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Insight into Pathologic Abnormalities in Congenital Semilunar Valve Disease Based on Advances in Understanding Normal Valve Microstructure and Extracellular Matrix

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Abstract

Congenitally diseased valves are relatively frequent causes of significant morbidity and mortality. Pathology descriptions of such valves have primarily focused on gross structural features including the number of leaflets or commissures (bicuspid/bicommissural valve) and alterations in the contour, thickness and consistency of the leaflets (dysplastic valve). Functional correlates of these pathologic alterations are valvar stenosis, insufficiency or both. Further characterization of the microstructural abnormalities seen in these malformed valves may not only provide insight into the correlation of distinct pathologies with their respective pathogenesis and clinical sequelae, but also may prove pivotal in uncovering new avenues for therapeutic interventions and prevention regimens. This review summarizes microstructural findings in congenital semilunar valve disease (CSVD) and discusses their relevance in light of recent advances in knowledge of normal valve microstructure, biology, and function. Specifically, the biological and mechanical roles of various matrix components and their interactions are discussed in the context of CSVD. Indeed, recent research in normal valves adds significant insight into CSVD, and raises many hypotheses that will need to be addressed by future studies.

Keywords

heart valve; congenital heart disease; extracellular matrix; bicuspid aortic valve

1. Introduction

Congenital heart disease (CHD) is the most common congenital anomaly, occurring in an estimated one out of every 100 live births [1]. In 2004 alone, 196,000 life years were lost before age 65 because of CHD, more than the sum of the life years lost to leukemia, prostate cancer, and Alzheimer's disease combined [2]. Of the many abnormalities seen in CHD, the majority involve the valves and/or septa [3]. Congenitally diseased valves are relatively frequent causes of morbidity and mortality, including valvar stenosis and/or insufficiency,

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ventricular dysfunction, cardiac chamber and/or arterial dilation, substrates for infection and thrombus formation, arrhythmias, and sudden death. Pathology descriptions of such valves have primarily focused on gross structural features such as the number of leaflets or commissures (bicuspid/bicommissural valve) and alterations in the contour, thickness and consistency of the leaflets (dysplastic valve). The nature of these abnormalities at the microstructural level has received limited detailed study. Instead, published reports on malformed valves have focused on associations with other cardiac and systemic anomalies, clinical diagnosis and evaluation, potential genetic and developmental causes, and the risks, benefits, and long-term outcomes of various therapeutic repairs. However, more in depth characterization of congenital semilunar valve disease (CSVD) may provide further insight into pathogenesis, mediators of disease progression, and new avenues for therapeutic intervention and prevention. This article reviews normal semilunar valve macrostructure, microstructure and extracellular matrix, then summarizes these same attributes in CSVD. The relevance of these findings will be discussed in light of recent advances in knowledge of normal valve microstructure, biology, and function. In fact, evidence suggests that some of the same mechanisms integral to normal valve physiology and development are at play in a range of valve pathologies [4], therefore in depth understanding of normal valve mechanobiology is paramount to our understanding of CSVD. Specifically, the role of valvular interstitial cells, the biological and mechanical roles of various matrix components, the interaction of these components with each other and bioactive molecules such as growth factors, and the role of mechanical stimulation in valve health will be discussed in the context of CSVD. Indeed, recent research in normal valve microstructure, developmental maturational changes, and mechanisms of acquired hemodynamic effects on valve structure adds significant insight into CSVD, and raises many hypotheses that will need to be addressed by further studies.

2. Spectrum of Congenital Semilunar Valve Disease (CSVD)

CSVD can involve any of the valve's components, including the cusps, annulus, commissures and sinuses. Many diseases involve multiple structures either as a primary defect or secondary acquired hemodynamic abnormality. The two extremes of CSVD are an atretic valve and absent valve. An atretic valve implies no luminal continuity from the ventricle to the great artery, often reflected by an outflow tract that ends blindly in a dimple with no valvular tissue, typical of aortic valve atresia (Fig. 1). Other forms of semilunar valve atresia include an imperforate small membrane or a dome-shaped, imperforate, unicuspid valve as seen in some cases of pulmonary valve atresia. Absent semilunar valve results in an unguarded valve orifice with either no valve tissue, a slight ridge, or very small nubbins of valve tissue at the annulus. A single semilunar valve is also seen with a persistent truncal valve in truncus arteriosus. In contrast to the absent or atretic valve where there are separate annuli for the aortic and pulmonary valves (albeit represented by a blind ending dimple), a persistent truncal valve indicates that there is a single, common valve annulus that never divided into aortic and pulmonary valves orifices. Truncal valves most often have 3 cusps; however bicuspid and quadricuspid valves are not uncommon.

The most common valve malformation is an abnormal number of cusps, usually a bicuspid valve. The two cusps may be of similar or unequal size, and one sinus will contain the abortive commissure or raphe. The unicuspid valve with its single commissure is inherently stenotic with a variably hypoplastic annulus and frequent associated complex constellation of cardiac malformations. Bicuspid or quadricuspid valves, with otherwise well formed cusps, are functionally normal at birth and may occur as isolated lesions. However, long-term altered hemodynamics, particularly for the bicuspid aortic valve, often lead to acquired stenosis requiring surgical intervention in adulthood. The quadricuspid valve may remain asymptomatic or develop valvar regurgitation [5].

