REVIEW

The hereditary basis of bicuspid aortic valve disease: a role for screening?

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Abstract: Over the past years, human and molecular genetic studies have provided new understanding of valve development and the molecular pathogenesis of bicuspid aortic valve (BAV) disease. BAV is an autosomal dominant disease with incomplete penetrance and is found to affect 1%–2% of the population. It can occur in isolation or coexists with other congenital heart diseases such as ventricular septal defect and tetralogy of fallot. BAV is a risk factor for premature cardiovascular disease and can lead to severe complications affecting the aorta and the valves. To date, *NOTCH1* and *GATA5* are the only genes linked to human BAV, and the genetic basis for most BAVs remains unidentified. Large-scale screening as well as whole exome sequencing studies hold promise for uncovering BAV-causing genes. Similarly, molecular analysis of valve development in animal models is needed for better insight of normal and pathologic valve formation. Together, these approaches will undoubtedly accelerate discovery of disease-causing genes opening the way for early diagnosis of BAV and prevention of valve degeneration and cardiovascular complications.

Keywords: congenital heart disease, valvulogenesis, genetic screening

General overview

The formation of the heart is a very complex process that begins at early stages of fetal development and is orchestrated by complex regulatory networks ensuring the formation of a fully mature four-chambered heart. The mammalian heart contains three layers: an inner endothelial cell lining called the endocardium, surrounded by a muscular component forming the majority of the heart called the myocardium, and an outer protective layer that envelops the myocardium called the pericardium. The unidirectional flow of blood into the whole body is ensured by the presence of thin structures, composed of leaflets opening and closing with each heart contraction. The valve leaflets originate from mesenchymal outgrowths known as the cardiac cushions. These cardiac cushions result from the transformation of endothelial cells into mesenchymal cells through a process called EMT (epithelial to mesenchymal transformation), where endothelial cells invade the cardiac jelly separating the outer myocardium from the inner endothelium, at the base of the outflow tract (OFT). The cushions containing mesenchymal-derived cells then elongate and undergo extensive remodeling to gradually mature and form thin leaflets known as valves. Mature valves are composed of a highly organized extracellular matrix (ECM) consisting of three distinct layers made of collagens (fibrosa), proteoglycans (spongiosa), and elastin (ventricularis).[1,2] The heart valves comprise the semilunar valves (aortic and pulmonary valves), controlling the systemic flow of blood; and the atrioventricular valves

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(mitral and tricuspid valves), controlling the flow of blood between the heart compartments. The semilunar valves arise from two types of cushions: the conotruncal and the intercalated cushions situated at the OFT. The mature aortic valve (AoV) is composed of three symmetrical leaflets (also called tricuspid aortic valve [TAV]): the right and left leaflets originate from the conotruncal cushions, and the posterior aortic leaflet is formed from the right-posterior intercalated cushion, adjacent to the conotruncal cushion.[3–5] The proper formation of the heart valves ensures the unidirectional flow of blood into the systemic circulation.

Semilunar valve malformations are very common and affect 2%–3% of the population causing a variety of valvular complications including valve stenosis and/or regurgitation.[6] There are two types of valve diseases: 1) congenital valve diseases that are present at birth such as bicuspid aortic valve (BAV), mitral valve prolapse, and tricuspid atresia and 2) acquired valve diseases that develop later in life such as valve calcification and dysfunction.[7] BAV is the most common congenital abnormality affecting 1%–2% of the population with a higher (3:1) male prevalence.[8] It results in the presence of two asymmetrical cusps or leaflets instead of three symmetrical ones with the presence or absence of a raphe (connecting ridge) on the larger leaflet marking the failure of separation between leaflets (Figure 1). Familial clustering studies have shown that BAV is a heritable trait with a prevalence of 9% among first-degree relatives and up to 24% in families where more than one member is affected. BAV follows an autosomal dominant mode of inheritance with incomplete penetrance suggestive of gene–gene and gene–environment interactions.[9–11] Leaflets orientation varies widely among BAV patients resulting in different types of BAVs classified according to the leaflets position relative to the right and left coronary arteries (Figure 1). The most frequent type in human is the RL-type BAV resulting from fusion of the right and left coronary cusps and representing 59% of all BAV cases. The RN-type results from fusion of the right and noncoronary cusps and is less frequent accounting for 37% of BAV cases.[12] A less common type, the LN BAV, results from a failure of separation between the left and noncoronary leaflets. Recent studies on animal models suggest that RL- and RN-type BAVs have distinct etiologies: RL-type BAV results from defective septation during valvulogenesis, while RN BAVs are caused by a defect in the formation of the OFT cushions.[13] Despite its importance, the underlying pathways leading to the different types of BAV remain undetermined.

