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Review Article

The pathology and pathobiology of bicuspid aortic valve: State of the art and novel research perspectives†

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†A report from the International Bicuspid Aortic Valve Consortium (BAVCon)

Abstract

Bicuspid aortic valve is the most prevalent cardiac valvular malformation. It is associated with a high rate of long-term morbidity including development of calcific aortic valve disease, aortic regurgitation and concomitant thoracic aortic aneurysm and dissection. Recently, basic and translational studies have identified some key processes involved in the development of bicuspid aortic valve and its morbidity. The development of aortic valve disease and thoracic aortic aneurysm and dissection is the result of complex interactions between genotypes, environmental risk factors and specific haemodynamic conditions created by bicuspid aortic valve anatomy. Herein, we review the pathobiology of bicuspid aortic valve with a special emphasis on translational aspects of these basic findings. Important but unresolved problems in the pathology of bicuspid aortic valve and thoracic aortic aneurysm and dissection are discussed, along with the molecular processes involved.

Keywords: bicuspid aortic valve; pathophysiology; calcific aortic valve disease; aorta dilation; thoracic aortic aneurysm

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Introduction

Bicuspid aortic valve (BAV) is a developmental abnormality that has an estimated prevalence of 0.5–2%, and a male predominance of about 3:1 [1]. BAVs usually exhibit normal function at birth and during early life, but can be associated with significant aortic valve disease prior adulthood. However, later in life BAV is associated with substantial morbidity [2]. Late complications of BAV include aortic stenosis or regurgitation, infective endocarditis, aortic dilatation and aortic dissection. In particular, BAVs are predisposed to progressive calcification, grossly identical to that occurring in tricuspid aortic valves. The increased propensity of BAV to calcific aortic valve disease (CAVD), relative to valves with a

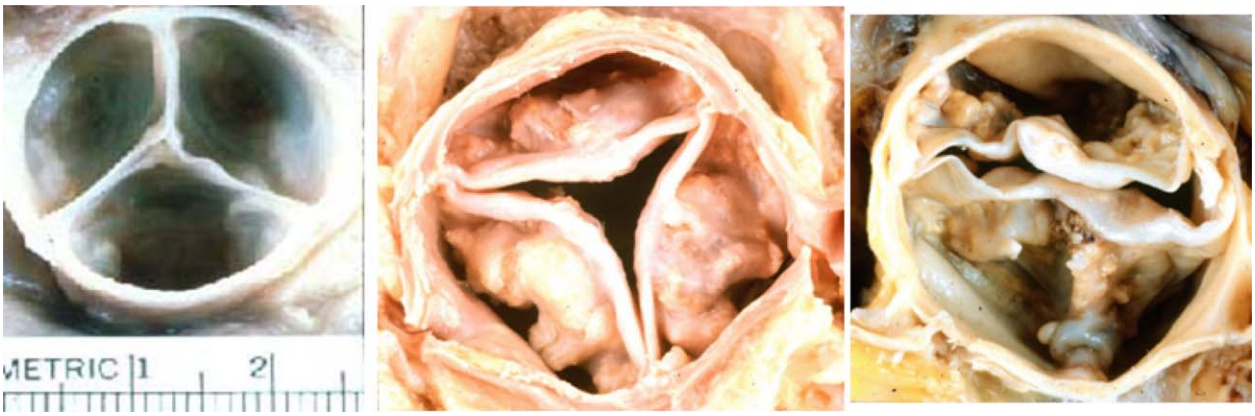

Figure 1. Non-mineralised tricuspid aortic valve (left) and stenotic mineralised tricuspid (middle) and bicuspid (right) aortic valves.

normal 3-leaflet configuration, is underscored by the data that calcified BAVs comprise 30–50% of cases of operated aortic stenosis in adults [3]. Moreover, calcific stenosis of a BAV is generally accelerated, appearing approximately a decade earlier than with TAV. Calcified or regurgitant BAVs often become clinically important in patients as young as 50 years old.

Morphology

Congenital BAVs have two functional leaflets, usually of unequal size, with the larger leaflet often having a midline *raphe*, resulting from incomplete commissural separation during development. Less frequently the leaflets are of equal size and the raphe is absent. Leaflet orientation varies widely among patients, with the most frequent BAV subtype being fusion of the right and left (R-L) coronary leaflets (59% of BAV) and fusion of the right and non-coronary (R-N) leaflets (37% of BAV).[4] Studies in $eNOS^{-/-}$ mice and an inbred Syrian hamsters suggest that the aetiologies of R-N and R-L BAVs appear to be distinct with the R-N BAV being caused by defective formation of the outflow tract (OFT) cushion whereas the R-L BAV is likely the result of defective OFT septation [5]. When compared to the R-L fusion, the R-N fusion is associated with a faster progression rate of aortic valve pathology (stenosis and insufficiency), especially in young patients [6].

