Valvular heart disease (VHD) is a major cause of morbidity and premature death from cardiovascular diseases, making it an important clinical entity. Despite a dramatic decline in the incidence of rheumatic heart disease in industrialized countries, VHD remains highly prevalent. Although many VHDs are acquired during adult life, familial clustering and heritability have been noted for common heart valve defects, such as bicuspid aortic valve and myxomatous mitral valve prolapse, denoting an underlying genetic basis. Over the past decade, advances in our understanding of the genetic basis of familial VHD have been made through the unraveling of gene network and molecular mechanisms regulating normal valve development. Important progress has also arisen from a series of elegant studies that have focused on linkage analyses of large families with VHD, transgenic animal models, in vitro studies, and, more recently, microRNA and transcriptomic assessment of diseased tissues. Identification of the genes and molecular pathways responsible for the development of VHD has important implications in terms of improving current therapeutic strategies, as well as guiding the management of at-risk family members, with the ultimate aim to reduce the health burden of VHD. This article will summarize the current state of knowledge regarding the genetic basis of familial VHD, the review will summarize the current state of knowledge regarding the genetic basis of 2 common familial VHDs, namely mitral valve prolapse and bicuspid aortic valve, and highlight some of the recent findings that shed light on the pathogenesis of these diseases.

Valvular heart disease (VHD) is a major cause of disability, diminished quality of life, and premature death from cardiovascular disease, making it an important clinical entity. Despite a dramatic decline in the incidence of rheumatic heart disease in industrialized countries, VHD remains highly prevalent. Although many VHDs are acquired during adult life, familial clustering and heritability have been noted for common heart valve defects, such as bicuspid aortic valve (BAV) and myxomatous mitral valve prolapse (MVP), implying an underlying genetic basis. These familial VHDs can occur in isolation (nonsyndromic) or present as part of a clinical genetic syndrome, such as Marfan, Turner, and Noonan syndromes. Traditionally, well-characterized multigeneration families have been invaluable for determining the genetic basis of disease, because they are amenable to marker-based genome-wide linkage analysis. However, the identification of causal genes in VHD has been hampered by complex genetic and phenotypic heterogeneity, incomplete penetrance, and the likely contribution of genetic modifier loci. Although in vitro studies and transgenic animal models that recapitulate the human phenotype have provided insights into the genetic basis of VHD, these findings do not always translate to humans. Therefore, concurrent studies on the molecular pathways regulating valvulogenesis have sought to highlight gene networks relevant to VHD.

This review article will focus primarily on the 2 most common isolated VHDs, ie, MVP and BAV. The review will summarize the current knowledge regarding the genetic basis of MVP and BAV and highlight some of the recent findings that shed light on the pathogenesis of these diseases. Although there is much more to discover about the genetic causes of VHD, it is timely to review the current state of knowledge in these important cardiovascular genetic disorders.

Overview of Development of Cardiac Valves

A key aspect of cardiac development is the formation and function of the cardiac valves. Cardiac valve development begins immediately after cardiac looping, at embryonic days 31 to 35 in humans, with the formation of endocardial cushions in the atrioventricular canal and outflow tract. Normal uridine diphosphate glucose dehydrogenase gene activity to synthesize glycosaminoglycan is critical for the cardiac cushion formation. The mesenchymal progenitor cells migrate into the intervening cardiac jelly and proliferate to form swellings of valve primordial, which eventually give rise to valvuloseptal structures and adult valvular interstitial cells. The mesenchymal progenitor cells migrate into the intervening cardiac jelly and proliferate to form swellings of valve primordial, which eventually give rise to valvuloseptal structures and adult valvular interstitial cells. The atrophicventricular cushions contribute to atrioventricular (mitral and tricuspid) valve leaflets, whereas the outflow tract cushions contribute to...
semilunar (aortic and pulmonary) valve leaflets. The development of the outflow tract and semilunar valve is complex and involves coordinated interactions between the endocardial-derived mesenchyme, cardiac, and smooth muscle progenitors from the anterior or secondary heart field and the cardiac neural crest. Whereas the secondary heart field progenitor cells contribute to the outflow tract myocardium, a population of neural crest-derived cells migrate into the distal (truncal) part of the outflow tract cushions, which subsequently divide the outflow tract into aortic and pulmonary trunks, and differentiate into the vascular smooth muscle layer of the aortic arch. A number of signaling pathways including Wnt/β-catenin, NOTCH, transforming growth factor β receptor (TGF-β), BMP, vascular endothelial growth factor, NFATc1 and MAPK, and transcription factors, including Twist1, Tbx20, Msx1/2, and Sox9, are active during this early stage of valvulogenesis. These pathways are collectively important in the regulation of cell migration, proliferation, and extracellular matrix expression in the developing valves.