BAV is a risk factor for premature valvulo-vascular disease

BAV occurs in isolation or coexists with other congenital heart diseases such as coarctation of the aorta, interruption of the aortic arch, and ventricular septal defects. Individuals with BAV are often asymptomatic but are at an increased risk of several life threatening events ranging from aortic stenosis and regurgitation, aneurisms, and dilation and rupture of the aorta to infective endocarditis.[12,14] So it is clear that BAV is a
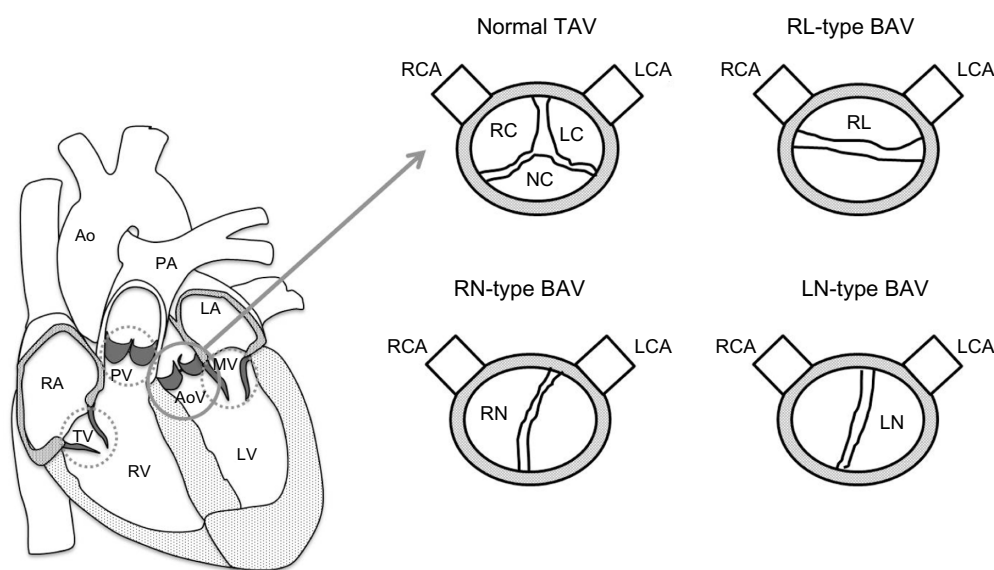

Figure 1 Representation of a normal TAV and different types of BAV.
Abbreviations: Ao, aorta; AoV, aortic valve; BAV, bicuspid aortic valve; LA, left atria; LC, left coronary; LCA, left coronary artery; LN, left-noncoronary; LV, left ventricle; MV, mitral valves; NC, noncoronary; PA, pulmonary artery; PV, pulmonary valves; RA, right atria; RC, right coronary; RCA, right coronary artery; RL, right-left; RN, right-noncoronary; RV, right ventricle; TAV, tricuspid aortic valve; TV, tricuspid valves.