Compared to TAVs, BAVs induce an abnormal, turbulent flow pattern and higher tissue stresses, which are concentrated in the abnormally large cusps and at the raphe. Calcium deposition and fibrosis predominate in the raphe and at the bases of the cusps, and the calcification may extend to the mitral annulus and anterior mitral leaflet. Once stenosis is present, the clinical course appears to be similar to that for calcific aortic stenosis in a 3-leaflet valve, in which the calcific deposits predominate at the cuspal bases (Figure 1).

Pathobiology

Mineralisation of the aortic valve: Basic concepts

CAVD is manifested as ectopic mineralisation and fibrosis, beginning initially in the extracellular matrix (ECM) and promoted by matrix vesicles produced by valvular interstitial cells (VICs) [7,8]. Histological analyses of surgically explanted stenotic aortic valves have revealed that calcific nodules are often surrounded by inflammatory infiltrates, new blood vessels and lipids [9,10]. Key controversies in the pathogenesis of CAVD with tri- or bicuspid valves relate to the extent to which its mechanisms are shared with those of aging and atherosclerosis, and how the mechanisms of initiation and progression of calcification are regulated, potentially actively [11]. In particular, for CAVD in BAV, it is uncertain whether abnormalities noted in clinically removed BAV tissues are primary or secondary, and what are the key differences that account for the accelerated and nearly ubiquitous formation of CAVD in the context of BAV. In CAVD, the increased mechanical stress on resident VICs induced by aging-related valvular remodelling, inflammation and other mechanical and biochemical processes could play an important role in early cell injury (apoptosis or necrosis) and osteogenic differentiation of VICs [12,13]. Apoptosis/necrosis-enabled dystrophic calcification mechanisms, in which cell injury is an important and early event, are exemplified by the

failure of glutaraldehyde-treated bioprosthetic substitute heart valves, in which calcification is initiated primarily within residual, non-viable porcine aortic valve or bovine pericardial cells [14]. Mineral found in CAVD is mostly hydroxyapatite of calcium (HAC), similar to bone mineral, which can be deposited by an apoptosis-mediated process or by osteogenic activity [15,16]. In some explanted stenotic aortic valves (~15%), well-differentiated osseous metaplasia is present, suggesting that a process analogous to bone ossification may occur during the development of CAVD [17]. Similarly, the expression of bone-related markers such as Runx2 (a transcription factor highly expressed during osteogenesis), bone morphogenetic protein 2 (BMP2), osteopontin, osteocalcin and osteonectin is increased in stenotic aortic valves when compared to non-mineralised aortic valves [15]. The presence of bone-related proteins and biomarkers of osteogenic pathways strongly supports an osteogenic program contributing actively to the mineralisation of the aortic valve. Crosstalk between different pathways may trigger an osteoblastic transition of VICs. In mineralised aortic valves, the level of Wnt3a is increased [18]. Wnt agonists bind to a membrane receptor formed by Lrp5/6 and Frizzled and inactivate a complex, which includes adenomatosis polyposis coli (APC), Axin and glycogen synthase kinase 3(GSK3). As a result, β-catenin is stabilised and translocates to the nucleus where it controls the expression of BMP2. In porcine VICs, Wnt3a-induced myofibroblast differentiation relies on TGF-β1 [19]. TGF-β1 was shown to induce the nuclear transclocation of β-catenin on matrices with fibrosa-like stiffness. The latter finding may explain the observation that the calcific nodules initiating CAVD develop in the fibrosa layer.

Several enzymes and transporters of the phosphate pathway, such as alkaline phosphatase (ALP), ectonucleotide pyrophosphatase/phosphodiesterase 1 (NPP1) and the phosphate transporter Pit1/SLC20A1 that are crucial regulators of mineralisation, are also highly expressed in mineralised aortic valves and regulate phosphate and pyrophosphate metabolism [20]. Pyrophosphate (PPi) is a powerful inhibitor of the nucleation of HAC whereas inorganic phosphate (Pi) has pro-mineralising properties. Both NPP1 and ALP promote mineralisation during CAVD by elevating the Pi/PPi ratio [16,21]. In this regard, ALP, which is highly expressed during the mineralisation of VICs, transforms PPi into Pi [22]. Intracellular channeling of Pi by Pit1/SLC20A1 contributes to increased expression of bone-related transcripts and to the promotion of apoptosis-mediated mineralisation [23]. A fundamental question is whether and to what extent the biological processes leading to valve calcification are different in BAV versus TAV.

Disorganised tissue architecture in bicuspic aortic valve: A contributor to inflammation and mineralisation

In non-mineralised BAV leaflets from newborn infants, the trilaminar architecture and compartmentalisation of valve interstitial cells (VICs) is lost and there is increased volume of proteoglycans (PG), glycosaminoglycans (GAG) and extracellular matrix (ECM) (Figure 2) [24]. Disorganised ECM in BAV may have an important impact on the development of CAVD later in life as increased PG/GAG content is a notable feature of CAVD [25]. In stenotic aortic valves, increased expression of PG promotes the retention of lipoproteins [26,27]. In turn, the accumulation of oxidised lipid species triggers the mineralisation of VICs [28]. Biglycan, which is highly expressed in mineralised aortic valves, stimulates Toll-like receptor 2 (TLR2) and NF-κB, which promotes the mineralisation of VIC cultures [29,30]. Also, oxidised-low density lipoprotein (ox-LDL) increases the synthesis of dermatan sulfate, which enhances the bioavailability of TGF-β1 [31]. Although the molecular mechanism is not clearly delineated, it is possible that the addition of GAG chain inhibits the normal sequestration of TGF-β1 by decorin [32].

