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 extracellular 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.
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,

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**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 into 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 — atri-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 SMADs 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.](image-url)
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 basis 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
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 Elt 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 Trunci 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 Colxa1 mutants [153]; and homozygous mutant Col3a1 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](https://example.com/figure.png)

**Figure. 4** *Gata5* is required for aortic valve formation. (A and B) Anatomic analysis reveals the presence of BAV in Tie2-cre*Gata5*+/+ 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*+/− 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 \textit{FBLN5} or \textit{FBLN4} corroborate these findings from animal models [166,167].

The best-studied model for defects in the extracellular matrix and changes in the TGF\textsubscript{β} signaling cascade is the Marfan syndrome caused by inherited or de novo mutations in \textit{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 \textit{FBN1}-deficient mice develop MVP [171]. In addition \textit{FBN2} mutations have been observed in patients with the Beal syndrome, an autosomal dominant disease with vascular anomalies including MVP [172,173].

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Two type of the Loeys–Dietz syndrome (LDS) inherited in an autosomal dominant pattern are caused by mutations in \textit{TGFBR1} also \textit{TGFBR2}. Type 1 shows arterial turttosity 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 \textit{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 \textit{FBN1} gene such as several MASS (mitral valve prolapse, upper limits of aortic root diameter, stretch marks of the skin and skeletal conditions) phenotypes. The Well–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 \textit{FBN-1} or one of the two metalloproteinases \textit{ADAMTS10} and \textit{ADAMTS17} [180,181]. No animal model has been established in WMS so far, but heterozygous \textit{Adams} 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 TGF\textsubscript{β} 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 \textit{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 \textit{ADAMTSL2} causes autosomal recessive geleophysic dysplasia (GD) going along with valvular thickening and atrial septal defects. The fact that \textit{ADAMTSL2} enhances TGF\textsubscript{β} 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).

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<tr>
<th>Gene</th>
<th>Locus</th>
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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)

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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.

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