially important with respect to phenotypes rooted in early development, which only become fully penetrant later in life. This knowledge will be of great benefit for counseling and treatment of patients with valve disease.

References

[1] Hoffman JI, Kaplan S and Liberthson RR. Prevalence of congenital heart disease. Am Heart J. 2004;147:425–439.
[2] Hoffman JI and Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol. 2002;39:1890–1900.
[3] Loffredo CA. Epidemiology of cardiovascular malformations: prevalence and risk factors. Am J Med Genet. 2000;97:319–325.
[4] Loffredo CA et al. Prevalence of congenital cardiovascular malformations among relatives of infants with hypoplastic left heart, coarctation of the aorta, and d-transposition of the great arteries. Am J Med Genet. 2004;124A:225–230.
[5] Hinton RB et al. Hypoplastic left heart syndrome links to chromosomes 10q and 6q and is genetically related to bicuspid aortic valve. J Am Coll Cardiol. 2009;53:1065–1071.
[6] Basso C et al. An echocardiographic survey of primary school children for bicuspid aortic valve. Am J Cardiol. 2004;93:661–663.
[7] Nistri S, Basso C, Marzari C, Mormino P and Thiene G. Frequency of bicuspid aortic valve in young male conscripts by echocardiogram. Am J Cardiol. 2005;96:718–721.
[8] Freed LA et al. Mitral valve prolapse in the general population: the benign nature of echocardiographic features in the Framingham heart study. J Am Coll Cardiol. 2002;40:1298–1304.

[9] Roberts WC and Ko JM. Frequency by decades of unicuspid, bicuspid, and tricuspid aortic valves in adults having isolated aortic valve replacement for aortic stenosis, with or without associated aortic regurgitation. Circulation. 2005;111:920.
[10] Billett J, Cowie MR, Gatzoulis MA, Vonder Muhll IF and Majeed A. Comorbidity, healthcare utilisation and process of care measures in patients with congenital heart disease in the UK: cross-sectional, population-based study with case-control analysis. Heart. 2008;94:1194–1199.
[11] Billett J, Majeed A, Gatzoulis M and Cowie M. Trends in hospital admissions, in-hospital case fatality and population mortality from congenital heart disease in England, 1994 to 2004. Heart. 2008;94:342–348.
[12] Pillutla P, Shetty KD and Foster E. Mortality associated with adult congenital heart disease: trends in the US population from 1979 to 2005. Am Heart J. 2009;158:874–879.
[13] Aristotle Historia Animalium. Book VI 3 translated by Beck 1970, (384AD).
[14] Masic I. On occasion of 800th anniversary of birth of Ibn al-Nafis–discoverer of cardiac and pulmonary circulation. Med Arh. 2010;64:309–313.
[15] Clayton M and Museum of Fine Arts, Houston; Windsor Castle. Leonardo da Vinci: the anatomy of man: drawings from the collection of Her Majesty Queen Elizabeth II. (Museum of Fine Arts; Little Brown and Co.: Houston; Boston, 1992).
[16] Tam PP, Parameswaran M, Kinder SJ and Weinberger RP. The allocation of epiblast cells to the embryonic heart and other mesodermal lineages: the role of ingression and tissue movement during gastrulation. Development. 1997;124:1631–1642.
[17] Garcia-Martinez V and Schoenwolf GC. Primitive-streak origin of the cardiovascular system in avian embryos. Dev Biol. 1993;159:706–719.
[18] Saga Y et al. MesP1: a novel basic helix-loop-helix protein expressed in the nascent mesodermal cells during mouse gastrulation. Development. 1996;122:2769–2778.
[19] Saga Y, Kitajima S and Miyagawa-Tomita S. Mesp1 expression is the earliest sign of cardiovascular development. Trends Cardiovasc Med. 2000;10:345–352.
[20] Saga Y et al. MesP1 is expressed in the heart precursor cells and required for the formation of a single heart tube. Development. 1999;126:3437–3447.
[21] Bondue A et al. Mesp1 acts as a master regulator of multipotent cardiovascular progenitor specification. Cell Stem Cell. 2008;3:69–84.
[22] Schoenebeck JJ, Keegan BR and Yelon D. Vessel and blood specification override cardiac potential in anterior mesoderm. Dev Cell. 2007;13:254–267.
[23] Prall OWJ et al. An Nkx2-5/Bmp2/Smad1 negative feedback loop controls heart progenitor specification and proliferation. Cell. 2007;128:947–959.
[24] Christoffels VM et al. Chamber formation and morphogenesis in the developing mammalian heart. Dev Biol. 2000;223:266–278.
[25] Brand T. Heart development: molecular insights into cardiac specification and early morphogenesis. Dev Biol. 2003;258:1–19.