The valve abnormality that is most likely to require therapeutic intervention in childhood is the dysplastic semilunar valve. Valvar dysplasia is characterized by variably thickened cusps with altered contours resulting in valvar stenosis or, less commonly, regurgitation. The dysplastic aortic or pulmonary valve may occur as an isolated lesion, with multiple involved valves (polyvalvular dysplasia), with another cardiac lesion such as coarctation of the aorta or as part of a more complex constellation of cardiac lesions such as tetralogy of Fallot or Shone syndrome. It is not uncommon to have a combined dysplastic and bicuspid or unicuspid semilunar valve. The dysplastic quadricuspid valve is a feature of truncus arteriosus. A regurgitant, dysplastic valve is also commonly seen with truncus arteriosus.

There are a number of differences between aortic and pulmonic CSVD. The most common form of isolated congenital pulmonary valvular stenosis is the domed deformity with fusion of the valve cusps around a central fixed orifice [6]. In the aortic valve stenosis, the bicuspid, dysplastic valve is more common. Differences have also been noted between the forms of “absent” CSVD. While in an “absent” pulmonic valve there is usually a remnant of dysplastic valve-like tissue [7,8], in the syndrome of “absent” aortic valve more often there is only an endothelial ridge at the level of the valve annulus [8]. Even within polyvalvular dysplasia, out of the four valves the aortic valve is the “least involved” [9].

CSVD affecting multiple valves is often a manifestation of a syndrome (Marfans syndrome, polyvalvular dysplasia associated with Noonan syndrome or certain trisomies) or a systemic metabolic storage disease (mucopolysaccharidoses). This review largely focuses on cases of CSVD not associated with more extensive extracardiac disease, with particular attention to the most common CSVD requiring therapeutic interventions: dysplastic semilunar valves and the bicuspid aortic valve (BAV).

3. Normal Semilunar Valve structure

3.1. Valve Macrostructure

The major components of the semilunar valves include the three half-moon shaped cusps, three commissures, sinuses, the annulus, and surrounding supporting structures (Fig. 2, see also review of normal valve biology, physiology, and pathologies by Schoen et al. [10]). Each cusp is anchored in place by a coronet shaped ring of fibrous tissue (the valve annulus) that separates the ventricle below from the arterial wall above. The part of the annulus that separates two immediately adjacent cusps is the commissure. The valve annuli and cardiac fibrous skeleton, which link all four cardiac valves, play essential roles in anchoring the valve cusps during the billions of times the valve opens and closes over a lifespan. For each semilunar valve the sinuses of Valsalva are created by the pockets formed between the valve cusps and the adjacent arterial wall. Blood filling these sinuses during diastole of the cardiac cycle causes the cusps to coapt against one another centrally. The cusps overlap one another for a distance extending several millimeters below the free edge, ensuring valve closure of the lumen. The area of overlap, or lunula, is demarcated at its lower margin by a ridge that becomes more accentuated with increasing age, and is more prominent on the aortic than pulmonary valve. The center of the lunula has a localized area of fibrous thickening forming a discrete nodule (nodule of Arantius) that further ensures a tight seal of the closed valve. With systolic opening of the valve, the cusps collapse against the wall of the valve sinus. The integral relationship between valve cusps, commissures, sinuses, annulus and supporting structures is essential for normal valve function. Abnormalities in the valves due to any of these components may cause valvular dysfunction and may impact cardiac physiology as a whole.

3.2. Valve Microstructure and Mechanics

Valves experience ongoing stresses imposed by a complex mechanical environment, namely a variety of forces that differ depending on the valve region and portion of the cardiac cycle. However, the composition and layered microstructure of the valves are built to withstand these continual tensile, bending, compressive, and shear forces. The semilunar valves have four layers named ventricularis, spongiosa, fibrosa and arterialis, aptly named as the ventricularis faces the ventricle and the arterialis faces the artery (Fig 3). Each layer has a distinct composition, mechanical behavior, and functional role. Elastic fibers are accentuated in the ventricularis and facilitate elastic recoil of the cusps [11]. The middle spongiosa layer has a highly hydrated matrix of proteoglycans (PGs) and glycosaminoglycans (GAGs), which provides compressive strength and allows shear between layers during flexure [12]. The fibrosa is the supporting backbone of the cusp, composed predominantly of circumferentially arranged dense collagen that merges with the fibrous tissue of the valve annulus and fibrous skeleton of the heart. The often overlooked arterialis is a thin, frequently imperceptible layer predominantly comprised of elastic fibers that merges with the intima of the adjacent artery. Its function has not been well characterized. The mechanical interaction of the different layers is important to valve function [13]; indeed, even when the tissue as a whole is not strained, internally the fibrosa layer is under compression while the ventricularis is held in tension [14]. The intervening spongiosa is thought to lubricate the adjacent layers and also decrease bending stress [15].

