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**Title:** Aortopathy in patients with a bicuspid aortic valve : determining susceptibility for aortic complications  
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1. BACKGROUND

Bicuspid aortic valve (BAV) is the most common congenital heart defect, being present in almost 1% of the general population (1-3). The prevalence of BAV depends on ethnicity and gender, as BAV is more common in Caucasians and males, however these factors have not always been considered in analyses of BAV prevalence (4;5). The male to female ratio of BAV is estimated to be approximately 2:1 (3;6).

BAV is associated with an increased risk of aortic valve stenosis, aortic regurgitation, as well as thoracic aortic dilation, occurring in approximately 40-60%, and aortic dissection, as compared to patients with a tricuspid aortic valve (TAV) (7;8). Aortic dilation was long thought to be secondary to the valve disease itself, but increasing evidence points to a common origin for both sites of pathology (3;6;9;10). Although the relative risk of aortic dissection is lower than in patients with Marfan syndrome (MFS), there are likely as many, if not more, dissections in patients with BAV given the significantly greater prevalence of this disease (11). Hence, BAV-associated aortic wall pathology has important public health implications and has therefore long been a subject of intensive clinical and basic research.

Optimal management of patients with BAV and associated ascending aortic dilation requires an approach, carefully assessing various risk factors of the aortic valve and wall and discerning individual indications for ongoing surveillance, medical management, and surgical intervention. Operative management of aortic dilation presents a complicated clinical problem given the unpredictable lifetime risk of morbidity and mortality related to aortic wall pathology in BAV and major surgical intervention required to address these risks (11-13). This is not a new clinical issue; the complexities of aortic wall disease have long been appreciated (14;15) but our understanding and ability to diagnose and intervene have also evolved considerably. Recommendations on when to intervene surgically for thoracic aortic dilation for BAV patients have been progressively expanded over the past 15 years. BAV is now widely considered to be an independent risk factor for an acute aortic event, which has led surgeons to consider whether an additional concomitant aortic procedure should be performed at the time of aortic valve replacement.

The fact that some BAV patients can present with severe forms of aortic wall pathology (16), with an early onset in life (17), and clear familiar inheritance
for aortic complications (10) has led to greater general aggressiveness towards all BAV-related vascular wall pathology. With some arguing that aortic dimensions indicating surgical intervention in BAV should be similar to those used for other genetic diagnosis with an increased risk for aortic complications, such as MFS (18-20). Absolute aortic diameter remains the most used clinical parameter to guide intervention, although indexed and non-size predictors have also been proposed (21-23). Guideline recommendations for surgical intervention based on the ascending aortic diameter have decreased from ≥5 cm (24-27) to ≥4.5 cm for patients with BAV undergoing concomitant aortic valve replacement, with others proposing even lower thresholds for intervention (21;28-30). However, these recommendations remain controversial (13;31;32) as there is still no clear understanding of how the dimensions of the ascending aorta change over long term in patients with BAV. A great number of BAV patients show a less severe natural clinical course (12). The debate will only calm down when our ability to stratify BAV patients will improve, so that indications will be less subjective and more individualized. Therefore among the most important tasks at hand for the research in this field is to define and validate tools and criteria for risk stratification, in order to more rationally guide surgical management.

To reach this, a detailed knowledge of the pathophysiology of aortic wall pathology in BAV and the clinical and genetic factors increasing the risk of aortic complications is necessary.

As an introduction to this thesis, an overview of the current status of knowledge of aortic wall pathology in bicuspid will be provided. First, the cardiac anatomy will be described, along with the embryologic development of the cardiac valves. Furthermore, the two most discussed possible pathogenetic mechanisms of aortic wall pathology in BAV: haemodynamics and intrinsic wall abnormalities will be discussed and compared. Finally, the aims of this thesis and the chapter outline are presented.

2. CARDIAC ANATOMY

The main function of the cardiovascular system is to transport nutrients and oxygen to the entire body. The heart can be thought of as two pumps in
series that send a fluid (blood) through tubes (vessels) that eventually return to the pump.

