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Genetics in Bicuspid Aortic Valve Disease: Where Are We?

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Abstract

Bicuspid aortic valve (BAV) is the most common congenital heart defect, found in up to 2% of the population and associated with a 30% lifetime risk of complications. BAV is inherited as an autosomal dominant trait with incomplete penetrance and variable expressivity due to a complex genetic architecture that involves many interacting genes. In this review, we highlight the current state of knowledge about BAV genetics, principles and methods for BAV gene discovery, clinical applications of BAV genetics, and important future directions.

Keywords

bicuspid aortic valve; genetics; aortic aneurysm; congenital heart disease

INTRODUCTION

Bicuspid aortic valve (BAV) is a heterogeneous disorder that is primarily inherited in an autosomal dominant pattern with incomplete penetrance and variable expressivity[1, 2]. BAV is a feature of some genetic syndromes, including Turner syndrome and Loeys-Dietz syndrome, as well as complex congenital heart defects that disrupt the left ventricular outflow tract (LVOT). In non-syndromic cases, BAV inheritance is best explained by a complex genetic architecture involving many different interacting genes. Thus, fully elucidating the genetic and epigenetic networks involved in the complex pathophysiology of BAV will be crucial for the development of personalized risk stratification approaches[3]. In this review we highlight the current state of knowledge about BAV genetics, principles and methods for BAV gene discovery, clinical applications, and potential future directions.

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GENETIC SYNDROMES WITH BAV

1. Turner syndrome

Turner syndrome (TS) features the highest prevalence of BAV among all genetic syndromes (Table 1)[4]. TS is caused by partial or complete absence of one X chromosome in women. BAV appears in more than 30% of women with TS, and the prevalence of associated coarctation of the aorta (CoA) and thoracic aortic aneurysm (TAA) exceeds non-syndromic BAV cases[5]. In combination with the higher prevalence of BAV in 46,X,Y men (3:1), this observation led to the hypothesis that copy number reduction of X chromosome genes may predispose to BAV formation[2, 6, 7]. Specific dosage-sensitive genes on the short arm of the X chromosome, including *KDM6A* and *TIMP1*, were implicated by recent genetic studies[8, 9]. In addition, genetic and non-genetic modifiers appear to influence the prevalence of BAV in TS. Impaired fetal thoracic lymphatic drainage causing LVOT obstruction during cardiac development may predispose individuals with TS and a history of central lymphedema to BAV and anomalous pulmonary venous drainage[10]. Potential autosomal genetic modifiers include polymorphic genomic duplications of 12p13.31 (*SLC2A3*, *SLC2A14*, *NANOGP1*) and rare deleterious variants of the *TIMP3* gene in 22q, which are significantly associated with BAV, CoA and aortic dissections in TS, independently of karyotype[7]. These data imply that copy variation of X chromosome gene(s) and other genetic variants may interact to modify susceptibility to BAV.

2. Loeys-Dietz syndrome

Approximately 10% of patients with Loeys-Dietz syndrome (LDS) have BAV, making it the second most highly associated genetic syndrome after TS[11]. LDS is caused by dominantly inherited mutations of TGF-β pathway genes, including ligands (*TGFB2* and *TGFB3*), receptors (*TGFBR1* and *TGFBR2*) and downstream effectors (*SMAD3*), Pathogenic mutations in *TGFBR1* or *TGFBR2* are also rare causes of heritable non-syndromic thoracic aortic aneurysms and dissections[12–18]. While there are no specific anatomic features that distinguish BAV in LDS from BAV in non-syndromic cases, valvular disease tends to manifest at younger ages in patients with LDS, who frequently present with aortic regurgitation due to proximal aortic dilation, and may have other cardiac and vascular malformations or conduction abnormalities[19]. In LDS patients with *TGFBR1* mutations, male sex and increased arterial tortuosity are more highly predictive of dissection and death than syndromic features[20]. However, the burden of rare variants in LDS genes is not increased in non-syndromic BAV patients, even among those with aortic dilatation[21].

3. Velocardiofacial syndrome (VCFS)

BAV and aortic dilation are also more prevalent in VCFS[22]. VCFS patients have recognizable syndromic features, including learning disabilities, hypoparathyroidism, autoimmune problems and conotruncal heart malformations, including ventricular septal defects (60%), interrupted aortic arch (40%) and patent ductus arteriosus (30%)[4]. Large genomic deletions of 22q11.2 cause VCFS, and haploinsufficiency of *TBX1* is strongly associated with cardiac abnormalities[23]. However, other genes in the commonly deleted VCFS region, including *HIRA*, *UFD1L*, *CRKL* and *DGCR6*, have been shown to regulate cardiac development and probably interact to explain the burden of conotruncal defects in

VCFS patients[24, 25]. Mutations of single VCFS genes do not appear to be prevalent in non-syndromic BAV cases[26], but smaller distal deletions adjacent to the commonly deleted VCFS region are evident in some patients with BAV and overlapping clinical features. The same polymorphic 12p13.31 duplications that are associated with BAV in TS are also associated with heart defects in VCFS, reinforcing the hypothesis that multiple genetic lesions interact to cause BAV[7].