3.3. Valve Cells

Two major cell types are found in cardiac valves, the surface lining valvular endothelial cells and the valvular interstitial cells (VICs) dispersed throughout the leaflet layers. VICs, the predominant and most studied valve cell type, produce the collagen, elastin, PGs, and GAGs that form the extracellular matrix, as well as matrix metalloproteinases and their inhibitors that break down and remodel the matrix [16]. Other reported VIC cellular functions include the production of cytokines, chemokines, and growth factors [16]. VICs are a heterogeneous population with a range of cell phenotypes, some of which appear more fibroblast-like and others more myofibroblast-like [17]. The fibroblast-like cell population consists of cells that contain organelles for matrix synthesis and secretion [16]. The more myofibroblast-like VIC subpopulation that expresses smooth muscle alpha-actin is considered an activated phenotype and are increased during physiologic and pathologic valve remodeling [18].

3.4. Extracellular Matrix Composition

The layered structural architecture of the semilunar valves is associated with layer specific composition of the extracellular matrix. Major components of the extracellular matrix include PGs, GAGs, collagen and elastin, as well as matrix metalloproteinases (MMPs) and various receptors and adhesion molecules. Studies of the extracellular matrix in the adult aortic valve reveal 60% collagen, 30% PG, and 10% elastin [19].

PGs and GAGs, also referred to as “mucopolysaccharides,” are components of the extracellular matrix throughout the body. The broad functional roles of PGs and GAGs, including those located in the heart valves, span a spectrum from embryologic development to tissue homeostasis; dysregulation of PGs and GAGs in valves has been associated with valvular disease [20,21]. PGs are comprised of a core protein with covalently attached chains of GAGs (dermatan and chondroitin sulfate) that resemble the bristles of a bottle brush [22]. The major GAG in adult aortic valves is hyaluronan (50–60% depending on age), which unlike the other GAGs, is not covalently attached to a protein core. Dermatan sulfate constitutes 19–24% of the valvular GAGs, followed by chondroitin sulfate (15–18%) and heparan sulfate (3–7%) [23].

The main PGs in valves are versican, decorin, and biglycan. Versican is a large PG that, with its highly hydrated chondroitin sulfate GAG chains, lubricate tissues and limit cell–matrix interactions [24]. The protein core of versican associates with hyaluronan and these combined aggregates have been suggested to be critical for the loose matrix that permits cell migration and proliferation during development, valve remodeling, and disease states [24]. Interaction of versican with microfibrillar proteins (fibulins and fibrillin) in other tissues also suggests a possible role for versican in tissue elasticity and/or elastic tissue formation [22,24]. Versican and hyaluronan are both most strongly expressed in the spongiosa layer of the valve [25].

Decorin and biglycan are small leucine-rich PGs composed of either one (decorin) or two (biglycan) chondroitin sulfate/dermatan sulfate GAG chains [26]. Both PGs, but particularly biglycan, are most strongly expressed in the inflow, ventricularis layer of the semilunar valves and least in the fibrosa [25,27]. Studies investigating binding of biglycan, and to a lesser extent, decorin, to elastin and its precursor tropoelastin, suggest a role for these PGs in elastogenesis [26] and is consistent with co-localization of these proteins in the ventricularis. Decorin is key to collagen fiber formation [28] and is the most abundant PG in the collagen rich fibrosa valve layer [25].

The predominant collagen subtype in heart valves is collagen I, which is concentrated in the fibrosa. When not under strain, the collagen fiber bundles in the fibrosa are crimped, held by elastic fibers and interconnections with the ventricularis [15]. This configuration allows the collagen uncrimp and the valve to stretch during valve closure; elastic fibers then return the collagen to its crimped state [15]. These collagen fiber bundles provide critical tensile strength [29]. Collagen III is present in all valve layers and confers more flexibility than collagen I [27,30].

3.5. Maturational and Physiologic Valve Changes with Age

Heart valve structure changes at both extremes of the age spectrum. While a detailed account of these changes is beyond the scope of this manuscript, a fundamental understanding of their spectrum is essential. Knowledge of age appropriate microanatomy is important to distinguishing normal maturational and physiologic alterations from valve pathology, particularly in the context of CSVD.

Recent studies have demonstrated that valve composition and accompanying mechanical properties normally change with age [25,31,32]. Not only does collagen content and cross-linking increase, as previously well established [33–35], but specific GAG sulfation patterns change with age in a valve and valve-region-specific manner [25]. Mechanically valves become stiffer and less extensible with age [31]. These normal age-related valve changes complicate the study of CSVD where maturational aging is superimposed onto structural and biological abnormalities. Altered hemodynamics and mechanical strains are known to stimulate compositional changes in normal valves, including increased collagen synthesis, MMPs, and myofibroblast marker expression [36–38]. How a structurally and biologically abnormal valve responds to the altered hemodynamics and loading patterns commonly present in CSVD remains largely unknown.