Inflammation and neovascularisation of the aortic valve are thought to promote tissue remodelling and calcification. The normal aortic valve is avascular and the formation of neovessels participates in the development of CAVD [14]. To this end, stenotic BAVs demonstrate increased remodelling, neovascularisation and inflammatory infiltration compared to TAV, even when accounting for other risk factors for CAVD [33,34]. The expression of chondromodulin-1 is markedly decreased in BAV compared to TAV [35]. Chondromodulin-1, expressed in the aortic valve during development, inhibits cell proliferation and angiogenesis [36]. Mice deficient for chondromodulin-1 have thickened aortic valves with new blood vessels, which is one feature also observed in human mineralised aortic valves [35]. It is possible that increased neovascularisation in stenotic aortic valves may participate in the recruitment of circulating osteogenic progenitor cells (OPC) that increase mineralisation of the aortic valve [37,38]. The role of neovascularisation is not clearly defined, but it may also enhance inflammation [39]. Mineralised aortic valves are infiltrated by macrophages and T cells. In BAV, the density of inflammatory cells is higher when compared to TAV [33]. Studies indicate

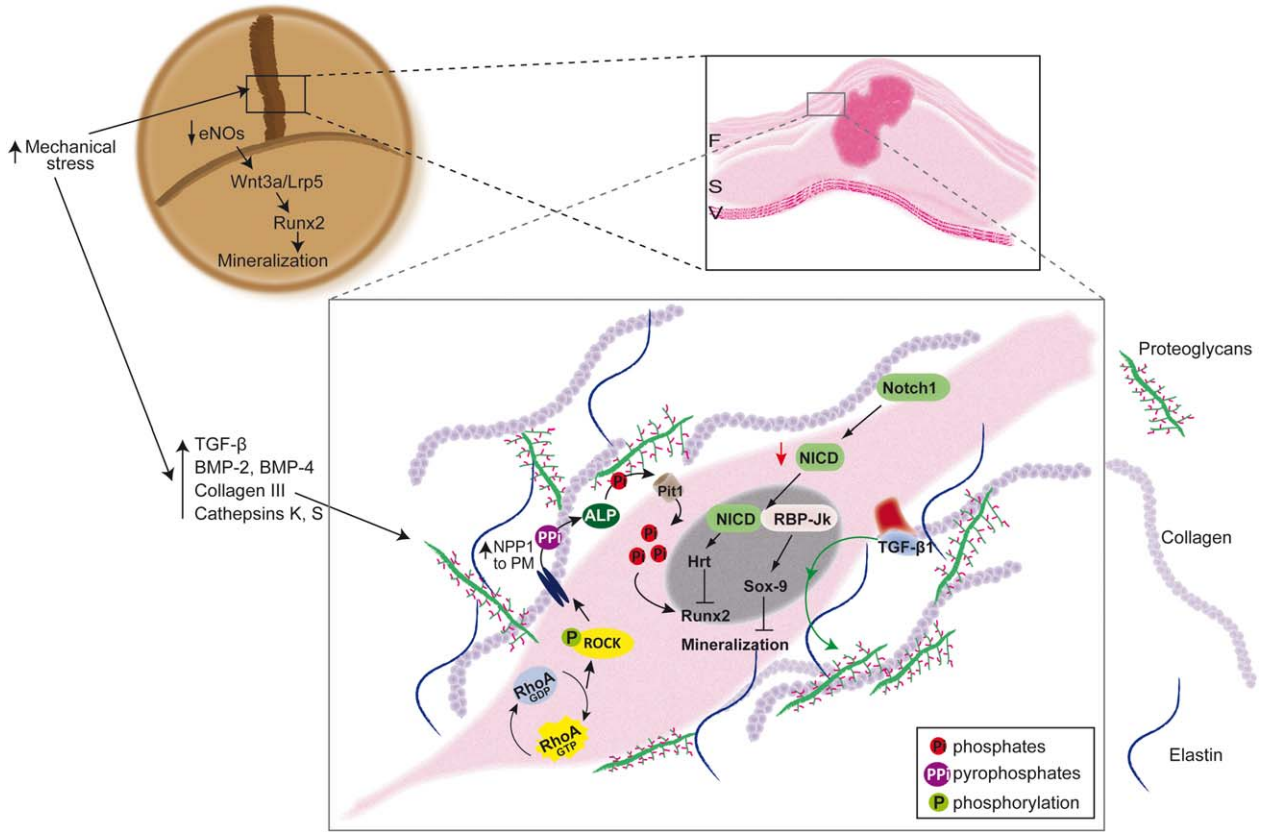

Figure 2. Schematic of pathophysiological mechanisms involved in bicuspid aortic valve (BAV). Dysregulation of NO signaling is suspected to play a role in the osteogenic transition of VICs through the Wnt pathway. Increased content of PGs and GAGs and disorganised tissue architecture could also promote lipid retention and increase the bioavailability of TGF-β1. In addition, elevated mechanical strain promotes the production of BMP2-4, collagen type III and cathepsins K, S, which participate in tissue remodelling in the BAV. TGF: transforming growth factor, BMP: bone morphogenetic protein, eNOS: endothelial nitric oxide synthase, Lrp5: low-density lipoprotein receptor-related protein 5, Runx2: runt-related transcription factor 2, NPP1: ecto-nucleotide pyrophosphatase/phosphodiesterase 1, ALP: alkaline phosphatase, ROCK: Rho-associated protein kinase, NICD: Notch1 intracellular domain, Hrt: Hairy-related family of transcription factors.

that chronic inflammation of the aortic valve is one important process involved in the ectopic mineralisation of valvular tissue [40]. The NF-κB cascade is activated in stenotic aortic valves with a high content of interleukin 6 (IL-6)[41]. VICs produce IL-6 during mineralisation and promote an osteogenic transition through a BMP2-dependent pathway [41]. Also, the production of TNF-α by macrophages promotes the mineralisation of vascular cells and VICs [42,43]. Hence, the increased inflammation and neovascularisation often observed in BAV may reflect a more aggressive pattern of mineralisation in these patients.