Later in embryonic valve development, cell proliferation decreases and the fused endocardial cushions remodel and thin out to form mature valve leaflets, which are characterized by increasing complexity and organization of the extracellular matrix, and compartmentalization of valvular interstitial cells. The molecular pathway that regulates this later stage of valvulogenesis is less well understood, but it involves periostin, a component of the extracellular matrix protein. The periostin gene has been identified as a bifunctional matrix protein. The periostin gene has been identified as a component of the extracellular matrix protein.

The process of valve compartmentalization and remodeling occurs at late gestation and continues into postnatal life, suggesting that mechanisms of prenatal valve development persist during postnatal valve growth and maintenance. Furthermore, evidence suggests that developmental signaling pathways are shared between heart valves and the precursors of cartilage, tendon, and bone, playing a critical role at this later stage of valve maturation/remodeling. These pathways include BMP-2–mediated induction of Sox9 and aggrecan, receptor activator of NFκB ligand–mediated induction of NFATc1, and FGF4-mediated induction of scleraxis and tenascin.

Cardiac valve development during embryogenesis is therefore complex, with an integrated mechanism of genetic factors, activation of signaling pathways, as well as external environmental factors, such as the physical forces of blood flow. Because many of the signaling pathways and transcription factors responsible for valve development are reactivated in pediatric and adult valve diseases, the insights gained from understanding normal valvulogenesis have provided a platform to identify potential candidate genes involved in familial VHDs.

**Mitrail Valve Prolapse**

**Clinical Significance**

MVP is a common disorder, which affects 2.4% of the general population and exhibits strong heritability. Because MVP is defined by the abnormal relationship of mitral leaflets to their surrounding structures, echocardiography has become a diagnostic standard to confirm its presence. In a pivotal 3D echocardiographic study in the late 1980s, Levine et al demonstrated that the mitral annulus was in fact saddle shaped, with the most superior aspects positioned anteriorly and posteriorly and the most inferior aspects positioned medially and laterally. This improved understanding of normal mitral valve anatomy has greatly enhanced the diagnostic specificity for MVP without loss of sensitivity. Because successful genetic studies rely on accurate phenotypic definition, the increased diagnostic specificity for MVP has been central in enabling the advances to be made in MVP genetics over the past few years.

MVP is characterized by fibromyxomatous degeneration of the mitral valve, leading to progressive thickening and expansion of the leaflet(s) and lengthening of the chordae tendineae, which results in superior displacement of the leaflet(s) into the left atrium during systole. The clinical phenotype of MVP is widely heterogeneous, ranging from a benign clinical course with normal life expectancy to adverse outcomes with significant morbidity and mortality resulting from the development of valvular insufficiency. Although most patients are asymptomatic, up to 13% of affected subjects develop serious MVP-related complications, attributed to significant mitral regurgitation, congestive heart failure, infective endocarditis, arrhythmias, and, in worst cases, sudden cardiac death. MVP is the leading cause of isolated mitral regurgitation, requiring surgical intervention in developing nations.

**Pathogenesis of MVP**

Histological examination of myxomatous MVP leaflets characteristically demonstrates activated interstitial myofibroblast-like cells, disorganized fragmentation of collagen and elastin fibers, and expansion of the spongiosa layer that have resulted from the accumulation of proteoglycans and glycosaminoglycans, which extend into the load-bearing fibrosa. This alteration of extracellular matrix synthesis and maladaptive remodeling by activated valvular interstitial cells result in a disruption in the mechanical integrity of the leaflet(s) which, together with normal wear and tear, leads to leaflet(s) stretching and expansion.

Although the pathological features of MVP are well known, the precise cellular and molecular mechanisms that contribute to the development of MVP are less clear. Isolated MVP is absent in newborn babies, suggesting that it may develop from a combination of genetic variation with age-dependent penetrance, postnatal disruption of cellular signaling, and environmental factors, including repetitive mechanical stress from normal physiological wear and tear (Figure 1). It is postulated that hemodynamic shear...
stress and impact-induced damage to the superficial lining layer of valvular endothelial cells leads to the release of proinflammatory cytokines, such as TGF-β and vasoactive substance; eg, endothelin-1 and prostanoids.\textsuperscript{11,32–35} This then activates the residing valvular interstitial cells, which transform into myofibroblast-like cells and secrete excessive levels of catabolic enzymes, including the collagenases MMP-1 and MMP-13, the gelatinases MMP-2 and MMP-9, cysteine proteases cathepsin C and M, and interleukin-1β, a cytokine that induces the secretion of proteolytic enzymes.\textsuperscript{34} Moreover, a proportion of valvular interstitial cells transform toward a hyperplastic CD34+ fibrocyte phenotype, which synthesizes MMP-9 and collagen-III, and seem to take part in leaflet remodeling (Figure 2).\textsuperscript{31,36} Together, these changes alter the metabolism and composition of the extracellular matrix and, although most pronounced in the spongiosa layer, also affect the fibrosa backbone layer, hence compromising the leaflet structural integrity and biomechanics. Ultimately, remodeling of the extracellular matrix and excessive glycosaminoglycan and water accumulation, give the diseased leaflets their classical myxomatous appearance.\textsuperscript{22}