[26] Davis CL. The cardiac jelly of the chick embryo. Anat Rec. 1924;27:201–202.
[27] Männer J, Wessel A and Yelbuz TM. How does the tubular embryonic heart work? Looking for the physical mechanism generating unidirectional blood flow in the valveless embryonic heart tube. Dev Dyn. 2010;239:1035–1046.
[28] Lee JS et al. Klf2 is an essential regulator of vascular hemodynamic forces in vivo. Dev Cell. 2006;11:845–857.
[29] Greulich F, Rudat C and Kispert A. Mechanisms of T-box gene function in the developing heart. Cardiovasc Res. 2011. doi:10.1093/cvr/cvr112.
[30] Kelly RG, Brown NA and Buckingham ME. The arterial pole of the mouse heart forms from Fgf10-expressing cells in pharyngeal mesoderm. Dev Cell. 2001;1:435–440.
[31] Torlopp A, Schlueter J and Brand T. Role of fibroblast growth factor signaling during proepicardium formation in the chick embryo. Dev Dyn. 2010;239:2393–2403.
[32] Cai CL et al. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. Dev Cell. 2003;5:877–889.
[33] Laugwitz KL, Moretti A, Caron L, Nakano A and Chien KR. Islet1 cardiovascular progenitors: a single source for heart lineages? Development. 2008;135:193–205.
[34] Waldo K et al. A novel role for cardiac neural crest in heart development. J Clin Invest. 1999;103:1499–1507.
[35] Jiang X, Rowitch DH, Soriano P, McMahon AP and Sucov HM. Fate of the mammalian cardiac neural crest. Development. 2000;127:1607–1616.
[36] Männer J, Schlueter J and Brand T. Experimental analyses of the function of the proepicardium using a new microsurgical procedure to induce loss-of-proepicardial-function in chick embryos. Dev Dyn. 2005;233:1454–1463.
[37] Cai C-L et al. A myocardial lineage derives from Tbx18 epicardial cells. Nature. 2008;454:104–108.
[38] Zhou B et al. Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. Nature. 2008;454:109–113.
[39] Christoffels VM et al. Tbx18 and the fate of epicardial progenitors. Nature. 2009;458:E8–9. discussion E9–10.
[40] Rosenthal N and Harvey RP. Heart Development and Regeneration. Academic. Amsterdam; Oxford. 2010.
[41] Person AD, Klewer SE and Runyan RB. Cell biology of cardiac cushion development. Int Rev Cytol. 2005;243:287–335.
[42] Schroeder JA, Jackson LF, Lee DC and Camenisch TD. Form and function of developing heart valves: coordination by extracellular matrix and growth factor signaling. J Mol Med. 2003;81:392–403.
[43] Combs MD and Yutzey KE. Heart valve development: regulatory networks in development and disease. Circ Res. 2009;105:408.

[44] Gross L and Kugel MA. Topographic Anatomy and Histology of the valves in the human heart. Am J Pathol. 1931;7:445–474.7.
[45] Hinton RB Jr et al. Extracellular matrix remodeling and organization in developing and diseased aortic valves. Circ Res. 2006;98:1431–1438.
[46] Lamers WH, Virágh S, Wessels A, Moorman AF and Anderson RH. Formation of the tricuspid valve in the human heart. Circulation. 1995;91:111–121.
[47] Gittenberger-de Groot AC, Vrancken Peeters MP, Mentink MM, Gourdie RG and Poelmann RE. Epicardium-derived cells contribute a novel population to the myocardial wall and the atrioventricular cushions. Circ Res. 1998;82:1043–1052.
[48] Chin C, Gandour-Edwards R, Oltjen S and Choy M. Fate of the atrioventricular endocardial cushions in the developing chick heart. Pediatr Res. 1992;32:390–393.
[49] Wessels A et al. The development of the atrioventricular junction in the human heart. Circ Res. 1996;78:110–117.
[50] Oosthoek PW et al. Development of the atrioventricular valve tension apparatus in the human heart. Anat Embryol. 1998;198:317–329.
[51] Verzi MP, McCulley DJ, De VS, Dodou E and Black BL. The right ventricle, outflow tract, and ventricular septum comprise a restricted expression domain within the secondary/anterior heart field. Dev Biol. 2005;287:134–145.
[52] de Lange FJ et al. Lineage and morphogenetic analysis of the cardiac valves. Circ Res. 2004;95:645–654.
[53] Lincoln J, Alfieri CM and Yutzey KE. Development of heart valve leaflets and supporting apparatus in chicken and mouse embryos. Dev Dyn. 2004;230:239–250.
[54] Snarr BS, Kern CB and Wessels A. Origin and fate of cardiac mesenchyme. Dev Dyn. 2008;237:2804–2819.