4. Genetic syndromes with less frequent BAV

Other genetic disorders that include BAV as an occasional feature include Down syndrome, caused by trisomy of chromosome 21, Alagille syndrome, caused by mutation of the *NOTCH* ligands *JAG1* or *JAG2*, Kabuki syndrome, caused by mutation of the epigenetic regulators *KMT2D* or *KDM6A*, and *FLNA* mutations that cause periventricular nodular heterotopia and mitral valve malformations[4, 27, 28]. All of these disorders are associated with distinctive extracardiac features and other left-sided obstructive cardiac lesions.

DEVELOPMENTAL ORIGINS OF BAV

1. Defective endocardial endothelial to mesenchymal transition (EndMT) during embryonic development

The aortic valve develops in the atrioventricular canal and outflow tract of the primitive heart tube, when signals from the myocardium induce endocardial-mesenchymal transition (EndMT) to create the endocardial cushions, in which proliferative mesenchymal cells are embedded in a loose extracellular matrix (cardiac jelly)[29]. This is followed by a complex sequence of cell proliferation, differentiation, migration, adhesion and apoptosis that results in elongation and remodeling of the cushions into the distinct layers of the mature valves[29–32]. Valve remodeling is dynamic and reciprocally regulated by blood flow and hemodynamic shear stress[33, 34].

EndMT is also required for late stages of aortic valve development, and defects in EndMT cause LVOT abnormalities and BAV (Figure 1). As demonstrated in mouse embryos, *NOTCH1* is highly expressed in the LVOT mesenchyme and endocardium at the location of the nascent valve cusps.[35] Homozygous *NOTCH1* mutations cause premature death due to vascular endothelial defects, but haploinsufficiency causes BAV and TAA, which is accentuated on a nitric oxide synthase (Nos3)-null background[30, 36–38]. BAV and TAA are highly correlated with impairment of EndMT in *NOTCH1*-deficient vascular cells[35, 36, 38–40].

2. Association between left-sided obstructive lesions and BAV

Due to the common embryologic origin of the aortic valve, LVOT and proximal aorta, BAV frequently co-exists with other left-sided congenital heart lesions, such as coarctation (CoA), Shone complex and hypoplastic left heart syndrome (HLHS). Approximately 50 to 85% of patients with CoA have BAV[41]. CoA consists of a discrete stenosis or hypoplastic segment located most often immediately after the origin of the left subclavian artery[42]. In humans, each subclavian artery derives from the 7th intersegmental artery from the right and left dorsal aortae immediately before they fuse into a single common aorta. Defective migration

of differentiation of cardiac neural crest cells into 4th pharyngeal pouch and 4th branchial arch derivatives that develop into the endocardial cushions, proximal aorta and aortic isthmus may explain this association[43]. Some human teratogens that affect neural crest derivatives, such as maternal phenylketonuria, have also been implicated in the development of aortic arch (including CoA) and conotruncal malformations[44].

Shone complex, characterized by mitral valve stenosis, LVOT obstruction and CoA, present with BAV in 71% of patients[45]. Additionally, up to 17% of patients with hypoplastic left heart syndrome (HLHS) have BAV[41, 46], suggesting an underlying common genetic defect between these conditions. This is supported by family-based genome-wide linkage analysis, where recurrence risk ratios of BAV in HLHS families was similar to that in BAV families[47].

Left ventricular noncompaction cardiomyopathy (LVNC) is an important cause of dilated cardiomyopathy due to impaired compaction of myocardial fibers during endomyocardial morphogenesis[48]. LVNC may be genetically linked to BAV through the Notch pathway, which is also essential to promote myocardial compaction and ventricular septation[37]. LVNC is found in conjunction with congenital heart defects in more than 10% of cases, predominately LVOT abnormalities including BAV[49]. In addition, LVNC may be more common in BAV patients than in the general population[50], and BAV patients with LVNC may develop earlier onset aortic valve disease or TAA with a more malignant course requiring surgical intervention[50, 51]. There is limited evidence that the same genetic mutations that cause isolated LVNC can also cause BAV, but the genetic causes of most LVNC cases remain unknown.

NON-SYNDROMIC BAV

Most people with BAV do not have syndromic features, but may have other congenital heart and vascular abnormalities with variable disease severity. The frequency of other left-sided lesions such as CoA (7%), patent ductus arteriosus (8.5%), mitral valve abnormalities (11%), ventricular septal defects (14%) and TAA (50%) are all significantly increased in BAV[52, 53]. Valvular aortic stenosis or regurgitation may eventually necessitate aortic valve replacement in up to 50% of BAV patients[54].

Complex inheritance is present in large families with non-syndromic BAV. The prevalence of BAV in first-degree family members is 10-fold higher than the general population[55, 56]. Inheritance is observed in more than half of the families if associated nonvalvular complications such as CoA, TAA, mitral valve or ventricular septal defects are included[1]. The heritability of BAV has been estimated to be as high as 90%, and multiple alleles can interact to cause BAV or other congenital heart defects without BAV in the same family[1, 57, 58]. With this basis, echocardiographic screening of first-degree family members is recommended in current guidelines[59, 60].