risk factor for many valvulo-vascular complications. Increased shear stress (effect of blood velocity and viscosity) is thought to play a crucial role in the pathogenesis of BAV-associated disease. The presence of BAV might be associated with pressure overload that could in part be due to the presence of malfunctioning valves; both conditions are risk factors for aortic dissection and rupture.[15] The most common phenotypic pattern in BAV disease is mid ascending aortic dilatation and is correlated with older age, whereas the other pattern, aortic root dilatation, is more associated with younger age and male sex.[16] The normal aortic wall consists of an internal intimal endothelial layer (intima), a medial layer (media) composed of smooth muscle cells with an ECM (rich in elastic and collagen fibers), and a covering protective layer rich in collagen fibers called the adventia.[17] BAV patients tend to develop aortic dilation, which is thought to be due to a disruption of the ECM by itself, caused by the lack of Fibrillin-1 and the presence of a cystic medial necrosis. Fibrillin-1 is a glycoprotein that ensures the strong interaction between vascular smooth muscle cells and elastin and collagen-rich matrix as well as maintaining the structural integrity of the aortic wall.[18] Studies have shown that regardless of the AoV function, BAV patients have a defect in the structure of the aortic wall that will eventually result in aortic disease.[19] BAV was found to be a pathological event since it was associated with accelerated degeneration of the medial layer of the aorta.[20] In the aorta of BAV patients, less elastic tissue loss and fragmentation, and less changes in the intimal layer were observed when compared to aortas from TAV patients.[21] Examination of specimens taken from BAV patients with aortic dissection showed a thinner medial layer due to elastic fiber necrosis (less vascular smooth muscle cells) leaving a greater distance between elastin fibers in those patients, which was not the case in TAV patients.[22] On the other hand, an elevation of matrix metalloproteinases, endogenous enzymes that degrade matrix components, has been reported to play an important role in the formation of aortic aneurysm in the aorta of patients with BAVs.[20] Beyond cardiovascular diseases, an increased risk of development of intracranial aneurysms was also found to correlate with the presence of BAV in a study done by Schievink et al. The study was done on a small population (61 BAV patients), so additional investigations are required before drawing any conclusions.[23] Together, these data raise the possibility that other subclinical genetic abnormalities in vascular endothelial or smooth muscle cells coexist with BAV and contribute to aortic disease. The development of animal models where BAV and TAV are present on a similar genetic background will help answer this important question.

With regard to the AoVs and their function, BAV is found to lead to severe valvular complications at late onset. The valve's ECM is regulated by valve interstitial cells (VICs) that are fibroblast like and quiescent in normal conditions. Changes in the hemodynamic environment are controlled by valve endothelial cells, which form a protective layer around the leaflets and communicate with the VICs through different molecular signals. In the presence of BAV, disruption of normal valve morphogenesis is accompanied by changes in the ECM composition, endothelial cell disruption, and VIC activation, eventually causing valvular dysfunction and calcification.[2,24] Valve disease is most commonly diagnosed as regurgitation, a leaking of the AoV that causes blood to flow in the reverse direction, or stenosis, a disease of the AoV in which the opening of the valves is narrowed. Both disease presentations are caused by disruptions in the structure and function of the valves. A study of 542 surgical BAV patients showed that 75% of the BAV cases had aortic stenosis; the same study showed that only 13% had aortic regurgitation. In fact, the regurgitation group had a higher rate of annular dilatation and a higher male to female ratio when compared to the stenosis group that had a higher prevalence of aortic calcification.[25] Studies have also shown that the presentation of valve disease varies among different BAV subtypes. RL-type BAVs are found to have more severe stenosis when compared to RN-type BAVs that develop more severe regurgitation.[26] In congenital bicuspid valve stenosis, aortic stenosis, which is secondary to the BAV, is found to lead to the development of calcification, occurring usually within the cusp's spongiosa.[27] This calcification in BAVs is due to the presence of an abnormal distribution of stress across the cusps in the deformed valves, leading to calcification earlier than in the case of TAV.[28] It can also be the result of mechanical disruptions (elastin and collagen fragments) that lead to the formation of nucleation sites for calcification.[29] In the case of calcified aortic valve disease, activation of the VICs is found to lead to the expression of bone development genes such as osteopontin, Runx2, and osteocalcin.[30] Once the valves are calcified, a surgical intervention is recommended in the case of severe valve dysfunction or the presence of comorbidities, which could further affect cardiac function and lead ultimately to heart failure.