[55] Nakamura T, Colbert MC and Robbins J. Neural crest cells retain multipotential characteristics in the developing valves and label the cardiac conduction system. Circ Res. 2006;98:1547.
[56] Zhou B, von Gise A, Ma Q, Hu YW and Pu WT. Genetic fate mapping demonstrates contribution of epicardium-derived cells to the annulus fibrosis of the mammalian heart. Dev Biol. 2010;338:251–261.
[57] Hinton RB and Yutzey KE. Heart valve structure and function in development and disease. Annu Rev Physiol. 2010. doi:10.1146/annurev-physiol-012110-142145.
[58] Brade T, Männer J and Kühl M. The role of Wnt signalling in cardiac development and tissue remodelling in the mature heart. Cardiovasc Res. 2006;72:198–209.
[59] Jiao K et al. Tgfbeta signaling is required for atrioventricular cushion mesenchyme remodeling during in vivo cardiac development. Development. 2006;133:4585–4593.
[60] Armstrong EJ and Bischoff J. Heart valve development: endothelial cell signaling and differentiation. Circ Res. 2004;95:459–470.
[61] Desgrosellier JS, Mundell NA, McDonnell MA, Moses HL and Barnett JV. Activin receptor-like kinase 2 and Smad6 regulate epithelial-mesenchymal transformation during cardiac valve formation. Dev Biol. 2005;280:201–210.
[62] Tavares ALP, Mercado-Pimentel ME, Runyan RB and Kitten GT. TGF beta-mediated RhoA expression is necessary for epithelial-mesenchymal transition in the embryonic chick heart. Dev Dyn. 2006;235:1589–1598.
[63] Ma L, Lu MF, Schwartz RJ and Martin JF. Bmp2 is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. Development. 2005;132:5601.
[64] van Wijk B, Moorman AFM and van den Hoff MJB. Role of bone morphogenetic proteins in cardiac differentiation. Cardiovasc Res. 2007;74:244–255.
[65] Romano LA and Runyan RB. Slug is an essential target of TGFbeta2 signaling in the developing chicken heart. Dev Biol. 2000;223:91–102.
[66] Romano LA and Runyan RB. Slug is a mediator of epithelial-mesenchymal cell transformation in the developing chicken heart. Dev Biol. 1999;212:243–254.
[67] Galvin KM et al. A role for smad6 in development and homeostasis of the cardiovascular system. Nat Genet. 2000;24:171–174.
[68] McCulley DJ, Kang J-O, Martin JF and Black BL. BMP4 is required in the anterior heart field and its derivatives for endocardial cushion remodeling, outflow tract septation, and semilunar valve development. Dev Dyn. 2008;237:3200–3209.
[69] Gitler AD, Lu MM, Jiang YQ, Epstein JA and Gruber PJ. Molecular markers of cardiac endocardial cushion development. Dev Dyn. 2003;228:643–650.
[70] Liebner S et al. Beta-catenin is required for endothelial-mesenchymal transformation during heart cushion development in the mouse. J Cell Biol. 2004;166:359–367.
[71] Hurlstone AFL et al. The Wnt/beta-catenin pathway regulates cardiac valve formation. Nature. 2003;425:633–637.
[72] Timmerman LA et al. Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic transformation. Genes Dev. 2004;18:99–115.
[73] Rutenberg JB et al. Developmental patterning of the cardiac atrioventricular canal by Notch and Hairy-related transcription factors. Development. 2006;133:4381–4390.
[74] Kokubo H, Tomita-Miyagawa S, Hamada Y and Saga Y. Hesr1 and Hesr2 regulate atrioventricular boundary formation in the developing heart through the repression of Tbx2. Development. 2007;134:747–755.
[75] MacGrogan D, Luna-Zurita L and de la Pompa JL. Notch signaling in cardiac valve development and disease. Birth Defects Res. Part A Clin Mol Teratol. 2011;91:449–459.
[76] Luna-Zurita L et al. Integration of a Notch-dependent mesenchymal gene program and Bmp2-driven cell invasiveness regulates murine cardiac valve formation. J Clin Invest. 2010;120:3493–3507.
[77] Watanabe Y et al. Activation of Notch1 signaling in cardiogenic mesoderm induces abnormal heart morphogenesis in mouse. Development. 2006;133:1625–1634.

[78] Rusanescu G, Weissleder R and Aikawa E. Notch signaling in cardiovascular disease and calcification. Curr Cardiol Rev. 2008;4:148–156.
[79] Fu Y et al. RUNX3 maintains the mesenchymal phenotype after termination of the Notch signal. J Biol Chem. 2011;286:11803–11813.
[80] de la Pompa JL et al. Role of the NF-ATc transcription factor in morphogenesis of cardiac valves and septum. Nature. 1998;392:182–186.