BAV is more frequent in first-degree relatives of patients with severe left-sided lesions, which include aortic valve stenosis, CoA, mitral valve stenosis, interrupted aortic arch type A, HLHS and Shone complex[61]. There is greater than 10 and 5.5-fold excess of BAV in first-degree relatives of patients with HLHS[62] and LVOT obstruction[63], respectively. Family-

based genome-wide linkage analysis have found that recurrence risk ratios of BAV in HLHS families are similar to that in BAV families, providing evidence that some HLHS and BAV are genetically related[47]. In these families, BAV likely represents a mild manifestation of the disease trait and thus implicates genetic pleiotropism, whereby environmental or stochastic factors play a significant role in the phenotypic expression[64].

Imaging studies show that first-degree relatives of BAV probands who have tricuspid aortic valves frequently have subtle root and ascending aortic dilation or valvular defects causing perturbed outflow[65]. These observations support the hypothesis that tricuspid aortic valve stenosis and other subclinical features may represent an underrecognized 'form fruste' of BAV[66].

IDENTIFICATION OF BAV GENES: PRINCIPLES

1. Distinguishing causation from phenotypic modification

For most Mendelian disorders, a single genetic mutation is sufficient to cause disease. The discovery of one rare pathogenic variant that segregates with disease in a family with clear and consistent inheritance of the phenotype usually provides adequate evidence to identify the causal gene[67]. In complex or polygenic disorders like BAV, a single genetic variant may increase the risk to develop disease, but is not sufficient to cause disease by itself. In many cases, environmental factors may interact with multiple genetic variants to modify the timing or penetrance of disease[68]. Thus, complex traits characteristically show reduced penetrance (same genetic variant but no disease) and variable expressivity (same genetic variant with different manifestations of disease). For example, interactions between genetic variants that cause BAV, genetic variants that accelerate aortic valve calcification and established clinical risk factors such as hypertension, dyslipidemia and smoking may increase the rate of progression of aortic stenosis in BAV patients[2, 69, 70].

2. Evidence framework to prove pathogenicity

According to the evidence framework developed by the Clinical Genome Resource[71], requirements to prove that a genetic variant is pathogenic, or disease-causing, include: 1) segregation, or co-inheritance of the variant with disease in a family[72]; 2) at least two independent observations of a loss of function variant in cases, in a gene that has no loss of function variants in normal controls; 3) multiple independent observations of rare missense variants in cases, in a gene that is highly invariant in controls (i.e., 'burden' tests of variants in cases vs controls must be significant in at least two independent cohorts); or 4) direct functional studies to prove that the genetic variant alters the function or expression of the encoded protein. Most of the genetic variants that were discovered in cohorts of BAV patients do not meet these standards. Few demonstrate familial segregation, and most were not replicated in independent populations or validated using functional genomic studies. Therefore, significant validation will be required before this genetic data can be used to develop genetic tests for BAV or make decisions about the clinical treatment of BAV patients based on genetic information.

3. Family-based vs population-based gene identification methods

In family-based strategies, the inheritance pattern of a disease is determined by careful phenotypic and genetic analysis of affected and unaffected relatives (Figure 2). Segregation of genetic mutations with disease traits in families remains the most effective approach to identify causative genes[73]. Population-based studies are based on the hypothesis that a few common genetic variants are shared by groups of people with the disease. Population-based genetic strategies are designed to compare the frequencies of common variants in a large sample of individuals with the disease to a control group. Instead of directly identifying causal genetic variants, population-based approaches detect indirect associations between polymorphic genetic markers (single-nucleotide polymorphisms -SNPs-) and disease. Common genetic variants that are more frequent in disease populations tend to have a modest and indirect effect on the likelihood of developing disease, but are more likely to modify the timing or severity of disease[74, 75]. Genetic studies of selected populations with 'extreme trait' characteristics that are more likely to have a genetic cause, such as early onset or severe manifestations of disease, may increase the power of association studies to identify clinically relevant genetic variants[76].

IDENTIFICATION OF BAV GENES: METHODS

1. Genome-wide association studies (GWAS)

Currently available 'chip' genotyping technologies capture SNP variants that are evenly distributed throughout the genome and detect common (present in >1% of the population) genetic variants that modify disease risk or increase susceptibility to disease, but in general do not cause disease[77]. Thousands of individuals must be genotyped in large GWAS studies to detect the relatively modest effect of most SNPs[75].

Several GWAS studies have focused on BAV, with variable results (Table 2). In 466 BAV patients who attended a cardiothoracic surgery clinic (83% TAA), GWAS identified more common noncoding and missense variants around the *GATA4* gene in BAV cases than in tricuspid controls[78]. In a separate cohort of 480 non-syndromic BAV patients, no individual gene attained genome-wide significance, but a pathway-based analysis identified variants in genes that regulate cilia. Functional studies in zebrafish confirmed that disruption of primary cilia can cause BAV and other left-sided cardiac lesions[79]. Other smaller GWAS studies were underpowered to find significant associations[80, 81].