Genetic basis of BAV: what we know from human and animal models
Even though the heritability of BAV is well documented, BAV causing genes remain largely unknown. Recent studies suggest that BAV is a complex process that can be caused by several genes, each with a distinct mode of inheritance, which

might explain why identifying the genetics of BAV through classical human genetic studies has been challenging.[10] To date, mutations in only two genes in human, *NOTCH1* (member of the Notch signaling pathway) and *GATA5* (member of the GATA family of transcription factors), were found to be linked to BAV. Based on DNA-sequencing of all coding exons and adjacent splice consensus sequences of the *NOTCH1* gene, missense mutations (p.T596M and p.P1797H) leading to amino acid substitution in a well-conserved region in *NOTCH1* were identified in patients with sporadic BAV, which were absent in 327 healthy individuals.[31] In another study, two novel mutations, p.P284L and p.Y1619X, a missense and a nonsense mutation, were found in the *NOTCH1* gene in two unrelated Italian families where the mutations segregated with the disease in both families.[32] Garg et al also identified two mutations in *NOTCH1*: the first mutation (p.R1108X) in a European-American five generation family showed linkage for BAV to a single locus on chromosome 9q34-35, whereas the second mutation (p.H1505del) cosegregated with the disease in three affected family members.[33] Consistent with a role for the BMP signaling in AoV formation, mutations in *SMAD6*, a component of the BMP intracellular signal transduction pathway, were reported in two out of 24 BAV patients with aortic stenosis and coarctation. Whether the mutations are causally linked with BAV remains uncertain since inactivation of *SMAD6* in mice did not cause BAV; instead, hyperplastic thickening of the valves and aortic ossification were observed.[34]

In order to further understand the complex process of valve morphogenesis, animal models lacking important regulators of valve formation were generated. Inactivation of key players during this process led to a wide variety of valvular malformations including BAV. One of the first models of BAV was reported in mice lacking NOS3 (endothelial nitric oxide synthase) where BAV was reported in five out of 12 mature NOS3-deficient mice (41.6%), all sharing the RN type with no BAV observed in any of the 26 wild-type littermates.[35] Another study by Bosse et al supports the important role of NOS3 in AoV formation by regulating NOTCH1 signaling and AoV disease prognosis. *NOS3−/− NOTCH1+/−* mice display 100% penetrance of BAV compared to the partial penetrance in the *NOS3−/−* (26%) and in the *NOTCH1+/−* (8%) single knockouts.[36] These results confirm a crucial role for the Notch pathway in AoV development and suggest that any deficiency in one or more of its key regulators could lead to valve defects including BAV.

In 2011, our group reported that targeted deletion of *GATA5* in mice leads to partially penetrant BAV (26%).

GATA5, a member of the GATA family of transcription factors crucial for heart morphogenesis, is restricted to endocardial cells during early embryonic development. Whether GATA5 is deleted in all cells or only in endocardial cells, partial penetrance of RN-type BAV is consistently observed. Mechanistically, GATA5 was found to regulate important pathways associated with endocardial cushion differentiation such as NOS3, NOTCH, BMP4, and TBX20.[37] *GATA5* mutations were later reported in several patients with BAV. A genetic analysis in 100 BAV cases identified four rare nonsynonymous *GATA5* variants (Gln3Arg, Ser19Trp, Tyr142His, and Gly166Ser) in GATA5 transactivational domain, pointing out a possible role for GATA5 in the pathogenesis of BAV. These mutations were in highly conserved and functional regions, which would affect protein function.[38] In another study, 78 BAV patients were analyzed using Sanger sequencing to screen for these *GATA5* variants. Two rare nonsynonymous heterozygote mutations, p.Gln3Arg and p.Leu233Pro, with a frequency of 2.6% (2/78), were found in patients with aortic coarctation and RN- or RL-type BAV.[39]