**Genetic Basis of MVP**

MVP can be sporadic, familial, or occur in the context of a syndrome, the latter occurring as part of heritable connective tissue disorders, which are often attributed to specific mutations in extracellular matrix genes. A summary of the genetic factors in MVP is provided in Table 1.

Mutations in fibrillin-1 (\textit{FBN-1}) and, less commonly, in TGF-β receptor-2 (\textit{TGFBR2}), cause Marfan syndrome Type I and Type II, respectively, in which MVP is common. Advances in understanding MVP pathogenesis have been made using an \textit{FBN1}-deficient mouse model that recapitulates human Marfan syndrome in a landmark study by Dietz and colleagues.\textsuperscript{32} Fibrillin-1 is a major structural component of the extracellular matrix microfibrils and a key regulator of TGF-β availability.\textsuperscript{37} TGF-β isoforms are synthesized in the intracellular compartment as large precursors that are cleaved into mature TGF-β and its propeptide, called latency-associated peptide, which are covalently bound to form inactive small latent complexes.\textsuperscript{38} This complex remains intracellular until it is bound
to latent TGF-β-binding protein, forming a large latent complex. Fibrillin-1 has been shown to bind to latent TGF-β-binding protein and the large latent complex, thus allowing fine control of TGF-β levels and activity by sequestering biologically active TGF-β into the extracellular matrix. Therefore, deficiency of fibrillin-1 results in increased TGF-β signaling within the extracellular matrix, as demonstrated by the increased levels of phosphorylated Smad2 and the expression of TGF-β-responsive extracellular matrix genes, such as Tgfb1, endothelin-1 (Edn1), and tissue inhibitor of metalloproteinases1 (Timp1). It is remarkable that TGF-β antagonism in vivo using neutralizing antibodies was able to rescue the valve phenotype of FBN1-deficient mice. Furthermore, Losartan, an angiotensin II type 1 receptor antagonist, prevents and possibly reverses the aortic root dilatation and MVP in mice with Marfan syndrome, probably by decreasing TGF-β-mediated ERK1/2 activation, a principal effector of disease in FBN1-deficient mice. These insightful findings suggest that myxomatous MVP may result from dysregulation of conserved signaling pathways and be amenable to therapeutic intervention. Although linkage between FBN1 and several of the collagen genes have not been demonstrated in autosomal dominant MVP, the established role of TGF-β in the pathogenesis of MVP in Marfan syndrome suggests that it may be relevant in nonsyndromic forms of MVP.

The contribution of genetic factors in nonsyndromic MVP is supported by family studies, which indicate genetic heterogeneity with autosomal dominant and X-linked modes of inheritance. Incomplete penetrance with age- and sex-dependent expressivity further contributes to the striking clinical heterogeneity even within the same family. X-linked myxomatous valvular dystrophy is a rare form of familial multivalvular myxomatous degeneration caused by mutations in the filamin A (FLNA) gene located on chromosome Xq28, in which MVP is a frequent manifestation. FLNA encodes a large cytoskeletal actin-binding protein that directly coordinates the localization and activation of Smad proteins, especially Smad2, and serves as a positive regulator of TGF-β signaling. Although defective Smad signaling caused by FLNA mutation has not been shown, understanding the role of FLNA in modulating TGF-β signaling has provided another clue toward an improved understanding of myxomatous MVP pathogenesis. Furthermore, genome-wide linkage analyses in large families have mapped MVP loci to chromosomes 16p11.2-p12.1 (MMVP1), 11p15.4 (MMVP2), and 13q31.3–31.2 (MMVP3), although the causative genes remain to be identified.