As these results illustrate, the significant genetic heterogeneity of BAV makes it difficult to detect the causal effect of individual genes[82]. Methods to reduce heterogeneity include limiting the analysis to strictly defined BAV subtypes based on anatomic classifications or disease presentations, where the effect of single gene mutations may be amplified, or a hybrid approach in which familial segregation is used to prioritize rare variants[73]. GWAS is also more likely to succeed in comparisons that identify more common variants that modify BAV disease presentation or complications. For example, genetic variants that influence vascular calcification may increase the risk for bicuspid aortic stenosis[83].

2. Next generation sequencing (NGS)

Current NGS technologies permit whole exome (WES) or whole genome (WGS) sequencing for approximately $1000[84]. NGS comprehensively detects unique or rare variants that are not accessible to other methods and can accelerate gene discovery. WES studies identified rare missense variants in *MAT2A*[85] and missense and loss of function variants in *ROBO4*[86] that segregate with non-syndromic BAV and TAA in families. In contrast, WES of unrelated, non-syndromic BAV cases has been less successful[83]. Rare loss of function variants in *GATA4*, *GATA5* and *GATA6* were each identified in more than one non-syndromic BAV cohort.[31, 32, 87]. More recent observations of rare, non-synonymous variants in *MIB1*, *ADAMTS5* and *ADAMTS19* have yet to be replicated[88]. Sequencing a panel of candidate genes based on prior information has been frequently used to validate their contributions to BAV in different contexts. In 441 non-syndromic BAV patients with TAA, targeted sequencing of 22 candidate genes identified recurrent loss of function mutations in *SMAD6* that account for 2% of cases[89]. These studies are much less expensive than genome or exome-wide approaches, but are much more limited in scope and generally lack data to corroborate the pathogenicity of candidate variants. For example, rare missense variants of candidate genes are generally not well correlated with specific valve or aortic phenotypes[90, 91].To date, *SMAD6* and *GATA6* have the most cumulative and consistent evidence for contribution to non-syndromic BAV based on recurrent deleterious mutations in multiple cohorts.

3. Rare genomic Copy Number Variants (CNVs)

A CNV is a contiguous genomic DNA segment that may be duplicated (≥3 copies) or deleted (≤1 copy) in comparison with the reference genome. While most CNVs are considered to be benign, more than 40 CNV loci are implicated in human diseases[92]. CNVs may account for up to 10% of BAV cases and are more likely to be found in patients that present at younger ages or with more severe valve or aortic complications[93]. Rare pathogenic CNVs in BAV patients affect a subset of genes that are mutated in syndromic and non-syndromic cases. A similar enrichment of rare CNVs was also observed in patients with related congenital lesions such as Tetralogy of Fallot and hypoplastic left heart syndrome[94, 95]. We identified rare and recurrent CNVs at 2q37.3 and 22q11.21 in patients with early onset BAV and TAA that are absent or extremely rare in controls and involve candidate genes that interact with each other during heart and vascular development[93].

GENOMIC LANDSCAPE OF BAV: RARE OR UNIQUE VARIANTS IN MANY GENES

Cumulative data demonstrate that BAV is caused by rare or private mutations in many different genes that each contribute to a small proportion of cases. Many BAV genes also regulate LVOT and aortic development and can influence cardiac and aortic traits besides - Tiif to do BAV, leading to reduced penetrance and variable expressivity of BAV phenotypes[86, 96]. In addition, epigenetic and common variation of other genes that are not directly involved in BAV development can influence the rate of disease progression, but do not cause disease[97, 98].

The power to identify BAV genes depends on the study population and approach. For example, rare or unique variants may be readily identified in single families where they may segregate with BAV. In contrast, large cohorts of unrelated probands may be needed to detect the relatively weaker associations between more common genetic variants and BAV. Thus, family-based exome and genome sequencing studies may be more appropriate for BAV gene discovery than population-based or GWAS approaches, which may identify disease modifiers rather than causal genes. The effect of single gene mutations may be more evident in pediatric, young adult and surgical cohorts with earlier onset and more aggressive valve or aortic complications than in older adults with later onset and more slowly progressive disease.

SINGLE GENES IN NON-SYNDROMIC BAV

Table 3 summarizes genes with the most extensive evidence for causative mutations in BAV patients. Many more potential candidate genes exist based on model organism data that have not been corroborated in human genetic studies.