Even though no human mutations linked to BAV in genes other than *NOTCH1* and *GATA5* were reported, potential candidate genes that could underlie the formation of BAV were discovered by examination of the AoVs of genetically engineered mice. Activin type I receptor (ALK2) is expressed in endocardial cells, and its role in endothelial EMT in the atrioventricular cushions is well established.[40] Deletion of ALK2 from the cushion mesenchyme after EMT using *GATA5*-Cre results in AoV defect (BAV) and development of aortic insufficiency and/or stenosis in some of the mutant mice. These results indicate an important role of ALK2 in AoV development and suggest that its absence can lead to AoV diseases.[41] No human mutations in *HOXA1* gene are observed in BAV patients, but its inactivation in mice was found to result in 24% penetrance of BAV, among other cardiac malformations. *HOXA1* null mice show defects such as interrupted aortic arch, tetralogy of fallot, and abnormal subclavian artery, pointing to an important role of HOXA1 in patterning of the OFT of the heart.[42] On the other hand, studies done on *NKX2-5* heterozygous mice showed an increased frequency of 8.2% of stenotic BAV with three out of 35 *NKX2-5* heterozygous mutant mice showing an increase in the aortic blood flow velocity and BAV with mild thickening upon autopsy. The presence of BAV in these mice was dependent on the genetic background confirming the presence of genetic modifiers as suggested in human genetic studies.[43]

All the mice models of BAV mentioned above displayed the RN-type BAV. A study by Sans-Coma et al in 1995

reported the first RL BAV model in inbred Syrian hamsters. In all, 16 out of 29 embryos had a fusion between the left and right cushions resulting in two abnormal valve cushions (a ventral and a dorsal).[44] To date, no genetically modified mouse model of RL BAV, the most common type of BAV observed in human, has been reported.

Given the lack of identification of BAV causative genes, studies have aimed at determining genetic loci associated with familial BAV. Early studies have identified genomic regions 17q29.3 (*KCJN2* gene) and 9q34.3 (*NOTCH1*) to be responsible for BAV in a small proportion of cases. A mutation in *KCJN2* gene, encoding the inward-rectifying potassium current, KIR2.1, was reported in patients with Anderson syndrome having sex-specific cardiac (BAV) and skeletal muscle phenotypes.[32,45] Genome-wide scan studies have identified three chromosomal regions, 18q, 5q, and 13q, associated with BAV, but the genes therein remain to be identified.[46] BAV is also manifested in some human syndromes such as Loeys–Dietz and Marfan. Loeys–Dietz syndrome is caused by a defect in the TGFB signaling pathway (mutations in *TGFBR1* [TGFB receptor 1] and *TGFBR2*) and is characterized by the presence of congenital heart disease and mental retardation among other abnormalities. Marfan syndrome is a connective tissue disorder characterized by cardiovascular, skeletal, and ocular manifestations. It is caused by a defect in the fibrillin-1 (*FBN1*) gene (essential for the formation of elastic fibers found in

connective tissues) localized on chromosome 15q21.1 and is inherited in an autosomal dominant manner.[47,48] Mutations in *HOXA1* gene were also found in Bosley-Salih-Alorainy as well as in Athabaskan brainstem dysgenesis syndromes, which are characterized by a wide range of heart defects including ventricular septal defects, tetralogy of fallot, and BAV.[49] A high prevalence of BAV is also observed in patients with Turner syndrome, caused by a deletion of the short arm of the X chromosome. This suggests that abnormal AoV and aortic arch development can also be caused by happloinsufficiency in Xp gene (Table 1).[50]

Is it important to screen?