In sporadic MVP, where family studies are not informative, associations with single-nucleotide polymorphisms in genes implicated in extracellular matrix remodeling, collagen metabolism, and the renin–angiotensin–aldosterone system (RAAS) have been examined (Table 1). The association of sporadic MVP with the G/G genotype of a polymorphism in exon 31 of the collagen IIIa1 gene (COL3A1) and 3 polymorphisms in the FBN1 gene have been reported. However, the generalizability of these results is restricted by the small number of patients, and is in contrast with the previously mentioned linkage analyses on several pedigrees that showed no linkage of these genes to autosomal dominant MVP. A polymorphism in the 3’ untranslated region of the urokinase–plasminogen activator gene, which plays a role in the pathogenesis of elastin and collagen degradation in arterial aneurysms, was also reportedly associated with MVP. It is interesting that in another study of MVP patients, an MMP-3 promoter haplotype-type 1612 5A/5A polymorphism was associated with severe mitral regurgitation and more pronounced left ventricular remodeling.
that the MMP-3 promoter 5A/5A polymorphism may result in increased MMP-3 expression in the myocardium, which then accentuates the adaptive response of the myocardium to the volume overload of mitral regurgitation and hence more adverse disease course. This suggests that genetic variation influences the disease course in MVP.

A subset of MVP patients present with a variety of symptoms that seem to be independent from their underlying valve disease, including chest pain, dyspnea, dysrhythmia, anxiety, and syncope, and this is collectively termed MVP syndrome. The misperception that these symptoms frequently occur concomitantly with MVP has led to the practice of obtaining screening echocardiograms on patients with atypical or nonspecific cardiovascular symptoms. Thus, MVP syndrome may be overdiagnosed as a result of 2 common things occurring together, without necessarily bearing any pathophysiological relationship. It is interesting that perturbations in autonomic, neuroendocrine, and renin–angiotensin–aldosterone system regulation have been reported among symptomatic patients with MVP. A number of studies have subsequently demonstrated genetic association between MVP syndrome and the components of the renin–angiotensin–aldosterone system regulation have been reported among symptomatic patients with MVP. A number of studies have subsequently demonstrated genetic association between MVP syndrome and the components of the renin–angiotensin–aldosterone system, namely an angiotensin I–converting enzyme insertion/deletion polymorphism in intron 16, the TT genotype of the angiotensinogen Met235Thr polymorphism, and the C allele of the angiotensin II type 1 receptor A1166C polymorphism, although these studies are limited by the relatively small cohort of patients with MVP syndrome.

Table 1. Summary of Gene Anomalies and Polymorphisms Associated With the Development of Mitral Valve Prolapse

<table>
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<th>Genetic Anomalies and Polymorphisms Associated With MVP Development</th>
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<td>Stickler syndrome</td>
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<td>Collagen type II and XI mutations</td>
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<td></td>
<td>Collagen type I mutation</td>
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<td>Pseudoxanthoma elasticum</td>
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<td>ATP-binding cassette protein ABC26 mutation</td>
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MVP indicates mitral valve prolapse; FBN, fibrillin; MMP, matrix metalloproteinases; CFA, canine chromosome; MMVP, myxomatous mitral valve prolapse; Chr, chromosome; PLAU, urokinase–plasminogen activator; ACE-I, angiotensin-converting enzyme inhibitor; and TGF[R], transforming growth factor [R] receptor.
However, further studies of asymptomatic patients with MVP have failed to show evidence of abnormal autonomic or neuroendocrine function, either at rest or during tilt testing. Therefore, although abnormal autonomic function might be responsible for symptoms in some patients with MVP, it remains unclear whether their MVP is directly related or incidental.

Furthermore, studies in animal models have also highlighted genetic factors involved in MVP pathogenesis. Proteoglycan accumulation is a hallmark of MVP, and mice haploinsufficient for the versican-specific protease, Adamts9, display abnormal valve morphogenesis and subsequently develop myxomatous mitral valve leaflets during adulthood as a result of versican accumulation. In addition, enzymes that degrade the extracellular matrix, such as the gelatinase MMP-2, may mediate myxomatous degeneration of the mitral valve leaflet, and the cardiac-specific transgenic expression of ADAMTS-1 in mice reproduces many of the pathological features of MVP.

Recently, gene expression microarray technology was used to gain a more unbiased, global view of the genetic signature of prolapsed mitral valve tissue from patients with idiopathic MVP, compared with control valve tissue from transplant donors. The study identified decreased expression of the metallothioneins 1 and 2 (MT1/2), which protect against oxidative stress, and ADAMTS-1, an abundant aggrecanase in the mitral valve leaflets that is implicated in proteoglycan degradation. Subsequent in vitro silencing of the expression of metallothioneins 1 and 2 in valvular interstitial cells culture resulted in the upregulation of TGF-β2 activity and TGF-β2 secretion, with consequent downregulation of ADAMTS-1, leading to versican accumulation and remodeling of the extracellular matrix, recapitulating the features of human MVP.

Collectively, these findings provide an insight into the genetic basis of MVP, which is complex, highly heterogeneous, and most likely involves causative gene mutations with genetic modifier loci that influence the disease course.