1. NOTCH1

NOTCH1 encodes a single-pass transmembrane receptor and functions in a highly conserved pathway that promotes the endothelial to mesenchymal transition and plays a critical role in cardiac valve development[35, 99, 100]. The *NOTCH1* pathway is also required to maintain vascular integrity by regulation of endothelial and vascular smooth muscle cell differentiation and proliferation[101–103]. Moreover, *NOTCH1*-dependent pathways can accelerate aortic valve calcification[104], which develops early in the course of BAV[105]. Familial studies demonstrate that loss of function mutations in *NOTCH1* are responsible for less than 1 WES identified rare and novel protein-altering variants in Notch pathway genes (*NOTCH1*, *ARHGAP31*, *MAML1*, *SMARCA4*, *JARID2*, *JAG1*) that co-segregate with LVOT obstructive phenotypes, including BAV and CoA, in French-Canadian families[106]. Rare variants of *JARID2*, a regulator of *NOTCH1* expression, were found in WES of 4593 individuals with left-sided obstructive lesions and in families with BAV and aortic dilatation[89, 107]. To date, rare variants of *NOTCH1* are the most commonly reported genetic variant in non-syndromic BAV cohorts[6, 108], but in most cases familial segregation or functional studies confirming the pathogenicity of these variants is lacking[13,86,91].

2. GATA factors

GATA (GATA binding protein) genes 4–6 encode zinc finger transcription factors that regulate early cardiac gene expression and cardiac cell lineage differentiation[109, 110]. *GATA4* is required for the early stages of heart development, and rare variants of *GATA4* are enriched in patients with Tetralogy of Fallot and isolated ventricular septal defects[111]. *GATA5* is expressed in the endocardium and deletion of *GATA5* in mice causes partially penetrant BAV[112]. *GATA6* is more directly required for aortic valve development by regulating a conserved semaphorin-plexin pathway in cardiac neural crest cells[113]. Common variants of *GATA4* are associated with BAV, and rare variants of *GATA4*, *GATA5* and *GATA6* were identified in candidate gene studies of BAV cohorts and in some families with autosomal dominant inheritance[32, 78, 114, 115].

3. SMAD factors

Smad (Mothers against decapentaplegic homolog) proteins are intracellular mediators of signal transduction by TGF-β and bone morphogenetic protein ligands[116]. Mutations in *SMAD3* cause a subtype of Loeys-Dietz syndrome that features a relatively late onset presentation with TAA, BAV and osteoarthritis. Recurrent rare missense and loss of function variants in *SMAD4* and *SMAD6* were also identified in non-syndromic probands with BAV and TAA[52,100,117].

4. ROBO4 in patients with BAV with TAA

WES and familial studies identified recurrent rare variants of the *ROBO4* (Roundabout homolog 4) gene in non-syndromic patients who were ascertained due to BAV and TAA. *ROBO4* is expressed in endothelial cells, and mutation or targeted silencing of *ROBO4* results in vascular defects and EndoMT in animal models[86]. Rare *TBX20* and *ADAMTS19* variants were identified in the same cohort, but each potentially accounts for less than 1% of cases[26].

5. Familial TAA without syndromic features

Mutations of the *ACTA2* gene, which encodes smooth muscle alpha-actin, are the most common cause (10–20%) of heritable non-syndromic TAA[118, 119]. *ACTA2* is required for actin filament assembly and smooth muscle cell contraction[120]. The prevalence of BAV (3%) is increased in *ACTA2* families, who may also manifest various features of a systemic smooth muscle dysfunction syndrome[120]. However, as with LDS genetic variants, *ACTA2* mutations are not frequent in non-syndromic BAV cases[121].

6. FBN1

The prevalence of BAV (4%) is also increased in Marfan syndrome, which is caused by mutation of the *FBN1* gene. *FBN1* encodes an extracellular glycoprotein (fibrillin-1) that is secreted by vascular smooth muscle cells and regulates the structural integrity of the aortic media. *FBN1* mutations disrupt smooth muscle cell attachments to arterial elastic laminae, resulting in progressive destruction of the media by matrix metalloproteinases and eventual TAA[122, 123]. In addition, common variation of *FBN1* is associated with non-syndromic TAA[124], and rare *FBN1* variants were identified in BAV patients who presented with aortic root aneurysms but had no features of Marfan syndrome[125]. Pathologic analysis of ascending aortic tissue from BAV patients demonstrated deficiency of fibrillin-containing microfibrils compared to tricuspid controls.

Current single gene mutations explain less than 10% of BAV cases. Several potential confounding factors may account for the missing heritability of BAV: 1) Multiple genetic variants in the same individual, affecting more than one candidate gene (compound heterozygotes) could explain up to 20% of BAV cases, based on recent analyses of other exome data; 2) Other types of variants (CNVs > noncoding > epigenetic) that are outside the exome may be discoverable by different technologies such as methylation arrays and whole genome sequencing; 3) The diversity of BAV phenotypes may cause the prevalence of BAV to be underestimated, if some affected individuals are not recognized.

CLINICAL APPLICATIONS OF BAV GENETIC INFORMATION

Family-based screening:

Based on the heritability of BAV in families, the current thoracic aortic disease guidelines recommend echocardiographic screening of all first-degree relatives of BAV probands[59, 60]. Affected relatives should receive a comprehensive clinical evaluation that includes complete imaging of the heart and thoracic aorta. Genetic tests may be appropriate for BAV patients when recognizable features of single-gene disorders or syndromes are present. If no external features of a genetic syndrome are present, genetic testing is generally reserved for BAV patients with high-risk clinical or imaging features, such as other congenital heart lesions, aneurysms or dissections beyond the proximal aorta, or a family history of dissection or sudden death. If genetic testing is contemplated, genetic counselors can facilitate screening, testing, clinical follow up and surveillance imaging of relatives.