[81] Ranger AM et al. The transcription factor NF-ATc is essential for cardiac valve formation. Nature. 1998;392:186–190.
[82] Lange AW and Yutzey KE. NFATc1 expression in the developing heart valves is responsive to the RANKL pathway and is required for endocardial expression of cathepsin K. Dev Biol. 2006;292:407–417.
[83] Combs MD and Yutzey KE. VEGF and RANKL regulation of NFATc1 in heart valve development. Circ Res. 2009;105:565–574.
[84] Dor Y et al. A novel role for VEGF in endocardial cushion formation and its potential contribution to congenital heart defects. Development. 2001;128:1531–1538.
[85] Krenz M et al. Role of ERK1/2 signaling in congenital valve malformations in Noonan syndrome. Proc Natl Acad Sci USA. 2008;105:18930–18935.
[86] Fragale A, Tartaglia M, Wu J and Gelb BD. Noonan syndrome-associated SHP2/PTPN11 mutants cause EGF-dependent prolonged GAB1 binding and sustained ERK2/MAPK1 activation. Hum Mutat. 2004;23:267–277.
[87] Araki T et al. Noonan syndrome cardiac defects are caused by PTPN11 acting in endocardium to enhance endocardial-mesenchymal transformation. Proc Natl Acad Sci USA. 2009;106:4736–4741.
[88] Meyer D and Birchmeier C. Multiple essential functions of neuregulin in development. Nature. 1995;378:386–390.
[89] Sibilia M et al. Mice humanised for the EGF receptor display hypomorphic phenotypes in skin, bone and heart. Development. 2003;130:4515–4525.
[90] Jackson LF et al. Defective valvulogenesis in HB-EGF and TACE-null mice is associated with aberrant BMP signaling. EMBO J. 2003;22:2704–2716.
[91] Chen B et al. Mice mutant for Egfr and Shp2 have defective cardiac semilunar valvulogenesis. Nat Genet. 2000;24:296–299.
[92] Chan R, Hardy WR, Laing MA, Hardy SE and Muller WJ. The catalytic activity of the ErbB-2 receptor tyrosine kinase is essential for embryonic development. Mol Cell Biol. 2002;22:1073–1078.
[93] Sanchez-Soria P and Camenisch TD. ErbB signaling in cardiac development and disease. Semin Cell Dev Biol. 2010;21:929–935.
[94] Zhou H-M et al. Essential role for ADAM19 in cardiovascular morphogenesis. Mol Cell Biol. 2004;24:96–104.
[95] Horiuchi K, Zhou H-ming, Kelly K, Manova K and Blobel CP. Evaluation of the contributions of ADAMs 9, 12, 15, 17, and 19 to heart development and ectodomain shedding of neuregulins h1 and h2. Dev Biol. 2005;283:459–471.
[96] Scherz PJ, Huisken J, Sahai-Hernandez P and Stainier DYR. High-speed imaging of developing heart valves reveals interplay of morphogenesis and function. Development. 2008;135:1179–1187.
[97] Hove JR et al. Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis. Nature. 2003;421:172–177.
[98] Yashiro K, Shiratori H and Hamada H. Haemodynamics determined by a genetic programme govern asymmetric development of the aortic arch. Nature. 2007;450:285–288.
[99] Vermot J et al. Reversing blood flows act through klf2a to ensure normal valvulogenesis in the developing heart. PLoS Biol. 2009;7:e1000246.
[100] Markwald RR, Norris RA, Moreno-Rodriguez R and Levine RA. Developmental basis of adult cardiovascular diseases: valvular heart diseases. Ann N Y Acad Sci. 2010;1188:177–183.
[101] Wirrig EE and Yutzey KE. Transcriptional regulation of heart valve development and disease. Cardiovasc Pathol. 2011;20:162–167.
[102] Cripe L, Andelfinger G, Martin LJ, Shooner K and Benson DW. Bicuspid aortic valve is heritable. J Am Coll Cardiol. 2004;44:138–143.
[103] Hinton RB et al. Hypoplastic left heart syndrome is heritable. J Am Coll Cardiol. 2007;50:1590–1595.
[104] McBride KL et al. Inheritance analysis of congenital left ventricular outflow tract obstruction malformations: segregation, multiplex relative risk, and heritability. Am J Med Genet A. 2005;134:180–186.
[105] Freed LA et al. A locus for autosomal dominant mitral valve prolapse on chromosome 11p15.4. Am J Hum Genet. 2003;72:1551–1559.
[106] Disse S et al. Mapping of a first locus for autosomal dominant myxomatous mitral-valve prolapse to chromosome 16p11.2-p12.1. Am J Hum Genet. 1999;65:1242–1251.