**Bicuspid Aortic Valve**

**Clinical Significance**

BAV occurs when the aortic valve has 2 cusps, rather than 3, and represents the most common form of congenital cardiac malformation, affecting ≈1.4% of the general population. BAV has a male predominance of ≈3:1, and at least 35% of those affected develop serious complications including aortic stenosis and regurgitation requiring valve replacement, endocarditis, ascending aortic aneurysm, and dissection. BAV underlies 70% to 85% of stenotic aortic valve in children and at least 50% of aortic stenosis in adults. Furthermore, BAV carries an 8-fold increased risk of aortic dissection, and over a 25-year period, the risk of valve replacement is 53%, aneurysm formation is 26%, and aortic surgery is 25%. As such, BAV represents a greater burden of disease than all other congenital heart diseases combined.

Disorders of aortic valvulogenesis tend to be regarded in the context of a phenotypic continuum, ranging from aortic valves with a single leaflet to those with 4 leaflets. Within this continuum, BAV exists and demonstrates a wide morphological variation, as seen in human cases of BAV (Figure 3) and observed in BAV of Syrian hamsters. BAV morphological phenotypes range from the typical form with 2 unequal-sized leaflets and the larger leaflet having a central raphe that has resulted from commissural fusion (ie, functionally bicuspid), to the less common form with 2 approximately symmetrical leaflets without a raphe (ie, anatomically bicuspid). Fusion of the right and left coronary cusps is the most common pattern of BAV and is associated with the coarctation of the aorta, whereas the fusion of the right and noncoronary cusps, the second commonest form, is associated with more cuspal pathology.

Like MVP, BAV can occur sporadically as an isolated birth defect, can be familial, and can occur as part of a syndrome with more global clinical manifestations, such as Turner, Williams-Beuren, and Andersen syndromes. Furthermore, BAV is also recognized to coexist with other congenital cardiovascular malformations, most commonly

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**Figure 3.** Different phenotypic spectrum of BAV morphology. **A,** Trileaflet aortic valve with complete fusion of the right and left (R-L) coronary cusps; **B,** Trileaflet aortic valve with complete fusion of the R-L coronary cusps and partial fusion of the right and noncoronary cusps; **C** and **D** Bileaflet aortic valve with extensive raphe in the fused leaflet; **E,** Bileaflet aortic valve with a vestigial raphe; **F,** True bicuspid aortic valve without raphe. Figures **A** and **B** are classified as functionally bicuspid and the remaining are classified as anatomically true bicuspid.
with coarctation of the aorta, and with cardiac septal defects or the genetically related hypoplastic left heart syndrome. This suggests that BAV is not only a disorder of valvulogenesis, but it also represents a more complex coexistent genetic disease of the aorta and cardiac development.

**Pathogenesis of BAV**

Despite its high prevalence, the pathogenesis of BAV is largely undetermined, although gene mutations leading to alterations in cell migration and signal transduction, in conjunction with nongenetic factors such as blood flow during valvulogenesis, may contribute to its formation prenatally. Because the relationship of the individual valve cusps to specific endocardial cushion progenitors is not known, BAV could result from a failure of separation of primordial cusps. Meanwhile, the pathogenesis of aortic valve dysfunction in BAV is thought to be the result of dysregulation in the complex molecular hierarchies controlling late valve development, which continues into the postnatal life and results in the chronic process of valve tissue remodeling, leading to abnormal leaflet architecture, valvaral thickening, and abnormal biomechanics (Figure 4). With increasing age, BAV leaflets are prone to develop premature fibrosis and calcification in response to hemodynamic shear stress, an active process that involves inflammation, endothelial dysfunction, and lipoprotein and calcium deposition, and results in the ossification of the leaflets and valvular stenosis. Histological examination of BAV tissue from pediatric patients revealed excessive extracellular matrix production and trilaminar matrix disorganization, with fragmentation and reduction in elastin content, and accumulation of collagen and proteoglycan. BAV leaflets also show valvular interstitial cell disarray and varying degrees of inflammatory cell infiltrates, which secrete proteolytic enzymes implicated in collagen and elastin degradation in abdominal aortic aneurysm, such as MMP-2 and MMP-9. Supporting this, tissue microarray and immunohistochemical analysis of BAV leaflets demonstrated higher levels of MMP-2 and MMP-9, and lower level of the MMP inhibitor, TIMP1. The increased MMP activity extends to the aortic anulus and ascending aorta and may contribute to the related aortopathy.