Each ‘pump’ in the heart is made up of two chambers; an atrium and a ventricle, giving the heart a total of four chambers. The atria receive blood returning from the circulation and pass it to the ventricles. The ventricles make up most of the heart’s volume, with the left ventricle being the larger of the two. The ventricles receive blood from the atria and pump it through arteries to the body.

The heart uses a series of valves to ensure that blood flows in one direction into and out of the heart. Heart valves are made of tough, flexible fibrous tissue that are oriented in such a way that blood can only go through the valve in one direction. A valve only opens when the blood exerts enough pressure on it, forcing it to open for blood to flow through. When this pressure drops, the valve returns to its originally closed position, preventing blood from flowing in the wrong direction. A pressure gradient is developed as blood flows through the body, and blood only flows from a high pressure to a lower one.

Like the heart chambers there are four heart valves, two atrioventricular valves and two semilunar valves. An AV valve is located between each atrium and ventricle, with the tricuspid valve on the right and the mitral valve on the left. The valve opens when the atrial pressure is greater than ventricular pressure. When ventricular pressure exceeds atrial pressure, the valve closes again. The area of the valve cusps are about twice that of the passageway they cover, creating a large overlap of the cusps when they close. This overlap helps to prevent the backflow of blood (regurgitation) into the atrium.

A semilunar valve is located between the right ventricle and pulmonary trunk (pulmonary valve) and the left ventricle and the ascending aorta (aortic valve). Similar to the AV valves, when left or right ventricular pressure exceeds aortic or pulmonary artery pressure, the valve opens. When ventricular pressure decreases, the three cusps of the valves close, preventing blood from flowing back into the ventricle.

The heart has two atria and two ventricles because there are two different blood flow circulation paths. The circulation path controlled by the right side of the heart is a low-pressure system known as the pulmonary circulation. Oxygen depleted blood enters the right atrium through the caval veins and coronary sinus and is pumped to the lungs by the right ventricle through the pulmonary trunk. The blood receives oxygen in the lungs and is sent back to
the left side of the heart through the pulmonary veins and is returned to the left ventricle through the left atrium. The left ventricle pumps the oxygen-rich blood through the aorta to the rest of the body. As the blood flows farther from the body, oxygen concentration is diminished by exchange with CO2 in the body tissues and organs. The blood returns to the heart through the caval veins in the right atrium, depleted of oxygen, completing the cycle. This circulation path is known as the systemic circulation. Due to the large distance the blood travels, this is a high-pressure system. After entering the right atrium, the blood repeats the two circulatory paths.

Figure 1. Schematic overview of the heart.
AA: ascending aorta; AoV: aortic valve, LA: left atrium; IPA: left pulmonary artery; LV: left ventricle; MV: mitral valve; PT: pulmonary trunk; PV: pulmonary valve; Pv: pulmonary vein; RA: right atrium; rPA: right pulmonary artery; RV: right ventricle; TV: tricuspid valve; VCI: vena cava inferior; VCS: vena cava superior.
Throughout the cardiac cycle, blood pressure increases and decreases. The aorta is an elastic artery (with a large number of collagen and elastin filaments in the tunica media), which gives it the ability to stretch in response to each pulse to maintain a relatively constant pressure in the aorta despite the pulsating nature of the blood flow. This is called the ‘Windkessel effect’ of the elastic artery.