3.6. Use of Animal Models in the Study of Valve Physiology and Pathology

A number of animal models have been used to study heart valve physiology and pathology, largely based on anatomic similarity. The ability to manipulate the genetics of mice has proven very enlightening about the roles of different matrix components in valve disease [39]. Although the specific disruption of a single matrix component is clearly a powerful technology, the size of murine valve leaflets presents challenges to their analyses. Among

larger animal models, porcine and ovine valves are commonly employed to investigate various aspects of valve biology, physiology and pathology. In terms of shape and dimensions, it was shown that there was no significant difference in shape between porcine, bovine, and ovine valves, although ovine valves were significantly thinner and all of these animal valves had increased myocardial support underlying the left and right aortic valve leaflets compared to human aortic valves [40]. Among porcine, bovine, and ovine valves, only porcine valves were found to have a fibrous attachment of the non-coronary leaflet, making them more suitable for use in bioprosthetic valves [40]. Although studies of valvular extracellular matrix composition have found that these animal models generally demonstrate comparable microstructure to human valves, they have reported differences in expression of MMP1 between porcine and human valves [4,32], as well as differences in PG distribution [25,41]. An investigation of GAGs in porcine mitral and aortic valves of different ages found that GAG composition was similar to human mitral valves, although the specific GAG types hyaluronan and 4-sulfated iduronate were increased with age in porcine mitral valves, unlike human mitral valves [25,42].

With respect to animal models of valve diseases, several important distinctions between different species have been noted. For instance, in models of aortic valve calcification, pigs develop valvular lesions spontaneously, whereas other species such as rabbits and mice need a stimulating factor [43]. Another issue that must be considered in the use of animal models is choosing the most appropriate age animal for the given disease studied. Cells from 6 month-old pigs are commonly used in the study of aortic valve calcification, but given that there are profound age-related changes in the extracellular matrix composition of porcine valves and that the age-equivalency of the 6 month-old pig is a young adult humans, an older pig may be more appropriate for use in modeling human calcific valve disease [25,32].

In the future, it is likely that advanced approaches offering the ability to track the impact of specific matrix components on tissue biology and mechanics throughout development of the semilunar valves in animal models, coupled with continued investigation into the signaling and genetics involved in valvulogenesis, may reveal important insights and ultimately therapeutic interventions for patients with congenital valve disease. The effective application of animal models in these endeavors will require further understanding of differences between these animal models and human valve biology and pathology, as well as recognition of the limitations for each animal model.

4. Macroscopic Characterization of Congenital Semilunar Valve Disease (CSVD)

4.1. Bicuspid Aortic Valve (BAV)

The two cusps of the bicuspid aortic valve (BAV) are supported by two commissures; therefore the valve may be described as bicuspid/bicommissural (Fig. 4). A third abortive commissure or "raphe" is frequently present evidenced by a ridge within one sinus, either confined to the aortic wall or extending a variable distance onto the base of the cusp. The raphe should not be confused with an acquired fused commissure between conjoined cusps in a tricuspid aortic valve. A BAV raphe does not extend to the free margin of the cusp and its aortic attachment sits beneath the horizontal level of the cusp [44]. Although histologically the raphe often contains abundant elastic fibers, similar to the adjacent aortic wall, this is not a reliable distinguishing feature [44]. The two cusps of a BAV may be of similar or unequal size; however the raphe typically partitions the cusp into similarly sized components [44]. In patients younger than 18 years of age, the right and left coronary cusps are most often (70%) conjoined, with the coronary artery ostia on either side of the raphe [45].

At birth, the BAV cusps are most often thin and delicate, similar to the normal tricuspid aortic valve, and in this setting the valve is functionally asymptomatic. BAVs that become symptomatic earlier in childhood are often both bicuspid and dysplastic [46]. In contrast to the greater frequency of right-left coronary cusp fusion in bicuspid aortic valves, those valves with fusion of the right and noncoronary cusps have more significant and progressive valvar stenosis and regurgitation in childhood requiring therapeutic intervention [45].

4.2. Dysplastic Semilunar Valves

Semilunar valve dysplasia implies congenital abnormalities in the valve morphology reflected by alterations in the size, thickness, contour and/or consistency of the cusps. In severe dysplasia, the cusps appear as nodularly thickened, myxoid warty protrusions (Fig. 5). The spectrum of changes in milder forms of valve dysplasia includes more localized areas of myxoid nodularity (Fig. 6). Alternatively, the valve may be diffusely, uniformly thickened with redundancy at the free margins creating an irregular contour. The dysplastic valve may have 1, 2, 3 or 4 cusps and some degree of commissural thickening may be present.

While such macroscopic characterization may be adequate for making a clinical diagnosis of dysplastic CSVD, with additional knowledge of matrix components and microstructure abnormalities, avenues for possible treatments and prevention may become evident.

4.3. Acquired Macroscopic Valve Changes

Although potentially thin and delicate at birth, the altered biology and physiology of a bicuspid valve results in progressive fibrous thickening, often apparent by the second decade of life, with increasing rigidity of the cusps [47]. In adults, calcification is also common, within the cusps and forming nodular masses within the sinus, often involving the raphe [48]. Calcification is a later manifestation of the dysfunctional BAV, becoming increasing more apparent in the fourth and subsequent decades [47].