Contribution of mechanical factors to the mineralisation of BAV

Why is mineralisation of the aortic valve accentuated in BAV? This is a key unresolved issue that deserves further attention. Present data suggest two non-mutually exclusive possibilities underlying the increased susceptibility of BAV to mineralisation. The morphology of the BAV increases the mechanical stress in the valve tissue and alters blood flow patterns. In addition, it is possible that the genetic variants that cause BAV formation *in utero* may contribute to increased mineralisation due to defective cell differentiation.

Computational modelling and magnetic resonance imaging suggest that BAVs show greater cuspal deformation and blood flow turbulence compared to TAVs [44]. Local stress certainly enhances mineralisation of the aortic valve [45]. Mechanical strain has been shown to promote the expression of collagen type III by VICs [46], and is increased in the area of the conjoined leaflets where calcification is often extensive [47]. Furthermore, cyclic stretch in VICs

promotes the expression of cathepsins K and S [48,49]. In apoE$^{-/-}$ mice, deficiency of cathepsin S prevented fragmentation of elastin and the development of CAVD [50]. Although the exact molecular process remains to be elucidated, elastin fragments induce the expression of alkaline phosphatase and promote the mineralisation of cell cultures [51]. These findings suggest that remodelling of the aortic valve could be, at least in part, promoted by mechanical cues, which may exacerbate tissue remodelling in BAV. Also, stretch-dependent expression of transforming growth factor-beta 1(TGF-β1) and BMP-4 has been shown in VICs [52]. In the latter study, stretch-induced mineralisation of valve tissue was inhibited by noggin, suggesting that signaling through the TGF-β superfamily of proteins is an important pathway leading to the mineralisation of the aortic valve under mechanical stress. Recently, Bouchareb et al. showed that cyclic stretch of VICs promoted activation of the RhoA pathway and intracellular transport of ecto-nucleotidase to the plasma membrane where it triggered the production of spheroid mineralised micro-particles [53]. Of interest, the presence of spheroid mineralised micro-particles has been recently demonstrated in human aortic valves [54]. It is suspected that the coalescence of spheroid mineralised micro-particles leads to the formation of larger mineralised structures. By using scanning electron microscopy and energy dispersive x-ray, it has been documented that mineralised micro-particles are abundant in the area of conjoined leaflets where ecto-nucleotidases are overexpressed [53]. These findings suggest that remodelling of the aortic valve may be initiated or augmented by haemodynamic stress created by the BAV anatomy, which may exacerbate mineralisation of valvular tissues.