3. CARDIAC EMBRYOLOGY

a. Semilunar valves
The heart is derived from the anterior splanchnic mesoderm. It forms from two crescent-like cardiogenic plates. After fusion of these plates in the midline, a primary heart tube is formed (33) that shows peristaltic contraction at 3 weeks of development in a human embryo. The primary cardiac tube is lined on the inside by endocardium and on the outside by myocardium consisting of about two cell layers. A thick basement membrane is sandwiched in between referred to as cardiac jelly, containing water-binding extracellular matrix molecules including hyaluronic acid. At a later stage the cardiac jelly is restricted to endocardial cushions lining the myocardial outflow tract and the atrioventricular canal (34). The myocardium-lined primary heart tube initially consists of a small atrial component (connected to the sinus venosus), an atrioventricular canal, a ventricular inflow tract and a small outflow tract (connecting to the aortic sac). On the borderline of the ventricular inflow tract and the outflow tract a bulboventricular or primary fold is present. These cardiac components are derived from the mesoderm of the first heart field. The addition of dorsal cardiac mesoderm positioned between the primary heart tube and the primitive gut, the so-called second heart field (SHF) mesoderm, is essential for the subsequent development of all cardiac components. This SHF mesoderm can reach the heart tube at both the arterial (anterior SHF) and venous poles (posterior SHF). At the arterial pole the anterior SHF-derived mesoderm supplies the myocardium of the right ventricle up to the right side of the ventricular septum. The SHF also contributes to the semilunar valves and the walls of the great arteries. For subsequent remodeling, septation, valve formation and coronary vascular development two other cell populations are added to the heart, being the neural crest cells and the epicardium. Semilunar valve formation
includes remodeling of the distal end of the endocardial outflow tract cushions that derive from the cardiac jelly and receive mesenchymal cells from the overlying endocardium. During normal development, separation of the arterial orifice level results in three semilunar valve cusps in the aortic and pulmonary orifices. Abnormal development includes both the occurrence of deficient numbers (as well as excessive numbers) of valve cusps (35). Several gene mutations have now been reported in the human population to play a role in the development of BAV, which will be further discussed in the paragraph ‘Genes related to BAV in animal models and human population’. However, the exact cause of BAV has not been elucidated, there are even indications that the anterior SHF is involved and that not only the aortic valve is abnormal but the wall of the ascending aorta is also included (35). The mature cusps of the aortic valve are usually less than 1 mm thick and consist of three layers: the collagen-rich fibrosa located at the aortic side of the valve, the ventricularis with an abundance of elastin and located at the ventricular side of the valve, and, in between these, the spongiosa, which rich in proteoglycans.

b. Ascending aorta
Recent lineage tracing studies in transgenic mice with Nkx2.5 (5) have shown that SHF progenitor cells give origin to three specific cell lines: (1) outflow tract and right ventricular myocardium, (2) endothelial-derived endocardial cushion cells, which are in part derived from the endothelium and (3) vascular smooth muscle cells (VSMCs) of the great arteries (36). Recently, Harmon et al presented data on the boundary where SHF-derived VSMCs meet neural crest cell-derived VSMCs at the base of the aorta (5). The SHF contribution to the aortic media then forms a vertical seam complementary with neural crest-derived VSMCs (36). Next to contributing to the vascular wall, a population of neural crest cells migrates to the outflow tract cushions where they are important for semilunar valve formation and outflow tract septation (37;38). Preliminary data show a contribution of the arterial epicardium to the VSMCs of the ascending aorta (39).

The wall of the ascending aorta consists of three basic layers: the internal layer, tunica intima, which contains a single layer of endothelial cells and a subendothelial thin layer of connective tissue and VSMCs; the middle layer, tunica media, which mainly contains VSMCs, elastic fibers and collagen; and the outer layer, tunica adventitia, which predominantly contains loosely
organized collagen fibers, small blood vessels and fatty tissue. The intima and the media are separated by the lamina elastica interna and the media is separated from the adventitia by the lamina elastic externa. The aortic media is arranged in lamellar units where two elastic lamellae enclose VSMCs, which are surrounded by extracellular matrix that contains microfibrils, small elastic fibers, collagen, and proteoglycans. The extracellular matrix interconnects the two elastic lamellae as well as connecting the elastic lamellae with the VSMCs (40-42). Elastin fibers from the lamellae protrude between thick collagen fibers (containing collagen I, III, and V) and together with microfibrils of fibrillin-1 and collagen VI (also containing some fibronectin) facilitate the VSMCs-elastin interaction (43).