Asymptomatic individuals with BAV may go undetected for a lifetime and many will be disease free. However, a significant proportion will develop cardiovascular events that could be life threatening, requiring surgical intervention in some cases. A recent study showed that 25% of BAV subjects had to undergo premature valvular or aortic surgeries.[51] Whether the BAV is the direct cause of aortic events or whether subtle defects in vascular cells – caused by the same genetic alterations – are responsible for BAV-associated cardiovascular complications remain to be determined. Be it as it may, BAV is a cardiovascular risk factor as also evidenced in the ASTRONOMER study.[52] It is now well established that early detection of affected individuals allows for better

Table 1 Genes associated with human and animal (mouse) BAV phenotypes

Genes	Human valve phenotype	Animal model valve phenotype (mouse)	Reference
Transcription factors			
NKX2.5	Not reported	Partial penetrance of BAV (8.2%) with aortic aneurism	43
H0XA1	Not reported	Partial penetrance of BAV (24%)	42
GATA5	BAV with aortic stenosis and regurgitation	Partial penetrance of BAV (30%)	32,37–39
Enzymes			
NOS3	Not reported	Partial penetrance of BAV (41.6%)	35
Channels			
KCNJ2	BAV with coarctation of aorta	Not reported	45
Ligands			
NOTCH1	BAV with aortic aneurism, calcification (5%)	Partial penetrance of BAV	31–33,35
Receptors			
TGFBR2	BAV with aortic aneurism	Not reported	32
ALK2	Not reported	BAV with aortic insufficiency and/or stenosis	41
Chromosomal regions			
Loci 5q-gene unknown	Partial penetrance of BAV		46
Loci 5q-gene unknown	Partial penetrance of BAV		46
Loci 5q-gene unknown	Partial penetrance of BAV		46
Xp gene	BAV		50
Signal transducer			
SMAD6	BAV with mild aortic stenosis	Not reported	34
	BAV with mild aortic stenosis and coarctation of the aorta		

Abbreviation: BAV, bicuspid aortic valve.

monitoring and preventative care. Echocardiography and magnetic resonance imaging have been reliable techniques so far to detect BAVs and dilated aortas, but the infrastructure and cost of these tests make them impractical for large-scale population screening. Moreover, valvular problems and dilated aortas caused by BAV are not detected at childhood, and the late identification of BAV often occurs when AoV disease has become irreversible.

Given the established familial contribution to BAV, the use of next-generation technologies such as whole exome sequencing (WES) and whole genome sequencing should be considered. Given the high cost of whole genome sequencing, WES is currently a more viable approach to determine the protein-coding sequence variations. In previous studies, WES has proven to be useful in revealing genetic variants in individuals affected with rare forms of heart diseases such as BAG3 gene in familial dilated cardiomyopathy.[53] Other genes causing severe hypercholesterolemia (ABCG) and familial combined hypolipidemia (ANGPTL3) could not have been identified without the availability of this technique.[54,55] In a not so distant future, WES and eventually WEG might well become routine techniques for sequencing due to accelerated developments that are resulting in rapid drop in cost. Investments in those technologies will accelerate discovery of BAV causing genes, ultimately enhancing patient management and treatment.[56] Recent studies have shown that WES is useful for disease diagnosis, which informs patient treatment. Since most BAVs diagnosed to date usually have a familial history, it is important to screen first-degree relatives of a patient with BAV. WES has helped in the diagnosis of Crohn's disease due to a mutation in the X-linked inhibitor of apoptosis gene in a 15-month old boy. The patient successfully underwent a hematopoietic progenitor cell transplant.[57] Discovery of BAV causing genes will also help develop genotype–phenotype correlations and open the way to personalized treatment. For example, knowledge of which BAV genotype might be predictive of valvulo-vascular complications will decrease unnecessary interventions such as unnecessary aortic aneurism repair surgeries while pointing to individuals who require closer cardiovascular monitoring. Therefore, it is important to accelerate the search for new causative genes that will allow for genetic screening as well as a better understanding of the molecular basis of BAV formation and degeneration. This holds great promise for secondary prevention and might open new therapeutic avenues.

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Disclosure

The authors report no conflicts of interest in this work.

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