Pattern of gene expression in BAV and relationship with calcification

Familial clustering of BAV and left ventricular OFT malformations [55] has been associated with *NOTCH1* receptor mutations [56]. The Notch signalling pathway is involved in formation of the OFT and in endocardial-mesenchymal transition (EndMT), both of which are important in development of the aortic and pulmonary valves [57]. Notch receptors (*NOTCH1-4* in mammals) interact with membrane ligands from neighbouring cells such as the delta-like (*DLL1, 3, 4*) and Jagged proteins (*JAG1, 2*). In addition to being associated with the genesis of BAV, *NOTCH1* variants with impaired function may increase Runx2 expression and mediate osteoblastic transition of VICs. Upon ligand binding, the Notch receptor undergoes cleavage by γ-secretase, which promotes production of the Notch intracellular domain (NICD). NICD then translocates to the nucleus where it associates with recombination signal binding protein for immunoglobulin κJ region (Rbpjκ) and promotes expression of the hairy-related family of transcription repressors (Hrt) [58]. Thus, signalling through Notch1 promotes the expression of Hrt, which represses the promoter of *Runx2*. Hence, decreased Notch1 signaling increases the expression of Runx2 and causes osteoblastic transition of VICs (Figure 2). Also, down-regulation of Notch signaling in VICs reduces Sox-9, a transcription factor of chondrogenic cells. Transfection of Sox-9 into VICs rescued the hypermineralising phenotype during Notch inhibition, suggesting that Notch signalling prevents mineralisation of the aortic valve in a Sox-9-dependent manner [59]. Mice haploinsufficient for the Rbpjκ transcription factor and supplemented with a cholesterol-rich diet and vitamin D develop CAVD but do not have BAV [60]. Intriguingly, GATA5$^{-/-}$ mice develop BAV ($\sim$25% of littermates) and have lower expression of Jag1 and higher levels of mRNA encoding for Rbpjκ in embryonic tissues, suggesting dysregulation of the Notch pathway in these mice [61]. Furthermore, the expression of endothelial nitric oxide synthase (eNOS), which has conserved GATA binding sites in its promoter, was significantly reduced in embryonic tissue of GATA5$^{-/-}$ mice. These data are of foremost interest considering that a similar proportion of both eNOS$^{-/-}$ and GATA5$^{-/-}$ mice develop the right-non-coronary (R-N) fusion type of BAV [62]. Recently, rare (4% of patients with BAV) non-synonymous variations within the transcriptional activation domains of GATA5 were documented in patients with BAV [63]. Worthy of note, levels of eNOS were found to be decreased in BAV leaflets [64]. Studies indicate that nitric oxide (NO) could modulate mineralisation and lower the expression of osteoblastic genes in vascular cells. In this regard, eNOS$^{-/-}$ mice under a cholesterol-rich diet develop CAVD and mice with BAV have higher levels of Wnt3a, Lrp5 and Runx2 [65]. These data suggest that eNOS-derived nitric oxide modulates the Wnt/Lrp5 pathway, which has been found to promote mineralisation of the aortic valve in patients with CAVD [18]. Hence, it is possible that complex interplay between GATA5, eNOS, Notch and Wnt/Lrp5 may promote early mineralisation of the aortic valve in BAV. These data suggest defective cellular differentiation in BAV that likely predisposes to mineralisation. Further investigations are needed to document the role of these pathways and how they may intersect with mechanical signals in promoting

mineralisation of BAV. Complicating the elegant interplay between these pathways and mineralisation of BAV is the current failure to identify a genetic cause of BAV in the vast majority of individuals.

Studies from the Encyclopedia of DNA Elements (ENCODE) project have revealed that, contrary to a previously held belief, a large portion of the non-coding genome is transcribed [66]. MicroRNAs (miRNAs) are short (~22 nucleotides) non-coding RNAs, which exert an important control over gene expression at the post-transcriptional level. They bind to target protein-coding RNA and induce degradation and/or prevent translational processes. Studies performed in the last several years have emphasised the role of microRNAs in different cardiovascular disorders. A transcriptomic analysis comparing microRNA expression in TAV vs. BAV has revealed that 34 of 1583 microRNAs examined in this study were differentially regulated. MicroRNA-141 was decreased by 14.5-fold in BAV and was shown to be an important negative regulator of BMP2 expression [67]. Different patterns of expression of microRNAs between stenotic and regurgitant BAV have also been observed. Stenotic BAVs had lower expression of microRNA-26a and microRNA-30b [68]. Both microRNA-26a and microRNA-30b were shown to be negative regulators of the osteogenic pathway and to lower the expression of BMP2. Hence, differential expression of microRNAs in BAV may contribute to increased osteogenic signals through a BMP2-dependent pathway. However, to date few studies have examined the role of non-coding RNAs in BAV and clearly further work is necessary in order to generate a comprehensive view of their role in the pathobiology of heart valve disorders.