When assessing CSVD, particularly in older children and young adults, it may be difficult to distinguish some milder forms of underlying valve dysplasia from superimposed pathological changes due to altered hemodynamics. These acquired alterations may include focal or diffuse thickening of the cusps in an environment of valvar stenosis and rolled, everted margins caused by the regurgitant stream on an insufficient valve. Other acquired changes to be considered include post-inflammatory effects of infective endocarditis and iatrogenic effects of interventional commissurotomy and balloon dilations.

A frequently related scenario encountered in CHD is the Ross procedure in which the normal PV is placed in the AV position to replace a malformed or otherwise deficient AV. Although in this case the PV is not malformed, it does expose the PV to increased pressures. A recent study has shown that these valves remodel in response to this altered mechanical environment [49], with increased MMPs and VIC activation particularly early after the Ross procedure.

5. CSVD Microstructure and its Implications

5.1 Microscopic Characterization of CSVD

Despite the variation in the macroscopic appearance of dysplastic semilunar valves, there is a unifying theme to the valve microstructure: a disrupted trilaminar structure. Increased acid mucopolysaccharides are evident with an expanded spongiosa that intermingles with variable amounts of collagen and is associated with decreased to absent valvar elastic tissue [9,50,51] (Fig. 7). Bharati and Lev also reported that when compared to age-matched normal

valves and normal valves exposed to altered hemodynamics, dysplastic valves showed an increase in the spongiosa layer with vacuolar and lacunar degeneration [9,50,51]. Similarly, Hyams' study of a broad group of dysplastic valves, including both isolated and polyvalvular cases from fetuses to young adults, reported myxomatous nodules devoid of elastic tissue with pockets of collagenous tissue [52]. Other authors studying various types of congenitally diseased aortic and pulmonic valves report the same essential findings [6,53,54]. One recent study of 6 explanted, bicuspid, stenotic aortic valves from infants and children aged 1 month to 14 years, further correlated thickening of the cusps with increased collagen and PGs [53]. VICs were also disorganized, focally forming clusters, although there was no increase in cell proliferation [53].

Ongoing studies demonstrate that VICs derived from CSVD valves, including isolated dysplastic valves, also demonstrate phenotypic differences compared to age-matched controls. As VICs produce many of the components of the extracellular matrix, abnormalities in these cells, including their response to developmental, physiologic and pathologic stimuli, are prime candidates for further study. It remains to be determined whether these VIC abnormalities are a primary or secondary phenomenon, and what *in utero* and/or postnatal factors modulate their effects on the valve architecture and composition.

5.2. Mechanical Implications of CSVD Structure

The observed microstructural changes seen in CSVD have important mechanical, and therefore functional implications. According to Bharati and Lev, the preponderance of the changes seen in CPVD was in the spongiosa layer [51], which based on the contribution the spongiosa makes to leaflet mechanics could affect leaflet bending stress [55] and the ability of the leaflet to bear compression [55]. Computational modeling indicates that the layered nature of the valve decreases the stress of bending [56], therefore the decreased leaflet layer delineation seen in many types of CSVD could decrease valve mobility, as well as increase stress on the leaflet. Furthermore, the complex interaction that exists between the layers of normal valves, which is important to valve mechanical behavior [13,14], is lost. The increased valve thickness reported in a number of types of CSVD [9,53] would also result in an increase in bending stress, according to computational analysis [57].

Increased collagen content, whether present in congenital BAV [53] or hemodynamically acquired, would be expected to increase leaflet stiffness as well as leaflet and chordal stress [58]. However, the valve has a complex, heterogeneous distribution and alignment of collagen that contributes to anisotropic, region-specific material behavior that is important to proper valve function [56,58,59]. Therefore, the specific consequences of increased collagen would depend on the exact location and orientation of this additional collagen. Disorderly arrangement of collagen is likely to be just as important as the amount of collagen in CSVD. Mechanical valve stresses also stimulate the activation of VICs, which may contribute to the altered distribution of VICs with further modification of valve matrix and perpetuation of altered valve mechanics.

Other changes in CSVD have functional and mechanical implications. The nodularity common to many types of CSVD [9,53] would disturb fluid flow and alter shear stress on the valve endothelial cells. Valve endothelial cells are well known to respond to shear stress [60] and endothelial cell injury may be a key step in calcification of the aortic valve [61,62]. Elastic fibers, which are decreased in a variety of congenitally diseased valves [9,50,52,53], also have important mechanical consequences as they return the collagen to its crimped configuration after each heart beat [29]. Based on studies in which functioning elastic fibers were removed from normal valves, loss of elastic fibers in CSVD likely causes reduced extensibility and increased stiffness [63].