[107] Nesta F et al. New locus for autosomal dominant mitral valve prolapse on chromosome 13: clinical insights from genetic studies. Circulation. 2005;112:2022–2030.
[108] Grau JB, Pirelli L, Yu P-J, Galloway AC and Ostrer H. The genetics of mitral valve prolapse. Clin Genet. 2007;72:288–295.
[109] Kyndt F et al. Mapping of X-linked myxomatous valvular dystrophy to chromosome Xq28. Am J Hum Genet. 1998;62:627–632.
[110] Klaus A, Saga Y, Taketo MM, Tzahor E and Birchmeier W. Distinct roles of Wnt/beta-catenin and Bmp signaling during early cardiogenesis. Proc Natl Acad Sci USA. 2007;104:18531–18536.
[111] Wang J, Greene SB and Martin JF. BMP signaling in congenital heart disease: New developments and future directions. Birth Defects Res Part A Clin Mol Teratol. 2011;91:441–448.
[112] Smith KA et al. Dominant-negative ALK2 allele associates with congenital heart defects. Circulation. 2009;119:3062–3069.

[113] Joziasse IC et al. ALK2 mutation in a patient with Down's syndrome and a congenital heart defect. Eur J Hum Genet. 2011;19:389–393.
[114] Wang J et al. Atrioventricular cushion transformation is mediated by ALK2 in the developing mouse heart. Dev Biol. 2005;286:299–310.
[115] Song L, Fässler R, Mishina Y, Jiao K and Baldwin HS. Essential functions of Alk3 during AV cushion morphogenesis in mouse embryonic hearts. Dev Biol. 2007;301:276–286.
[116] Andrabi S et al. SMAD4 mutation segregating in a family with juvenile polyposis, aortopathy, and mitral valve dysfunction. Am J Med Genet A. 2011;155:1165–1169.
[117] Howe JR et al. Mutations in the SMAD4/DPC4 gene in juvenile polyposis. Science. 1998;280:1086–1088.
[118] Gallione C et al. Overlapping spectra of SMAD4 mutations in juvenile polyposis (JP) and JP–HHT syndrome. Am J Med Genet A. 2010;152A:333–339.
[119] Iyer NK, Burke CA, Leach BH and Parambil JG. SMAD4 mutation and the combined syndrome of juvenile polyposis syndrome and hereditary haemorrhagic telangiectasia. Thorax. 2010;65:745–746.
[120] Moskowitz IP et al. Cardiac-specific transcription factor genes Smad4 and Gata4 cooperatively regulate cardiac valve development. Proc Natl Acad Sci USA. 2011;108:4006–4011.
[121] van de Laar IMBH et al. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat Genet. 2011;43:121–126.
[122] Garg V et al. Mutations in NOTCH1 cause aortic valve disease. Nature. 2005;437:270–274.
[123] McBride KL et al. NOTCH1 mutations in individuals with left ventricular outflow tract malformations reduce ligand-induced signaling. Hum Mol Genet. 2008;17:2886–2893.
[124] Mohamed SA et al. Novel missense mutations (p.T596M and p.P1797H) in NOTCH1 in patients with bicuspid aortic valve. Biochem Biophys Res Commun. 2006;345:1460–1465.
[125] McKellar SH et al. Novel NOTCH1 mutations in patients with bicuspid aortic valve disease and thoracic aortic aneurysms. J Thorac Cardiovasc Surg. 2007;134:290–296.
[126] Alagille D et al. Syndromic paucity of interlobular bile ducts (Alagille syndrome or arteriohepatic dysplasia): review of 80 cases. J Pediatr. 1987;110:195–200.
[127] Krantz ID et al. Jagged1 mutations in patients ascertained with isolated congenital heart defects. Am J Med Genet. 1999;84:56–60.
[128] Benson DW et al. Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. J Clin Invest. 1999;104:1567–1573.
[129] Goldmuntz E, Geiger E and Benson DW. NKX2.5 mutations in patients with tetralogy of fallot. Circulation. 2001;104:2565–2568.
[130] McElhinney DB, Geiger E, Blinder J, Benson DW and Goldmuntz E. NKX2.5 mutations in patients with congenital heart disease. J Am Coll Cardiol. 2003;42:1650–1655.
[131] Schott JJ et al. Congenital heart disease caused by mutations in the transcription factor NKX2-5. Science. 1998;281:108–111.
[132] König K, Will JC, Berger F, Müller D and Benson DW. Familial congenital heart disease, progressive atrioventricular block and the cardiac homeobox transcription factor gene NKX2.5: identification of a novel mutation. Clin Res Cardiol. 2006;95:499–503.
[133] Garg V et al. GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5. Nature. 2003;424:443–447.