Development of clinical genetic testing for BAV

When referring BAV patients for genetic counseling and testing, accurate phenotypic analysis of all available family members is essential for gene discovery. The variable expressivity of valve and aortic phenotypes in BAV families means that parents who have affected children and are assumed to harbor causative genetic mutations may not have BAV themselves or may have other cardiovascular abnormalities. Lifetime follow up of affected individuals is also essential, because the timing of BAV-related complications such as aortic valve disease or TAD may be very different between individuals, even within the same family.

Table 2 highlights genes that could potentially be included in a commercially available genetic test for BAV, based on current high-quality evidence. Those with familial BAV and TAD, as well as those with early onset complications of BAV, are most likely to have mutations in currently known TAD genes and therefore may represent the subset with the greatest potential benefit from genetic testing. Due to the frequency of rare CNVs in BAV cohorts, it may be reasonable to include SNP or chromosomal microarray analysis with single gene testing. The potential benefits of this approach to reduce healthcare costs by eliminating surveillance imaging of patients who test negative will need to be evaluated in future studies.

CONCLUSION

BAV is a complex disorder that is primarily inherited in an autosomal dominant pattern with incomplete penetrance and variable expressivity. Although BAV is a feature of some genetic syndromes and complex congenital heart defects, most cases are isolated and non-syndromic. Currently known single gene mutations do not explain most non-syndromic BAV cases. Alternative approaches to gene discovery using methods that account for multiple variants or interrogate the noncoding genome, family-based cohorts, and populations with highly penetrant disease may be necessary to discover new BAV genes. Until then, genetic testing should be considered for BAV patients who have features of genetic syndromes or heritable TAD.

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ABBREVIATIONS

BAV	bicuspid aortic valve
CoA	coarctation of the aorta
CNVs	copy-number variants
EndMT	endothelial to mesenchymal transition
GWAS	genome-wide association study
HLHS	hypoplastic left heart syndrome
LDS	Loeys-Dietz syndrome
LVNC	left ventricular noncompaction cardiomyopathy
LVOT	left ventricular outflow tract
NGS	next generation sequencing
SNP	single-nucleotide polymorphism
TAA	thoracic aortic aneurysm
TAD	thoracic aortic aneurysm and dissection
TS	Turner syndrome
VCFS	Velocardiofacial syndrome
WES	whole exome sequencing
WGS	whole genome sequencing

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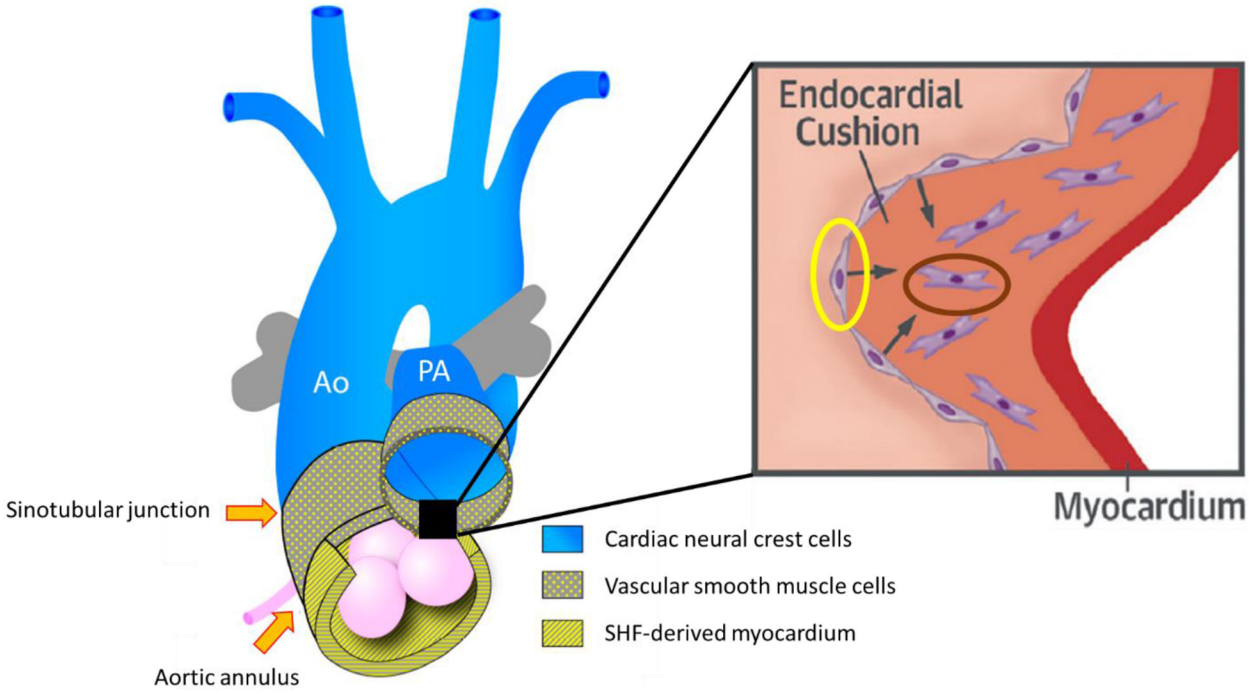

Figure 1. Cellular contributions to the formation of the outflow tract.