Aortopathy and BAV

Structural abnormalities of the aortic wall commonly accompany BAV, even when the valve is haemodynamically normal, and this may potentiate both aortic dilatation (the most common aortic complication of BAV) and aortic dissection. Moreover, patients with BAV have a higher rate of coarctation of the aorta, and left coronary arterial dominance [69,70]. Development of the aortic and pulmonary valves is intimately linked to OFT septation and aorta/aortic arch remodelling. Interactions between the second heart field (SHF) and neural crest patterning are important in orchestrating development of the OFT along with the aortic arch from the common arterial trunk [71]. Disruption of Notch signalling in the SHF of transgenic mice, by using a truncated form of mastermind-like protein (a transcriptional co-activator of Notch), was associated with defective neural crest cell patterning and unequal aortic valve leaflets with a bicuspid-like morphology [72]. Mice displayed enlarged leaflets and aortic arch abnormalities. Moreover, the mice mutant for Notch signalling had moderate to severe aortic insufficiency (AI) and showed disorganised aortic wall histology with dispersed vascular smooth muscle cells (VSMCs). It should be pointed out that mice with defective Notch signalling in the SHF had lower expression of fibroblast growth factor 8 (Fgf8) [73]. Deficiencies in Fgf8 in the third and fourth pharyngeal endoderm promoted the development of BAV [74]. These findings support the notion that cross-talk between Notch and Fgf8 may orchestrate neural crest and SHF interactions during normal development of the semilunar valves and aorta/aortic arch (Figure 3). Thus, the syndromic and non-syndromic associations between BAV and aortopathy may be based on embryologic patterning of neural crest cells. Neural crest cells contribute to the formation of VSMCs of the aorta and coronary arteries and intervene in the late phase of semilunar valve development (Figure 3) [75]. Interestingly, the aorta of patients with BAV shows a high level of apoptosis in neural crest-derived cells [76]. Hence, although not yet established firmly in humans, it is possible that one or more defects originating from the patterning of neural crest cells play a role in the pathophysiology of some BAVs. This may explain the higher prevalence of congenital head and neck defects in patients with coarctation and BAV [77]. In association with an elevated rate of apoptosis, the aorta of BAV patients shows fragmented elastic fibres with increased distance between elastic lamellae [78]. Furthermore, dilated aortas from patients with BAV have a higher metalloproteinase 2 (MMP-2) content and a lower level of tissue inhibitor of metalloproteinase 2 (TIMP-2) compared to TAV patients, indicating increased collagen turnover [79]. More recently, a defect in cross-linking of collagen associated with lower expression of lysine oxidase has been demonstrated in the dilated aortas of BAV patients [80]. Therefore, loss of elastin combined with increased collagen turnover and decreased collagen cross-linking may predispose to aneurysm formation in patients with BAV (Figure 4).

Patients with Marfan syndrome have mutations of the fibrillin-1 gene (*FBN1*), which results in higher signalling through the TGFβ-1 pathway with increased phosphorylation of Smad2/3. Interestingly, BAV aortic tissues have lower fibrillin-1 content coupled with higher TGFβ-1 levels [81,82]. Fibrillin-1 contributes to the elastomeric properties of the

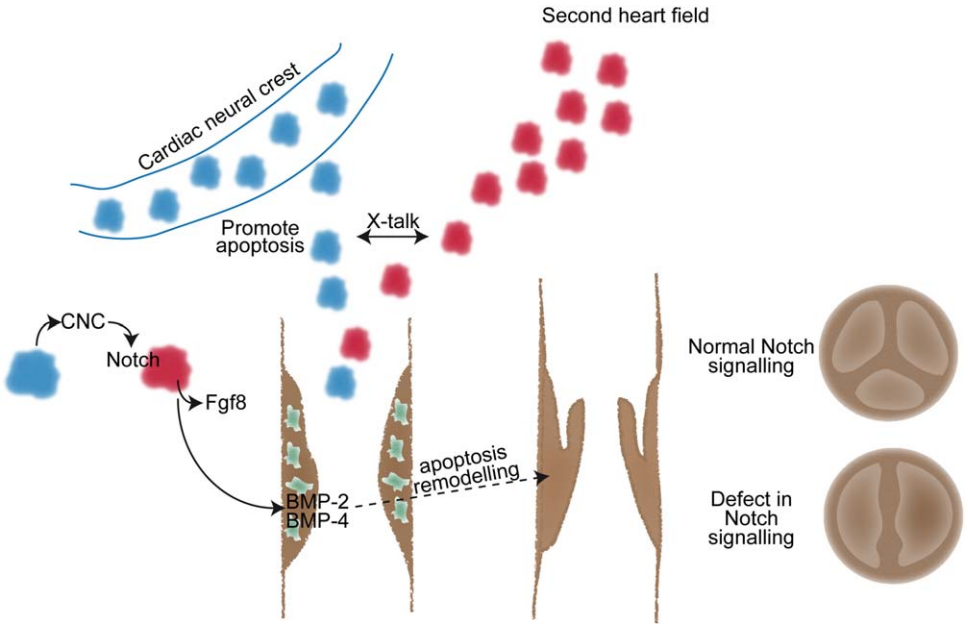

Figure 3. Signaling between cardiac neural crest cells (CNCCs) and the second heart field (SHF) is necessary for proper development of the aortic valve. Crosstalk between neural crest cells and the SHF ensures the production of fibroblast growth factor 8 (Fgf8) in a Notch-dependent manner. This step is essential as it contributes to the production of BMP2-4 and allows tissue reorganisation and loss of cellular components through apoptosis. Disruption of Notch signaling in the SHF leads to defective neural crest cell patterning and the formation of leaflets with a bicuspid-like morphology in mice.