5.3. Insight into Possible Pathogenesis of Dysplastic CSVD

Alterations in blood flow patterns within the heart have long been thought to contribute to cardiac morphology, and form the basis of the concept of "form follows function." This concept is best illustrated by the correlation of the degree of hypoplasia of a cardiac structure with the amount of blood flow required for it to carry (i.e., marked hypoplasia of the ascending aorta in the setting of severe aortic valve stenosis or atresia). Cardiac valve morphology has also been hypothesized to be impacted by other structural cardiac anomalies altering normal blood flow patterns during development. Experimental models show that indeed mechanical stimulation is a necessary part of valve development. In zebrafish, decreasing wall shear stress leads to dysmorphic valves [64] and decreasing heart contractility and blood flow before endocardial cushion development leads to failed valve development [65]. In mice, the hyaluronan and collagen composition of developing atrioventricular valve cushions correlated with the cushions' biomechanics and blood flow [66]. Although the mechanical environment contributes to normal valve development, there are other factors that contribute to valve dysplasia. Bharati and Lev specifically noted abnormalities in CPVD compared to both normal and hemodynamically altered aged-matched controls [9], suggesting that altered hemodynamics alone cannot explain CPVD. The issue of mechanical stimulation becomes complex in the case of congenitally diseased valves because these valves often occur in the context of "structural" cardiac defects that alter the hemodynamics and forces that the valves experience. One could speculate that the effects from this mechanical environment then interact with altered biological processes within valves, such as dysfunctional collagen turnover, to create the pathological microstructure that we see in congenitally diseased valves. The complexities of this process remain to be determined, but certainly future studies delineating the molecular mechanisms underlying the interactions between the mechanical environment and biological signaling cascades in proper valve maturation could lead to novel therapeutic strategies for congenitally diseased valves.

Observations in several congenital and acquired valvular disorders may provide further insight into the pathogenesis of dysplastic valves. Dysfunctional BAVs excised in childhood, and therefore likely to have been dysplastic, have been found to be deficient in fibrillin [67]. Fibrillin deficiency is also a feature of infantile Marfan's syndrome, which is attributed to mutations in the fibrillin-1 gene, and has macroscopic myxomatous valvular changes similar to dysplastic valves [68]. Fibrillin is a major component of the extracellular matrix, essential to normal elastic tissue formation. It is integrally involved in TGF β regulation [69], a growth factor essential to major signaling pathways including the epithelial (endocardial)-mesenchymal transition in atrioventricular valve development [70]. Fibrillin also influences the effects of TGF β on VICs [71]. TGF β expression is altered in the myxomatous valves in Marfan's syndrome [72] as well as in non-syndromic myxomatous mitral valve disease and degenerative aortic valvular disease in adults [73,74]. Alterations in fibrillin and TGF β during *in utero* valvular development would impact the composition and integrity of the extracellular matrix, potentially altering the manner in which the valve responds to mechanical and other stimuli, and ultimately distorting normal valve morphology.

Similarities between the microstructure of dysplastic valves and features of immature valves during normal development have led some investigators to postulate that dysplastic valves represent an arrested step in development [4,50,52]. Recent studies characterizing changes in specific valve matrix components and structure during fetal and postnatal valve development demonstrated that many of the matrix components in the first trimester were ubiquitous throughout the valve layers, but became localized to specific layers in later trimesters, with increasing elastic fiber content, paralleling delineation of layers that become evident in Movat stained valves [4,25,32]. Interestingly, expression of fibulin and fibrillin

correlate with the transition from immature mesenchymal tissue to mature valve, suggesting that the altered valve morphology in children with BAV deficient in fibrillin may in part relate to a persistent immature matrix [75]. Delineating the molecular mechanisms and signaling pathways involved in these maturation processes could lead to novel, pathway-specific interventions for particular congenital valve diseases.

Alterations in the interaction of VICs with the extracellular matrix may be operative in other forms of myxoid valvular heart disease. Mutations in filamins have been associated with myxomatous changes in familial mitral valve prolapse as well as a multi-system disorder with polyvalvular dysplasia [71,76]. Filamin has also been postulated to interact with the TGF β pathway [71]. The effects of these varied genotypic matrix alterations leading to heart valves with similar microstructure, suggest that valvar dysplasia, as currently defined, may reflect a final common morphologic pathway resulting from alterations of components of the extracellular matrix. The potential central role of TGF β and its interaction with VICs and matrix components remain to be defined.

Observations in transgenic mouse models of Marfans with fibrillin-1 mutations and mitral valve abnormalities also provide insight into potential therapeutic interventions. Perinatal administration of neutralizing antibodies to TGF β rescued the normal mitral valve phenotype [72]. Further delineation of molecular mechanisms of normal valve development and alterations in CSVD may provide pathways for other potential therapeutic interventions.

6. Microstructural Characterization of Bicuspid Aortic Valve (BAV) and its Implications

Like many different types of CSVD, BAV can range from valves that appear normal microscopically, except for the macroscopic configuration of the cusps, to dysplastic leaflets complete with loss of the internal layered structure. Typically patients with BAV that present with valvar dysfunction in early childhood have more dysplastic valves as compared to BAV presenting in adults with more acquired hemodynamic changes and superimposed calcific changes. Studies of the extracellular matrix of BAV in early infancy and childhood, prior to the interceding effects of prolonged hemodynamic abnormalities, may shed light on the role of altered matrix in BAV.