Over 50% of individuals with BAV also present with dilatation of the proximal aorta. Some controversies exist in the literature regarding the underlying pathogenesis of BAV-related aortopathy. These are of major clinical relevance because they will have an influence on the surgical management of the valve and the ascending aorta. One proposition is that aortic dilatation in BAV is a hemodynamic phenomenon, because the structurally defective valve alters blood flow within the aortic root, producing abnormal biomechanics inside the proximal aorta. The resulting shear stress and friction on the aortic endothelium subsequently lead to increased expression of MMPs and growth factors that regulate matrix degradation and vascular smooth muscle apoptosis. This proposition is supported by the increased expression of MMP-2 and a higher ratio of MMP-2 to TIMP1 activity in the tissue samples from aortic aneurysms in BAV patients. An alternate proposition is based on the observation that aortic dilatation can occur in patients with normally functioning BAV or after valve replacement. Moreover, an inherent structural alteration has also been described in the ascending aorta of BAV patients. The dilated aortas of BAV patients demonstrate degeneration of the aortic media with focal matrix disruption, fragmented elastic lamellae, and higher rates of vascular smooth muscle cell loss, which is also present in the no dilated aortas of BAV patients. This cell loss is similar to that observed in the aortas of Marfan syndrome patients, which may involve...

**Figure 4.** Proposed pathogenetic mechanism of bicuspid aortic valve (BAV). Although BAV is largely genetically determined, both environmental and stochastic factors play a contributory role in the determination of BAV morphogenesis and later manifestation of valve dysfunction and complication. It is likely that different BAV morphology have different etiologic entities. R-N indicates right and noncoronary; R-L, right and left coronary; L-N, left and noncoronary; A-P, anterior to posterior; and miRNA, microRNA.
the Bcl-2 mediator of apoptosis. Deficiency of fibrillin-1 has also been reported in BAV aortic tissue, and this may result in increased TGF-β signaling, as seen in Marfan syndrome. This suggests an underlying genetic defect in the pathogenesis of aortic dilatation in BAV, although both genetic and hemodynamic factors likely contribute to the development of osteopathy and subsequent disease progression.

**Genetic Basis of BAV**

Familial clustering demonstrates that BAV is heritable, with 9% prevalence in first-degree relatives of patients with BAV, and up to 24% in families with >1 person affected. Table 2 summarizes the key genetic findings in BAV identified to date. Genetic heterogeneity in BAV is demonstrated by the involvement of mutations in diverse genes encoding transcription factors, extracellular matrix proteins, and signaling pathways that regulate cell proliferation, differentiation, adhesion, or apoptosis.

To date, only the transcriptional regulator *NOTCH1* gene at chromosome 9q34.3 has been linked to the development and calcification process of nonsyndromic BAV in humans, in a limited number of familial cases and ~4% of sporadic cases. The Notch signaling pathway is highly conserved and plays a critical role in cell fate determination and differentiation during organogenesis. *NOTCH1* transcripts are abundant in the mesenchyme of the outflow tract and the developing aortic valve leaflets, which probably underlie the role of notch signaling in aortic valve development. There are 4 notch proteins in mammals (*NOTCH1*–4), which are large single-pass trans membrane receptors that are activated upon binding with legends expressed on neighboring cells, e.g., Delta-like proteins (DLL1, DLL3, and DLL4) and jagned proteins (JAG1 and JAG2). The ligand–receptor interaction results in 2 independent proteolytic cleavages of the notch receptor, first, by a metalloprotease followed by a presenilin, which releases the notch intracellular domain. The notch intracellular domain then translocates to the nucleus where it, together with its cofactors, activates transcription of downstream target genes, including members of the hairy/ enhancer of split (Hes/Hey) family of transcription factors. *NOTCH1* signaling also represses MMP-2 and the downstream osteogenic gene, *RUNX2*, a central transcriptional regulator of osteoblast cell fate. Recently, *NOTCH1* signaling has been shown to regulate the expression of Sox9, a key chondrogenic transcription factor and regulator of extracellular matrix genes that is required for normal valve development and maintenance. Although inhibition of *NOTCH1* signaling downregulates Sox9 expression and promotes valvular calcification in vitro, overexpression of Sox9 markedly attenuates the calcific process that occurs with *NOTCH1* inhibition. *NOTCH1* mutations are therefore associated with both defective development of the aortic valve and, later, derepression of an osteoblast gene program and dysregulation of valvular extracellular matrix genes, via a Sox9-dependent mechanism, leading to accelerated aortic valve calcification.