connective tissue and also interacts with the TGFβ family of proteins. Studies performed in the last several years have emphasised the concept that abnormal secretion of fibrillin-1 leads to activation of TGFβ-1 by freeing it from microfibril-bound large latent complex (LLC) [83]. Cell contraction following stimulation with different agonists such as angiotensin II, thrombin and endothelin-1 increase the release of TGFβ-1 from the extracellular matrix (ECM) [84]. It has been proposed that expression of α-smooth muscle actin (α-SMA) promotes cell contraction, which is transmitted to integrin bound to the RGD site of latency associated protein (LAP) leading to allosteric modification and liberation of TGFβ-1 (Figure 2). Also, TGFβ-1 induced expression of splice variant EDA of fibronectin is reduced in VSMCs from BAV aortic tissues, suggesting dysregulation of the TGFβ pathway in BAV compared to TAV aorta [85]. Recently, in thoracic aortic aneurysms (TAAs) of different aetiologies, including aortic dilatation associated with BAV, it was shown that expression and activation of Smad2 was independent of TGF-β1 activity [86]. Instead, increased histone methylation and acetylation of the Smad2 promoter of VSMCs from these aortas was associated with the overexpression of Smad2, indicating an epigenetic contribution to dysregulation of the TGFβ/Smad pathway. TGFβ levels and signalling are inhibited by the angiotensin II type 1 receptor blockers (ARBs), such as losartan, and in a mice model of Marfan syndrome administration of losartan reduced TGFβ-1 signalling and concomitantly prevented the development of aneurysm [87]. These promising findings have fuelled the development of several randomised trials to evaluate the effect of losartan upon aortic morbidity and mortality in patients with Marfan syndrome [88]. However, a randomised study has recently shown in 608 patients (children and young adults) with Marfan syndrome that losartan did not alter the rate of aortic root dilatation [89]. Whether angiotensin II type 1 receptors play a significant role in BAV-associated aortopathy remains to be investigated.

One key observation in BAV-associated aortopathy is the asymmetrical pattern of histological abnormalities, which is also linked to the expression of genes involved in tissue remodelling. Several studies have shown that elastic fibre fragmentation and apoptosis of VSMCs were mostly observed at the convexity of the aorta, but attenuated at the concavity of the aorta [90]. In addition, expression of collagen types I and III was reduced in the convexity when compared to the concavity [91]. Taken together, these findings suggest that mechanical stress could contribute to specific spatial alteration of the ECM in BAV. Of particular importance, the opening of BAVs is asymmetrical and alters flow, resulting in uneven wall stress distribution in the