[134] Reamon-Buettner SM and Borlak J. TBX5 mutations in non-Holt–Oram syndrome (HOS) malformed hearts. Hum Mutat. 2004;24:104.
[135] Sperling S et al. Identification and functional analysis of CITED2 mutations in patients with congenital heart defects. Hum Mutat. 2005;26:575–582.
[136] Reamon-Buettner SM and Borlak J. HEY2 mutations in malformed hearts. Hum Mutat. 2006;27:118.
[137] Guo DC et al. Mutations in smooth muscle alpha-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. Nat Genet. 2007;39:1488–1493.
[138] Zhu L et al. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. Nat Genet. 2006;38:343–349.
[139] Winston JB et al. Heterogeneity of genetic modifiers ensures normal cardiac development. Circulation. 2010;121:1313–1321.
[140] Laforest B, Andelfinger G and Nemer M. Loss of Gata5 in mice leads to bicuspid aortic valve. J Clin Invest. 2011. doi:10.1172/JCI44555.
[141] Ewart AK et al. Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome. Nat Genet. 1993;5:11–16.
[142] Li DY et al. Elastin is an essential determinant of arterial morphogenesis. Nature. 1998;393:276–280.
[143] Li DY et al. Novel arterial pathology in mice and humans hemizygous for elastin. J Clin Invest. 1998;102:1783–1787.
[144] Hinton RB et al. Elastin haploinsufficiency results in progressive aortic valve malformation and latent valve disease in a mouse model. Circ Res. 2010;107:549–557.
[145] Sage H and Gray WR. Studies on the evolution of elastin–I. phylogenetic distribution. Comp Biochem Physiol B. 1979;64:313–327.
[146] Rosenquist TH, McCoy JR, Waldo KL and Kirby ML. Origin and propagation of elastogenesis in the developing cardiovascular system. Anat Rec. 1988;221:860–871.
[147] Hinton RB et al. Extracellular matrix remodeling and organization in developing and diseased aortic valves. Circ Res. 2006;98:1431–1438.
[148] Majava M et al. A report on 10 new patients with heterozygous mutations in the COL11A1 gene and a review of genotype-phenotype correlations in type XI collagenopathies. Am J Med Genet A. 2007;143:258–264.

[149] Van Camp G et al. A new autosomal recessive form of Stickler syndrome is caused by a mutation in the COL9A1 gene. Am J Hum Genet. 2006;79:449–457.
[150] Ahmad N et al. Prevalence of mitral valve prolapse in Stickler syndrome. Am J Med Genet A. 2003;116A:234–237.
[151] Byers PH, Tsipouras P, Bonadio JF, Starman BJ and Schwartz RC. Perinatal lethal osteogenesis imperfecta (OI type II): a biochemically heterogeneous disorder usually due to new mutations in the genes for type I collagen. Am J Hum Genet. 1988;42:237–248.
[152] Superti-Furga A, Gugler E, Gitzelmann R and Steinmann B. Ehlers–Danlos syndrome type IV: a multi-exon deletion in one of the two COL3A1 alleles affecting structure, stability, and processing of type III procollagen. J Biol Chem. 1988;263:6226–6232.
[153] Lincoln J, Florer JB, Deutsch GH, Wenstrup RJ and Yutzey KE. ColVa1 and ColXIa1 are required for myocardial morphogenesis and heart valve development. Dev Dyn. 2006;235:3295–3305.
[154] Liu X, Wu H, Byrne M, Krane S and Jaenisch R. Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development. Proc Natl Acad Sci USA. 1997;94:1852–1856.
[155] Lucero HA and Kagan HM. Lysyl oxidase: an oxidative enzyme and effector of cell function. Cell Mol Life Sci. 2006;63:2304–2316.
[156] Hornstra IK et al. Lysyl oxidase is required for vascular and diaphragmatic development in mice. J Biol Chem. 2003;278:14387–14393.
[157] Mäki JM et al. Inactivation of the lysyl oxidase gene Lox leads to aortic aneurysms, cardiovascular dysfunction, and perinatal death in mice. Circulation. 2002;106:2503–2509.
[158] Hurtado PA et al. Lysyl oxidase propeptide inhibits smooth muscle cell signaling and proliferation. Biochem Biophys Res Commun. 2008;366:156–161.
[159] Atsawasuwan P et al. Lysyl oxidase binds transforming growth factor-beta and regulates its signaling via amine oxidase activity. J Biol Chem. 2008;283:34229–34240.
[160] Oleggini R, Gastaldo N and Di Donato A. Regulation of elastin promoter by lysyl oxidase and growth factors: cross control of lysyl oxidase on TGF-beta1 effects. Matrix Biol. 2007;26:494–505.