6.1. Proteoglycans (PGs) and Glycosaminoglycans (GAGs) in BAV

Studies assessing specific matrix components and their structure in BAV are limited. The previously described matrix composition of dysfunctional BAVs in childhood reflected increased PGs, correlating with the myxoid changes seen in dysplastic CSVD. In contrast, Baig's 1979 study found less acid mucopolysaccharide content in adult BAV compared to age-matched controls [77]. In terms of particular GAGs, there was more dermatan sulfate in the 21–40 year-olds, and less hyaluronan with increased chondroitin sulfate in the 41–60 year-olds in BAV compared to age-matched controls [77]. The increase in dermatan sulfate largely reflects the greater content of decorin, the PG important in collagen fiber formation [28,42], and there was a corresponding increase in valvar collagen in this adult population. Chondroitin sulfate is a component of several PGs, however it is most abundant with versican, suggesting a possible increase in this PG in the older BAV population. Versican is important to elastic fibrillogenesis [78], and alterations in this PG may relate to abnormal elastin [53] and fibrillin-1[67] content in BAV. Decreased hyaluronan with increased collagen would impact valvar mechanical behavior, conferring increased stiffness to the valve. Cell-matrix interactions are also likely to be impaired with possible effect on other processes such as cell proliferation that have been shown to be impacted by hyaluronan alterations in other non-cardiac tissues [79]. The contribution of years of altered

hemodynamics, created by BAV, to these varied matrix abnormalities remains to be determined. Nevertheless, altered GAG content has been implicated in the calcification of congenitally normal aortic valves [80] and may also contribute to increased calcification in BAV. Altered GAG content has also been implicated in bioprosthetic valve failure [81]. As we learn more about the biological and mechanical roles of these PGs and GAGs in normal valve function, these may become targets for therapeutic intervention in CSVD.

6.2. Fibrillar Proteins and Matrix Metalloproteinases in BAV

A recent study in BAV from children revealed a deficiency in fibrillin-1 [67]. Interestingly, expression of fibulin and fibrillin correlate with the transition from immature mesenchymal tissue to mature valve, suggesting BAV could involve a suspension of development [75]. Furthermore, as mentioned earlier, fibrillin is integrally involved in TGF β regulation [69], a growth factor essential to major signaling pathways including the endocardial-mesenchymal transition in atrioventricular valve development [70]. Potentially, the deficiency of fibrillin-1 observed in BAV [67] could result in altered growth factor regulation and matrix abnormalities in BAV.

Another study showed that BAV, compared both to normal and diseased tricuspid aortic valves, showed increased MMP2 and MMP9 expression [82]. MMP2 and 9 are both gelatinases that degrade elastic fibers; indeed, another study of BAV from pediatric patients reported fragmented and decreased abundance of elastic fibers [53]. As discussed earlier, this decrease in elastic fibers could have important implications for valvular mechanics. Increased MMP expression in BAV is particularly interesting in light of increased MMPs evident in normal valves experiencing altered hemodynamics [20,83] and valves undergoing remodeling [18,49], as well as the association of increased MMPs with other valve pathologies [73,84]. It may be that increased MMP expression in valves is a common pathway for various stresses.

6.3 Possible Pathogenesis of BAV

While a comprehensive discussion of signaling involved in valvulogenesis and genetics of BAV pathogenesis is outside the scope of this review article (for more thorough reviews see [85–88]), a limited discussion of pertinent data here yields additional insight. There is increasing evidence that the pathogenesis of BAV is complex with multiple factors influencing valvulogenesis, as well as post-natal valve structure and function. Notch signaling pathways have been found to be important in aortic valve development and mutations in the gene cause a spectrum of aortic valve anomalies, including BAV [89]. Notch1 is a transmembrane receptor involved in cellular signaling pathways for cell differentiation and cell fate, influencing cell ligand and receptor interactions of neighboring cells and regulating epithelial to mesenchymal transition in the cardiac cushions from which the valves develop [87]. Dysplastic atrioventricular and pulmonary valves have also been associated with mouse models in which target genes of NOTCH1 (HRT1 and HRT3) have been inactivated [90]. Furthermore, Notch1 has been associated with aortic valve calcification, possibly via its expression in endothelial and endocardial cells [87].

Neural crest cells have a key role in epithelial to mesenchymal transition with septation of the outflow tract of the heart, and these cells are also impacted by NOTCH1 signaling [91]. Neural crest cells have been suggested to contribute to semilunar and atrioventricular valve development, although confirmatory data is lacking [87,91]. It should be noted, however, that the cardiac anomalies typically associated with neural crest abnormalities (interrupted aortic arch and truncus arteriosus) often have either an associated BAV or abnormal number of valve cusps that may be dysplastic. Further investigation of these molecular genetic

pathways may provide insight into the pathogenesis of aortic valve anomalies and provide potential avenues for therapeutic intervention.