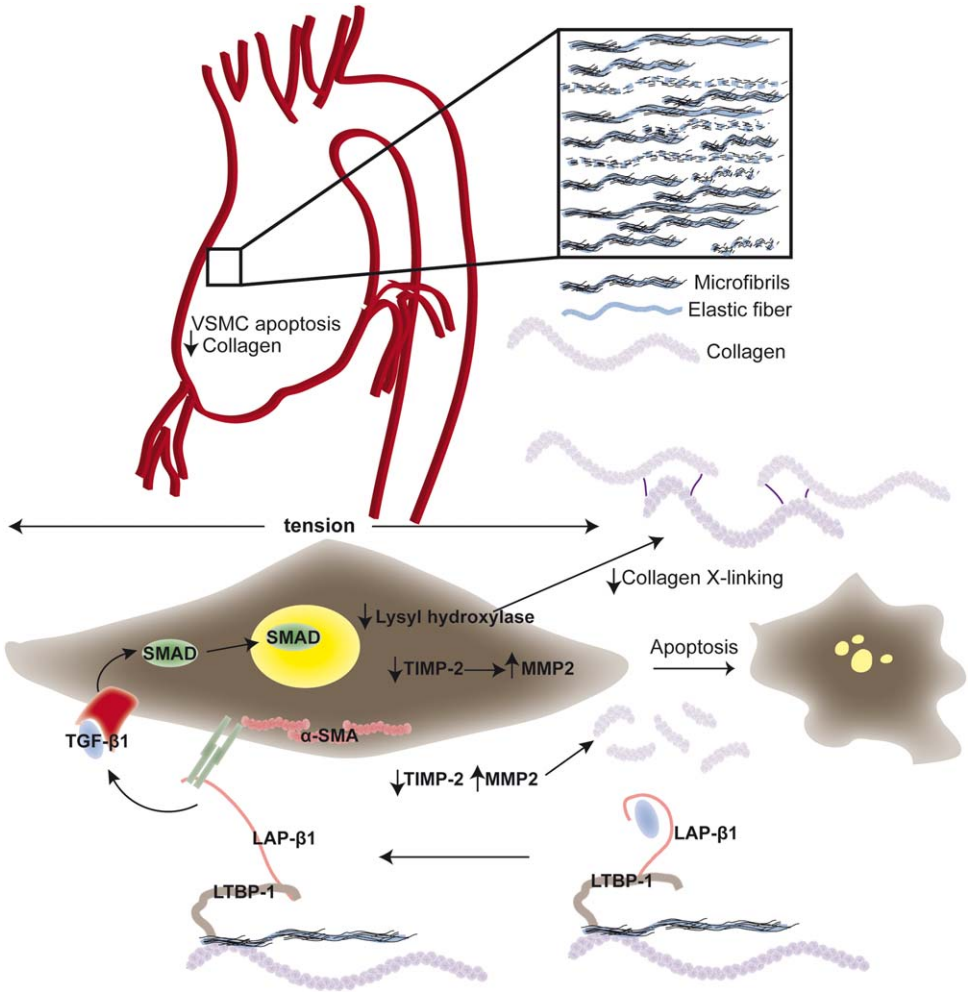

Figure 4. Schematic of the pathophysiological processes involved in the dilated aorta of BAV patients. Fragmentation of extracellular matrix components and decreased cross-linking between collagen fibres modify the biomechanical properties. Increased production of MMPs and lower expression of TIMPs contribute to remodelling of the arterial wall. Though it remains to be investigated in the context of BAV, it is possible that increased arterial wall tension is transmitted to VSMCs through integrin interactions. In turn, binding of integrin with the latency associated protein (LAP) may promote allosteric modifications that increased the bioavailability of TGFβ-1. VSMC: vascular smooth muscle cell, SMA: smooth muscle actin, LAP: latency associated peptide, LTBP: latent TGF-β binding protein, MMP2: matrix metalloproteinase 2, TIMP-2: tissue inhibitor of metalloproteinase 2.

aorta. The R-L type of fusion has been associated with a right anterior jet, whereas R-N fusion is related to an abnormal and eccentric left posterior jet. The specific flow patterns of different cusp configurations may explain the observation that L-R fusion is associated with asymmetrical enlargement of aorta at the convexity, whereas the R-N fusion is sometimes associated with tubular enlargement of the aorta, with extension into the aortic arch [92]. Hence, considering the non-homogeneous distribution of biomolecular changes within the BAV aorta it is likely that haemodynamic factors may contribute along with the genotype to the development of different phenotypes associated with BAV.

Unresolved questions and research perspectives

The morbidity of BAV is likely determined by genetic susceptibility, abnormal solid and fluid mechanical forces imposed on the aortic valve/aorta, and perhaps environmental risk factors [93]. BAV and its associated phenotypes have underlying genetic defects, which promote abnormal expression of proteins regulating ECM organisation and alter different signal transduction cascades, including NOTCH, Wnt/LRP5 and TGFβ pathways. In addition, BAV creates abnormal blood flow patterns, which may also contribute to the modification of cell signalling and tissue remodelling. Investigations in the last decade have shown that

key events during valvulogenesis are critical to understanding the pathobiology of BAV and its related complications. For instance, during valvulogenesis, including endocardial cushion development and cusp remodelling, several genes known for their role in osteogenesis are transiently expressed in the developing valves [94]. Hence, an altered pattern of gene expression during embryogenesis may have a lasting effect and may promote, amongst other mechanisms, maladaption to mechanical stimuli and premature mineralisation of the aortic valve. Thus, BAV-associated morbidity represents an exquisite example of complex gene–environment interactions. It also follows that elucidating the mechanisms underlying BAV complications poses several challenges. The development of animal models in which these complex gene–environment interactions can be manipulated, together with advances in human genetics, bio banking, cell and systems biology will be critical in providing much needed mechanistic insight. Hence, basic research related to the pathobiology of BAV should be integrated using a multidisciplinary team approach. We thus propose a list of key points for a research agenda which, although neither extensive nor exclusive, may help elucidate critical issues in BAV pathobiology: (1) Establish tissue banks of consistently and appropriately prepared and well-annotated specimens of aortic valves and aortas along with DNA of well-phenotyped patients undergoing surgery for BAV-related complications (and from autopsies of non-complicated patients who die of other causes); (2) Correlate key findings obtained from DNA studies (GWAS or candidate gene approach) with transcriptomics and functional assays in VICs and VSMCs; (3) Translate human investigations to animal models relevant to BAV embryology; (4) Develop animal models (including genetically modified mice) of BAV, which can recapitulate human morbidity; (5) Investigate the interrelationships between mechanical stress, gene expression and VIC/VSMC biology and (6) Identify novel key and pharmacologically approachable target(s) in early BAV and different BAV pathologies (e.g. CAVD, TAA). Creation of the International Bicuspid Aortic Valve Consortium (BAVCon) and large-scale collaborations between investigators of different but complementary expertise will help resolve underlying pathobiological processes in BAV, and may result in novel therapies for patients.

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Author Contributions

P.M. drafted the manuscript. M.C.B. and F.J.S. were involved in providing the figures. Y.B., G.S.H., A.D.C., P.P., H.I.M., G.L., M.C.B., A.E., E.B. R.C., S.C.B, M.N. and F.J.S reviewed the manuscript and were involved in the scientific contents.

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