Further evidence of specific gene mutations as a cause of BAV has come from genome-wide marker-based linkage analyses. Families showing autosomal dominant inheritance of BAV and associated cardiovascular malformations have shown linkage to chromosomes 18q, 5q, and 13q, whereas families with BAV and ascending aortic aneurysms have shown linkage to chromosome 15q25-26. The causal gene(s), however, are yet to be identified. Furthermore, mutations in *ACTA2* gene on chromosome 10q, which encode smooth muscle α-actin, cause thoracic aortic aneurysm and, in some instances, BAV. It has been suggested that the different phenotypes of BAV-associated aortopathy may be caused by unique pathogenetic mechanisms. A missense mutation in the *TGFB2* gene, c.1159G>A, which destabilizes the protein structure, was segregated in a family with nonsyndromic BAV with proximal aortic involvement, although the true incidence of *TGFB2* mutation in the overall BAV population is probably very low. Associations have also been reported between aneurysm risks in BAV patients with polymorphisms in *eNOS*, angiotensin I-converting enzyme, and *MMP-9* and *MMP-2* genes. Furthermore, downregulation of the ubiquitin fusion degradation 1–like gene, which is highly expressed in the cardiac outflow tract during embryogenesis, has also been noted in BAV, and may represent a candidate gene.

In addition to the above studies in humans, animal studies have shed light on the genetic basis of BAV. In mice, homozgyous deletion of the endothelial NO (eNOS) synthase gene

| Table 2. Gene Mutations in Human and Animal Models of Bicuspid Aortic Valve |
|---------------------------------|----------------------------------|
| **Human**                       | **Mouse**                        |
| Nonsyndromic (familial and sporadic) BAV:                         |                                   |
| *NOTCH1* missense and frame shift mutations                        | NOS3 (eNOS) deficiency           |
| Linkage to locus on Chr 5q, 13q, 18q                                | Nkx 2.5 haploinsufficiency        |
| GATA5 missense mutations                                            | Targeted deletion of GATA5       |
| **Syndromic BAV or BAV with other cardiovascular malformation:**   |                                   |
| Andersen syndrome—*KCNJ2* mutation (Chr 17q)                        |                                   |
| Thoracic aortic aneurysm and dissection syndrome (TAAD)—*ACTA2* mutation (Chr 10q) |                                   |
| Marfan syndrome—Fibrillin1 mutation (Chr 15q)                      |                                   |
| William Beuren syndrome—Elastin mutation (Chr 7q)                  |                                   |
| Turner syndrome (45X0 karyotype)                                   |                                   |

BAV indicates bicuspid aortic valve; eNOS, endothelial NO synthase; ACTA2, α-actin-2; and Chr, chromosome.
and haploinsufficiency in the cardiac homeobox gene Nkx2-5, are associated with a higher incidence of BA V.\(^{62,8}\) However, neither of these genes have been associated with BA V in humans.\(^{84,85}\) Furthermore, targeted deletion of the transcription factor Gata5 in mice leads to partially penetrant BA V with fusion of the right and noncoronary cusps.\(^{84}\) An essential role of GATA5 in cardiac morphogenesis and aortic valve development has only recently been reported, whereby it regulates several pathways involved in endocardial cell differentiation, including those directed by Bmp4, Tbx20, and, notably, eNOS and NOTCH1.\(^{84}\) Downregulation of eNOS expression and attenuation of the notch pathway are observed in Gata5\(^{−/−}\)mice,\(^{84}\) which may underlie the development of BA V as mutations in eNOS, and NOTCH1 are associated with BA V in mice and humans, respectively. It is interesting that a number of rare nonsynonymous variations within the highly conserved and functionally important GATA5 transcriptional activation domains have recently been reported in up to 4% of sporadic cases of human BA V.\(^{86}\) This provides support for the role of GATA5 as a potential candidate gene in modulating the pathogenesis of BA V disease in humans.

Animal studies are also at the forefront of research into the hypothesis that the different BA V morphologies have a different etiologic basis. Gata5\(^{−/−}\) mice, eNOS knockout mice, and inbred Syrian hamsters each display a high incidence of BA V, but with different valve morphologies. BA V with fusion of the right coronary and noncoronary leaflets may result from morphogenetic defects that happen before outflow tract septation, which probably relies on an exacerbated NO-dependent endothelial-to-mesenchymal transformation. BA V with fusion of right and left coronary leaflets may result from the anomalous septation of the proximal portion of the outflow tract, because of the distorted behavior of neural crest cells (Figure 4).\(^{62}\) This anomalous behavior of the cardiac neural crest is believed to explain the association of the right and left coronary leaflet fusion BA V morphology with coarctation of the aorta and the more pronounced aortic wall degeneration, compared with BA V with fusion of the right and noncoronary leaflets.\(^{62}\)