The outflow tract is formed by three cell lines: cardiac neural crest cells (blue), vascular smooth muscle cells (dotted yellow) and second heart field-derived myocardium (striped yellow). Inset shows endothelial (yellow oval) to mesenchymal (dark oval) transition in the endocardial cushions. Ao, aorta; PA, pulmonary artery; SHF, second heart field. Adapted from Martin et al and Kovacic et al[99, 126].

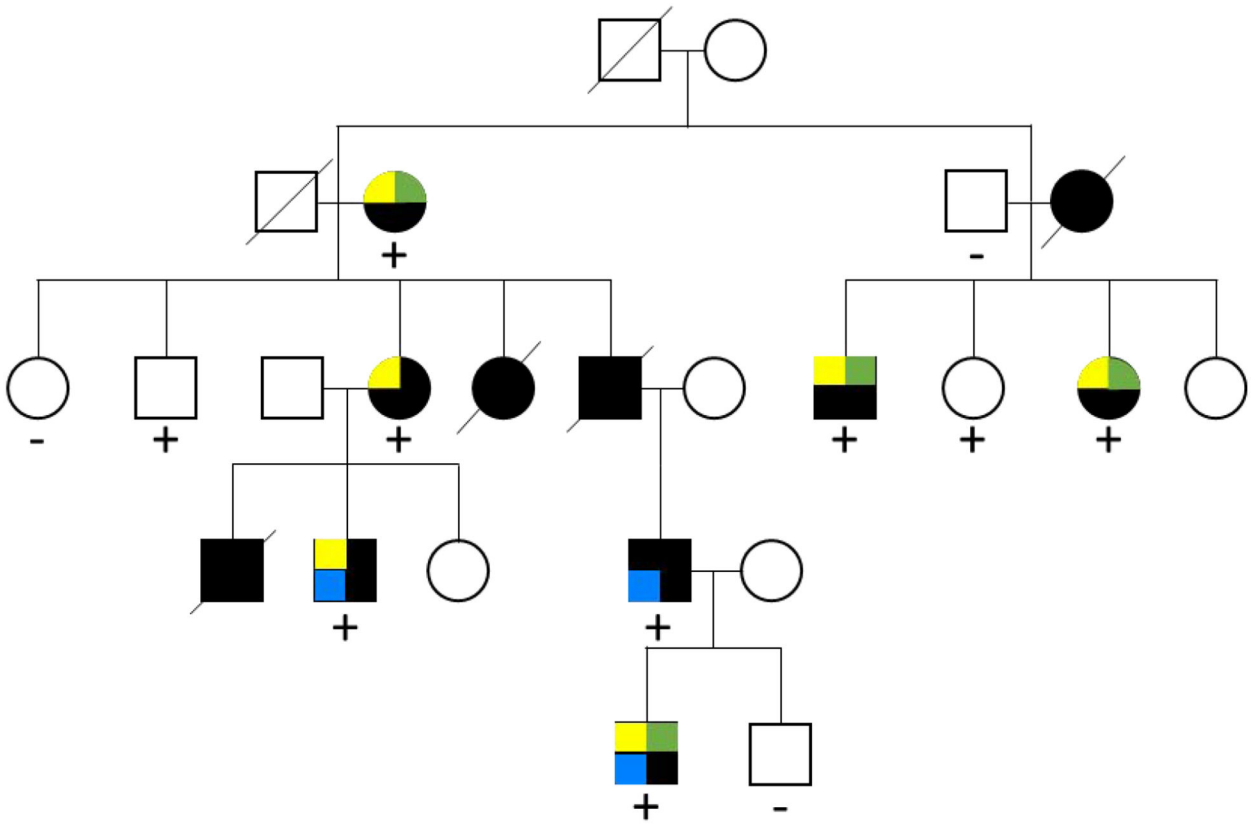

Figure 2. Family-based analysis

Kindred with five generations affected by bicuspid aortic valve (yellow square), coarctation of the aorta (green square) and thoracic aortic aneurysm (blue square) demonstrates reduced penetrance and variable expressivity that is characteristic of BAV pedigrees. Squares, males; circles, females; slashed, deceased family members; dark filled, affected; +, presence of mutation; −, absence of mutation. Adapted from Garg et al[35].

Table 1.

Syndromes with BAV

Syndrome	Gene(s)	Other features
Loeys-Dietz	*TGFBR1, TGFBR2, TGFB2, TGFB3, SMAD3*	Bifid uvula HTAD
Multisystemic Smooth Muscle Dysfunction	*ACTA2*	Mydriasis HTAD
Down	21 duplication	Atrioventricular septal defects
Turner	X monosomy	Short stature Coarctation of the aorta
Velocardiofacial	22q11.2 del	Truncus arteriosus Tetralogy of Fallot

HTAD: hereditary thoracic aortic aneurysm and dissection.