[161] Giampuzzi M et al. Lysyl oxidase activates the transcription activity of human collagene III promoter. possible involvement of Ku antigen. J Biol Chem. 2000;275:36341–36349.
[162] Liu X et al. Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. Nat Genet. 2004;36:178–182.
[163] McLaughlin PJ et al. Targeted disruption of fibulin-4 abolishes elastogenesis and causes perinatal lethality in mice. Mol Cell Biol. 2006;26:1700–1709.
[164] Hirai M et al. Fibulin-5/DANCE has an elastogenic organizer activity that is abrogated by proteolytic cleavage in vivo. J Cell Biol. 2007;176:1061–1071.
[165] Hanada K et al. Perturbations of vascular homeostasis and aortic valve abnormalities in fibulin-4 deficient mice. Circulation research. 2007;01:
[166] Hucthagowder V et al. Fibulin-4: a novel gene for an autosomal recessive cutis laxa syndrome. Am J Hum Genet. 2006;78:1075–1080.
[167] Loeys B et al. Homozygosity for a missense mutation in fibulin-5 (FBLN5) results in a severe form of cutis laxa. Hum Mol Genet. 2002;11:2113–2118.
[168] Pyeritz RE and McKusick VA. The Marfan syndrome: diagnosis and management. N Engl J Med. 1979;300:772–777.
[169] Dietz HC et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature. 1991;352:337–339.
[170] Dietz HC, Loeys B, Carta L and Ramirez F. Recent progress towards a molecular understanding of Marfan syndrome. American Journal of Medical Genetics Part C: Seminars in Medical Genetics. 2005;139:4–9.
[171] Ng CM et al. TGF- β-dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. J Clin Invest. 2004;114:1586–1592.
[172] Gupta PA et al. Ten novel FBN2 mutations in congenital contractural arachnodactyly: delineation of the molecular pathogenesis and clinical phenotype. Hum Mutat. 2002;19:39–48.
[173] Putnam EA, Zhang H, Ramirez F and Milewicz DM. Fibrillin-2 (FBN2) mutations result in the Marfan-like disorder, congenital contractural arachnodactyly. Nat Genet. 1995;11:456–458.
[174] Chaudhry SS et al. Mutation of the gene encoding fibrillin-2 results in syndactyly in mice. Hum Mol Genet. 2001;10:835–843.
[175] Gansner JM, Madsen EC, Mecham RP and Gitlin JD. Essential role for fibrillin-2 in zebrafish notochord and vascular morphogenesis. Dev Dyn. 2008;237:2844–2861.
[176] Loeys BL et al. Aneurysm syndromes caused by mutations in the TGF-beta receptor. N Engl J Med. 2006;355:788–798.
[177] Attias D et al. Comparison of clinical presentations and outcomes between patients with TGFBR2 and FBN1 mutations in Marfan syndrome and related disorders. Circulation. 2009;120:2541–2549.
[178] Stheneur C et al. Identification of 23 TGFBR2 and 6 TGFBR1 gene mutations and genotype-phenotype investigations in 457 patients with Marfan syndrome type I and II, Loeys–Dietz syndrome and related disorders. Hum Mutat. 2008;29:E284–295.
[179] Kosaki K et al. Molecular pathology of Shprintzen–Goldberg syndrome. Am J Med Genet A. 2006;140:104–108. author reply 109–110.
[180] Morales J et al. Homozygous mutations in ADAMTS10 and ADAMTS17 cause lenticular myopia, ectopia lentis, glaucoma, spherophakia, and short stature. Am J Hum Genet. 2009;85:558–568.
[181] Dagoneau N et al. ADAMTS10 mutations in autosomal recessive Weill–Marchesani syndrome. Am J Hum Genet. 2004;75:801–806.
[182] Kern CB et al. Reduced versican cleavage due to Adamts9 haploinsufficiency is associated with cardiac and aortic anomalies. Matrix Biol. 2010;29:304–316.

[183] Jungers KA, Le Goff C, Somerville RPT and Apte SS. Adamts9 is widely expressed during mouse embryo development. Gene Expression Patterns. 2005;5:609–617.
[184] Carta L et al. Fibrillins 1 and 2 perform partially overlapping functions during aortic development. J Biol Chem. 2006;281:8016–8023.
[185] Ohno-Jinno A et al. Versican and fibrillin-1 form a major hyaluronan-binding complex in the ciliary body. Invest Ophthalmol Vis Sci. 2008;49:2870–2877.
[186] Camenisch TD, Biesterfeldt J, Brehm-Gibson T, Bradley J and McDonald JA. Regulation of cardiac cushion development by hyaluronan. Exp Clin Cardiol. 2001;6:4–10.