4. GENETIC BASIS OF BAV

In 1866 it was already suggested by Peacock that BAV is congenital in origin (44). It has since then been confirmed that BAV is indeed related to other cardiac malformations. BAV is reportedly found in 25-85% of patients with
aortic coarctation (45). BAV further is found co-existing with hypoplastic left heart syndrome and these two conditions are to some extent genetically linked (46). Shone’s syndrome is characterized by a supravalvular mitral ring, parachute mitral valve, subaortic stenosis, and aortic coarctation. Bolling et al. reported that 63% (19/30) of their patients with Shone’s syndrome had a BAV (47). William’s syndrome is a neurodevelopmental disorder that is also associated with cardiovascular conditions such as supravalvular stenosis, peripheral pulmonary artery stenosis, aortic coarctation, and BAV (48). BAV is found in about 25% of individuals with Turner’s syndrome and is present in 95% of patient with Turner’s syndrome who suffer from aortic dissection (49;50). BAV has been noted in conjunction with ventricular septal defects (51), patent ductus arteriosus, (52;53) and atrial septal defects. BAV is also associated with left coronary artery dominance (54;55). Heritability of BAV formation is suggested to be as high as 89% (56). BAV has an inheritance consistent with an autosomal dominant pattern with reduced penetrance (57;58) and about 9% of first-degree relatives of BAV individuals will also have the malformation (56;59). BAV formation has been linked to several different chromosomes and gene mutations, which suggests complex inheritance (57;60-62).

5. GENES RELATED TO BAV IN ANIMAL MODELS AND HUMAN POPULATION

Since BAV is a congenital malformation, it is reasonable to expect that mutations in genes encoding transcription factors, extracellular matrix components, and proteins of signaling pathways that are implicated in valvulogenesis are important in BAV formation (Table 1) (63-67). Linkage analyses revealed an association between BAV and chromosome 9q34-35 and subsequently NOTCH1 in humans (60). NOTCH1 encodes a transmembrane receptor and its signaling pathway is important in developmental processes and organogenesis. NOTCH signaling is highly conserved in evolution and, regardless of what animal model is used, perturbations in the pathway inevitably lead to developmental abnormalities (review (68). NOTCH1 is expressed in endocardial cells found in the common outflow tract in mouse embryos (69) and NOTCH signaling is suggested to have a role in BAV formation and valve calcification (60). Smad6
Table 1 Genes related to valvulogenesis in BAV formation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acvr1</td>
<td>Activin A receptor, Type 1 (Alk2)</td>
<td>Member of the TGFβ superfamily</td>
</tr>
<tr>
<td>EGF superfamily</td>
<td>Epidermal growth factor</td>
<td>Signaling pathway</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide, NOS3</td>
<td>Signaling pathway</td>
</tr>
<tr>
<td>ErbB</td>
<td>V-erb-b2 erythroblastic leukemia viral oncogene homolog</td>
<td>Epidermal growth factor receptor signaling pathway/family of receptor tyrosine kinases</td>
</tr>
<tr>
<td>GATA5</td>
<td>GATA binding protein 5</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>KCNJ2</td>
<td>Inward-rectifying potassium channel Kir2.1</td>
<td>Voltage-gated potassium channel activity involved in cardiac muscle action potential repolarization</td>
</tr>
<tr>
<td>NF-1</td>
<td>Neurofibromin 1</td>
<td>Ras signaling pathway</td>
</tr>
<tr>
<td>NFATc1</td>
<td>Nuclear factor of T cells cytoplasmic 1</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Nkx2-5</td>
<td>NK2 homeobox 5</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>NOTCH1</td>
<td>Family of transmembrane receptors</td>
<td>Signaling pathway</td>
</tr>
<tr>
<td>Smad6</td>
<td>SMAD family member 6</td>
<td>Signal transducer and transcriptional modulator in BMP signaling</td>
</tr>
<tr>
<td>Sox9</td>
<td>SRY (sex determining region Y)-box 9</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>TGF-β superfamily</td>
<td>Transforming growth factor beta</td>
<td>Signaling pathway</td>
</tr>
<tr>
<td>Tbx20</td>
<td>T-box 20</td>
<td>Transcription factor</td>
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<tr>
<td>Twist-1</td>
<td>Twist homolog 1</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
<td>Signaling pathway</td>
</tr>
<tr>
<td>Wnt/β-catenin Canonical Wnt signalling pathway</td>
<td>Wingless type MMTV integration site family/cadherin-associated protein beta</td>
<td>Wnt, growth factor; β-catenin, co-activator of transcription factors</td>
</tr>
</tbody>
</table>