It is interesting that Sans-Coma et al\(^{87}\) recently demonstrated that BA V in Syrian hamsters express a quantitative trait, subject to polygenic inheritance, with reduced penetrance and variable expressivity. Furthermore, examination of aortic valve morphology in virtually isogenic Syrian hamsters produced by systematic inbreeding through full-sib mating, with the probability of homozygosity being 0.999 or higher, demonstrated the presence of BA V in up to five sixths of the hamsters studied, whereas the remaining one sixth had a trileaflet aortic valve. No significant association was noted between the valvular phenotype in the parents and the offspring produced by crossing genetically alike hamsters, with the probability of homozygosity of at least 0.989. This suggests that a single underlying genotype may predispose to BA V and account for the whole range of valve morphology, and implies that nongenetic factors are acting during embryonic life, creating “developmental noise,” to influence the definitive anatomic configuration of the valve.\(^{87}\) This finding supports a complex inheritance pattern, as seen in human BA V, and may explain the relatively low recurrence rate of BA V in first-degree relatives, despite its common prevalence within the general population.\(^{87}\) The findings also provide a potential explanation regarding aortic valve anatomy in monozygotic twins where BA V was present in 1 twin, but tricuspid aortic valve in the other.\(^{88}\)

Although specific gene mutations can cause BA V in mice and humans, the variability in BA V morphology and its disease manifestations cannot be entirely explained. MicroRNAs (miRNAs) are a class of evolutionarily conserved small, noncoding RNAs that regulate gene expression in development and disease. Reduced expression of miR-26a, miR-30b, and miR-195 in stenotic BA V associated with calcification, compared with a regurgitant phenotype, has been reported.\(^{89}\) In vitro transfection of each of these miRNAs into human aortic valvular interstitial cells indirectly modulated the expression of several calcification-related genes, including RUNX2, BMP-2, alkaline phosphatase (ALPL), SMAD1, and SMAD3.\(^{89}\) Patients with BA V-associated aortic stenosis have also been shown to have attenuated the expression of miRNA-141, a repressor of BMP-2.\(^{90}\) Together, these studies suggest that miRNA dysregulation contributes to aortic stenosis in BA V, and that miRNAs represent a potential new therapeutic target to limit progressive aortic valve calcification and dysfunction.

Most recently, global gene expression levels have been compared in aortic tissue from patients with a bicuspid or tricuspid aortic valve, with and without thoracic aortic aneurysm.\(^{91}\) Only 7 genes were differentially expressed between the nondilated BA V and nondilated tricuspid groups; the 4 upregulated genes were: left–right determination factor 2, a TGF-β family member; Fraser syndrome 1, a member of the extracellular matrix family of protein; Src homology 2 domain containing family, member 4;and death-associated protein kinase 3, a proapoptotic gene; and the 3 downregulated genes were: VEGFC (a member of the vascular endothelial growth factor family); NFASC (neurofascin homolog, a member of the L1 family of cell adhesion molecule); and LSP1 (lymphocyte-specific protein 1). Given the possible shared genetic underpinnings of BA V and related aortopathy, an investigation of these genes in BA V is warranted. The study also showed that immune responsive genes were upregulated in patients with tricuspid valve and with a dilated aorta, compared with those with a dilated aorta and BA V, suggesting that immune response genes are not directly involved in BA V-related aneurysms. Rather, BA V patients with aortic dilation showed an almost exclusive expression of the TGF-β-binding proteins LTBP3/4, ADAMTS11, and an alternatively spliced isoform of fibronectin-1 (FN1).\(^{91}\)

Collectively, these findings support a genetic basis for BA V, the pathogenesis of which involves a complex interplay between specific gene mutations, environmental influences, and stochastic factors. The balance of this interaction is likely to have major influence on disease progression and severity, including valve dysfunction and aortic complications.
Conclusions and Future Directions

Current evidence points to heritable and familial VHD being a final common pathway for a wide variety of altered molecular and genetic defects. Determination of the genetic basis underlying these disorders is complex. Not only that there are large number of genes involved in valvulogenesis (genetic heterogeneity) but epigenetic, environmental, and stochastic factors are also important in modulating phenotype expression and thus contributing to the wide spectrum of disease manifestations (clinical heterogeneity). Recent advances in the fields of valve development and familial VHD, combined with major developments in genetic analysis technologies, place the field at the precipice of accelerated discovery into the causative and pathogenetic mechanisms responsible for common heart valve defects.

Furthermore, the present limitation of pharmacological therapy in delaying or stopping disease progression has left surgical therapies as the most effective cure for many cases of familial VHD. Improved understanding of the genetic basis, molecular pathways, and cellular mediators involved in the pathogenesis of VHD will create great opportunities for the development of novel pharmacological treatment and prevention strategies in VHD. This knowledge has the potential to also be used to stratify treatment modalities, time surgical intervention, and guide management of at-risk family members, in terms of diagnostic, therapeutic, and prevention strategies, with the overall aim of reducing the health burden of VHD. With the advancement of genetic technologies, the increasing availability of next-generation sequencing, coupled with well-characterized families with VHD, we are hopeful that many of the key genes and elusive pathways in VHD will be identified in coming years.

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Disclosures

None.

References


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