6.4. Aortic Wall Abnormalities in BAV

The association of the BAV with a number of aortic wall abnormalities, including aortic aneurysm and dissection, led to speculation that matrix abnormalities in these patients are not limited to the aortic valve. Approximately 20–50% of patients with BAV have an associated cardiovascular complication [52], the most common being coarctation of the aorta and interrupted aortic arch. In fact, 50% of patients with both BAV and coarctation of the aorta have a dissection of the aorta [47]. In the past it was thought that dilation and/or dissection of the ascending aorta were secondary to the altered hemodynamics caused by BAV, (i.e., post-stenotic dilatation), but multiple studies now point towards an intrinsic defect in the aortas of BAV patients. Significant dilation of the aorta, even in childhood, may occur independent of whether or not the valve is stenotic or regurgitant [92]. Furthermore, after valve replacement for aortic stenosis, dilation of the aorta may continue to increase in patients with BAV, as compared to a decrease in size for tricuspid aortic valves [93].

Microscopic findings in the aorta in BAV include decreased collagen I and III, elastic fiber fragmentation, decreased number and wider spacing of elastic lamellae, and cystic medial necrosis [94–96]. Just as fibrillin-1 has been found to be decreased in the BAV, fibrillin-1 has also been reported to be decreased in the aortas of BAV adults compared to controls [75]. Some postulate that the BAV and aortic complications occur by a common mechanism of cellular apoptosis [97] based on increased apoptosis of smooth muscle cells and higher expression of pro-apoptotic markers found in the aortas of BAV patients [98]. Others suggest that dysregulation of MMPs leads to both BAV and its aortic complications [99]. However, in non-dilated aortas from BAV, no increase in gene expression for these MMPs was identified, suggesting an important role for hemodynamic alterations for the effects of these MMPs [100]. It appears likely that a combination of genetic and hemodynamic alterations contribute to the pathogenesis of BAV and its associated pathologies. Considerable work remains to further elucidate the specific factors and mechanisms involved. Parallels between the clinicopathologic cardiac findings of Marfan's syndrome and BAV, particularly with respect to fibrillin and TGF β , provide a promising basis for pathogenetic and therapeutic interventional studies.

Characterizations of matrix composition abnormalities in BAV certainly add considerable insight into BAV, but they also may provide insight into other types of congenital valve diseases. For instance, given that BAV matrix abnormalities are more widespread than just the valve, it could be hypothesized that other, less studied congenital valve diseases also demonstrate matrix abnormalities in other portions of the heart and aorta. Evidence to support this hypothesis was provided by a review of atrioventricular valve dysplasia reporting concomitant myxomatous areas of the interatrial septum [101]. Another study reported both aortic and pulmonic valve atresia to be associated with myocardial disarray in the interventricular septum [44]. The preponderance of concomitant cardiac anomalies for congenital valve diseases could also be considered an argument for a broader, underlying pathological mechanism.

7. Conclusion

This article has summarized the microstructural findings of CSVD and examined these in light of recent advances in the knowledge of normal valve biology, microstructure, and mechanics. This discussion not only yields further insight, but also presents important

questions and directions to be addressed in future research to elucidate the pathogenesis of CSVD.

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Fig. 1.
Atretic, domed shaped pulmonic valve from a 2 year-old with 3 centrally fused cusps.

A.

B.

Fig. 2.
A) Normal aortic valve from an 8 year-old viewed from the left ventricular outflow track. B) Aerial view of a normal aortic valve from a 13 year-old demonstrating thin, delicate cusps.

Fig. 3.

Movat pentachrome of a normal aortic valve from a 2 year-old illustrating the histological layers. In Movat pentachrome stain yellow indicates collagen, blue/green indicates proteoglycans and glycosaminoglycans (PGs/GAGs), black indicates elastic fibers, and red-purple indicates muscle. The annular attachment region of the leaflet is circled in red, the direction of the leaflet free edge is indicated by the red arrow. The elastic fiber-rich ventricularis layer, PG/GAG-rich spongiosa layer, and collagen-rich fibrosa layer are all labeled with arrows. The thin arterialis layer is the barely perceptible layer lining the fibrosa that merges with the intima of the aorta and is labeled with an arrow. Scale bar indicates 500 μm .

Fig. 4. Bicuspid stenotic aortic valve from a 40 year-old with relatively well-formed, but diffusely thickened cusps and the raphe (arrow).

Fig. 5. Severely dysplastic, nodularly thickened aortic valve (arrow) with associated left ventricular hypertrophy from a 2–3 month-old.

Fig. 6. Mildly to moderately dysplastic aortic valve from an 8 month-old with localized areas of nodular thickening (arrow).

Fig. 7. Dysplastic pulmonic valve from a 2 year-old (top) compared to a normal (control) PV from a 2 year-old (bottom). Movat stain demonstrates loss of the normal layered leaflet structure in dysplastic valve compared to control. Arrow points to elastic fibers in normal PV (bottom) that are absent in the dysplastic PV (top). Asterisks (“*”) highlight areas of mucopolysaccharides in both valves; these areas are increased in the dysplastic PV (top) compared to the normal PV (bottom). Carrot (“^”) highlights collagen in the fibrosa in the normal PV (bottom) that is absent in the dysplastic valve (top). Scale bar in upper panel indicates 1 mm; scale bar in lower panel indicates 500 μ m