[187] Camenisch TD, Schroeder JA, Bradley J, Klewer SE and McDonald JA. Heart-valve mesenchyme formation is dependent on hyaluronan-augmented activation of ErbB2–ErbB3 receptors. Nat Med. 2002;8:850–855.
[188] Kern CB et al. Proteolytic cleavage of versican during cardiac cushion morphogenesis. Dev Dyn. 2006;235:2238–2247.
[189] Kern CB et al. Versican proteolysis mediates myocardial regression during outflow tract development. Dev Dyn. 2007;236:671–683.
[190] Azeloglu EU, Albro MB, Thimmappa VA, Ateshian GA and Costa KD. Heterogeneous transmural proteoglycan distribution provides a mechanism for regulating residual stresses in the aorta. Am J Physiol Heart Circ Physiol. 2008;294:H1197–1205.
[191] Ramirez F, Sakai LY, Rifkin DB and Dietz HC. Extracellular microfibrils in development and disease. Cell Mol Life Sci. 2007;64:2437–2446.
[192] Kenagy RD et al. Accumulation and loss of extracellular matrix during shear stress-mediated intimal growth and regression in baboon vascular grafts. J Histochem Cytochem. 2005;53:131–140.
[193] Le Goff C et al. ADAMTSL2 mutations in geleophysic dysplasia demonstrate a role for ADAMTS-like proteins in TGF-beta bioavailability regulation. Nat Genet. 2008;40:1119–1123.
[194] Le Goff C and Cormier-Daire V. Genetic and molecular aspects of acromelic dysplasia. Pediatr Endocrinol Rev. 2009;6:418–423.
[195] Allali S et al. Molecular screening of ADAMTSL2 gene in 33 patients reveals the genetic heterogeneity of geleophysic dysplasia. J Med Genet. 2011;48:417–421.
[196] Dietz HC. New therapeutic approaches to mendelian disorders. N Engl J Med. 2010;363:852–863.
[197] Brooke BS et al. Angiotensin II blockade and aortic-root dilation in Marfan's syndrome. N Engl J Med. 2008;358:2787–2795.
[198] Habashi JP et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science. 2006;312:117–121.
[199] Habashi JP et al. Angiotensin II type 2 receptor signaling attenuates aortic aneurysm in mice through ERK antagonism. Science. 2011;332:361–365.
[200] Holm TM et al. Noncanonical TGFβ signaling contributes to aortic aneurysm progression in Marfan syndrome mice. Science. 2011;332:358–361.
[201] Hirayama-Yamada K et al. Phenotypes with GATA4 or NKX2.5 mutations in familial atrial septal defect. Am J Med Genet A. 2005;135:47–52.
[202] Sarkozy A et al. Spectrum of atrial septal defects associated with mutations of NKX2.5 and GATA4 transcription factors. J Med Genet. 2005;42:e16.
[203] Reamon-Buettner SM and Borlak J. GATA4 zinc finger mutations as a molecular rationale for septation defects of the human heart. J Med Genet. 2005;42:e32.
[204] Pehlivan T et al. GATA4 haploinsufficiency in patients with interstitial deletion of chromosome region 8p23.1 and congenital heart disease. Am J Med Genet. 1999;83:201–206.
[205] Kirk EP et al. Mutations in cardiac T-box factor gene TBX20 are associated with diverse cardiac pathologies, including defects of septation and valvulogenesis and cardiomyopathy. Am J Hum Genet. 2007;81:280–291.
[206] Postma AV et al. A gain-of-function TBX5 mutation is associated with atypical Holt–Oram syndrome and paroxysmal atrial fibrillation. Circ Res. 2008;102:1433–1442.
[207] Hart AW et al. Cardiac malformations and midline skeletal defects in mice lacking filamin A. Hum Mol Genet. 2006;15:2457–2467.
[208] Kyndt F et al. Mutations in the gene encoding filamin A as a cause for familial cardiac valvular dystrophy. Circulation. 2007;115:40.
[209] Dietz HC et al. Four novel FBN1 mutations: significance for mutant transcript level and EGF-like domain calcium binding in the pathogenesis of Marfan syndrome. Genomics. 1993;17:468–475.
[210] Kojuri J, Razeghinejad MR and Aslani A. Cardiac findings in Weill–Marchesani syndrome. Am J Med Genet A. 2007;143A:2062–2064.
[211] Gould DB et al. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. Science. 2005;308:1167–1171.
[212] Richards AJ et al. Variation in the vitreous phenotype of Stickler syndrome can be caused by different amino acid substitutions in the X position of the type II collagen Gly-X-Y triple helix. Am J Hum Genet. 2000;67:1083–1094.
[213] Richardson L et al. EMAGE mouse embryo spatial gene expression database: 2010 update. Nucleic Acids Res. 2010;38:D703–709.