Table 2.

Genes associated with BAV

Gene	Locus	OMIM disease	Function	Size and Type of Cohort	References
NOTCH1	9q34.3	Adams-Oliver syndrome 5, Aortic valve disease 1	Endocardial cushion development, aortic valve calcification	Family-based genome-wide scan, including 14 BAV individuals	35
SMAD6	15q22.31	Aortic valve disease 2	Cardiac valves development and outflow tract septation	Targeted resequencing in 441 BAV/TAA cohort	89
GATA4	8p23.1	Atrial septal defect 2, atrioventricular septal defect 4, tetralogy of Fallot, ventricular septal defect 1	Myocardial differentiation and function	Sequencing of 150 nonsyndromic BAV individuals	31
GATA5	15q25-q26.1, 20q13.13	Congenital heart defects, multiple types, 5	Extracellular matrix remodeling and morphogenesis of valve leaflets	Sequencing of 110 nonsyndromic BAV individuals	87
GATA6	18q11.2	Tetralogy of Fallot, patent ductus arteriosus, atrial septal defect 9, atrioventricular septal defect 5	Outflow tract and subpulmonary myocardial development	Family-based sequencing study including 152 BAV individuals	32
ROBO4	11q24.2	Aortic valve disease 8	Outflow tract development and integrity of ascending aorta	Family-based targeted sequencing including 10 BAV individuals	86
MAT2A	2p11.2		Smooth muscle cell function and development	Family-based whole exome sequencing including 8 individuals with TAA with or without BAV	85
ADAMTS19	5q23.3		Perturbs shear stress signaling in valvular endothelial cells, increasing cellularity and proteoglycan deposition	Family-based exome sequencing, including 8 affected individuals with early-onset valvular heart disease	127

BAV: bicuspid aortic valve; TAA: thoracic aortic aneurysm.

Table 3.

Genome-wide association and next-generation sequencing studies of BAV

Author	Type	Cases	Controls	Findings	Reference
Hanchard	GWAS	778 non-syndromic left-sided lesions cases	2756 patients without left-sided lesions from the high-density SNP association analysis of melanoma: case-control and outcomes investigation and from the GWAS of Parkinson disease: genes and environment	Locus 20q11 associated with BAV, *MYH7B* and *MIR499A* as candidate genes	61
Yang	GWAS	466 non-syndromic BAV (83% TAA) attending cardiac surgery clinic at the University of Michigan Frankel Cardiovascular Center	4660 age, sex and ethnicity matched controls from the Michigan Genomics Initiative	Noncoding variants near *GATA4* associated with BAV	78
Fulmer	GWAS	2131 non-syndromic BAV cases	2728 patients without BAV from the Framingham Heart Study cohort	15 SNPs associated with BAV, including *EXOC4*	79
Helgadotir	GWAS	208 non-syndromic BAV (Iceland, Sweden, USA)	25139 controls (Iceland deCODE database, Stockholm POLCA/Olivia study, Michigan Genomics Initiative, Framingham Heart Study and National Institute of Neurological Disorders and Stroke)	*PALMD* intergenic and *TEX41* intronic variants associated with BAV	128
Bjornsson	GWAS	120 Icelandic non-syndromic CoA cases (75% with BAV)	355166 disease-free individuals randomly selected from Icelandic genealogical databases at deCODE	Rare missense mutations of *MYH6* in BAV cases	81
LeMaire	GWAS (3 stages)	765 non-syndromic TAD, 385 non-syndromic TAD (192 BAV), 163 non-syndromic TAD (157 BAV)	1355, 159 and 476 controls from Wellcome T rust Case-Control Consortium 1958 Birth Cohort and US National Institute of Neurological Disorders and Stroke	Common *FBN1* variants associated with TAD, with or without BAV	124
Wooten	Modified GWAS	68 non-syndromic BAV	830 controls from Illumina iControlDB and 7 BAV negative familial controls	Rare *AXIN1/PDIA2* haplotype associated with BAV	80
Gago-Dlaz	Population-based NGS	565 Spanish non-syndromic BAV cases (none with cardiac or ascending aortic surgery)	484 controls attending primary health care centers in Galicia and from Plataforma en Red Banco Nacional de ADN Carlos III	No significant associations with BAV	83
Guo	Family-based NGS	34 family members with TAA (BAV 47%)		*MAT2A* mutations segregate with BAV and HTAD	85
Gould	Family-based NGS	286 family members with BAV/TAA	193 unrelated controls	*ROBO4* mutations segregate with BAV and HTAD and are enriched in non-syndromic cases.	86

BAV: bicuspid aortic valve; GWAS: genome-wide association study; NGS: next generation sequencing; SNP: single-nucleotide polymorphism; TAA: thoracic aortic aneurysm; TAD: thoracic aortic aneurysm and dissection.