family member 6) encodes a protein that functions as a signal transducer and transcriptional modulator in BMP signaling (bone morphogenetic protein, a member of the TGF-β superfamily). Smad6 is expressed in the embryonic outflow tract in mice and genetic variants of Smad6 predispose for BAV in humans (70). BAV has also been linked to chromosomes 18q22, 5q21 and
13q34; however which specific genes within these loci are associated with BAV is unknown (61). The empirical association of BAV and hypoplastic left heart syndrome has also been confirmed genetically (46). The mutation of the inward-rectifying potassium channel Kir2.1 (KCNJ2) found in patients with Andersen’s syndrome (a combination of characteristic physical features and arrhythmias) has also been suggested in BAV disease (62). A number of other genes are implicated in BAV formation in mice but their relevance in humans remains to be shown. These include Nkx2-5 (NK2 homeobox 5, transcription factor) (71), eNOS (endothelial nitric oxide, Nos3) (72), GATA5 (GATA binding protein 5, transcription factor) (73), and Acvr1 (activin A receptor, Type 1, alias Alk2, member of the TGF-β superfamily) (74). There is a marked phenotypic variability in individuals with BAV; it is not always familial and/or associated with other cardiac malformations. Furthermore, not all individuals with BAV develop valve and/or aortic disease and therefore might remain unidentified (75-77).

6. BAV PHENOTYPE

It is now recognized that BAV should not be considered as one single entity, but that distinct morphological phenotypes are distinguishable based on the presence and number of raphe, as classified by Sievers et al. (6) Most BAVs consist of one free and two cusps that are conjoined (or have failed to separate during embryonic development). The term ‘raphe’ defines the conjoined area of the two underdeveloped cusp turning into a malformed commissure between both cusps (6). Variable orientation of the raphe/fused commissure in relation to the sinus are seen: fusion of the right (RCC) and left coronary cusp (LCC) with a raphe (RCC/LCC), fusion of the right and non-coronary (NCC) cusp with a raphe (RCC/NCC), fusion of the left and non-coronary cusp with a raphe (LCC/NCC). This orientation of the raphe defines the subcategory in the classification of Sievers et al. referring to three types: type 0, valve with no raphe; type 1, valves with one raphe; and type 2, valves with two raphe. In our pathology papers we used a modified classification: type 1: RCC/LCC, type 2: RCC/NCC, type 3: LCC/NCC (Fig. 3). BAV phenotype is suggested to have an incomplete heritability (78) i.e. families with several BAV individuals will not all have the same BAV phenotype. The etiology of the different BAV phenotypes seems to differ. Mice
The RCC/NCC phenotype is thought to be the result of perturbations in the formation of endocardial cushions of the common outflow tract that occurs before septation of the outflow tract (79). By contrast, the RCC/LCC phenotype is suggested to be the result of an abnormal septation of the common outflow tract and to be linked to neural crest cell function on the basis of studies in inbred Syrian hamsters (79).

7. BAV AND AORTIC WALL PATHOLOGY

a. General introduction

BAV is usually asymptomatic in children and young adults and is commonly an incidental finding. Symptoms and physical findings associated with BAV are mainly related to the associated valvular dysfunction, being aortic stenosis, aortic regurgitation or a combination of both conditions. Ascending aortic dilation is rarely symptomatic whereas aortic dissection is usually associated with an acute onset of severe chest and/or back pain and is accompanied by signs of organ dysfunction and shock.

Some BAVs have an accelerated progression of valve thickening and calcification which is generally evident in the fourth decade of life (77;80).
(5;81;82). The valvular changes frequently progress into clinically significant valve stenosis (83). Excessive folding and creasing as well as asymmetrical and turbulent flow patterns due to a morphological 'stenosis' are thought to cause increased stress and subsequently early regurgitation, (not secondary to infective endocarditis) (77;80) Furthermore, BAV individuals are predisposed to infective endocarditis (77;80). The risk of bacterial colonization is greater owing to increased shear stress and subsequent endothelial damage which in turn leads to platelet aggregation and fibrin deposition. Microorganisms tend to adhere to and multiply in these platelet-fibrin vegetations (84-86) (review (87)). BAV phenotype has been proposed to affect the susceptibility for valve disease; BAV individuals with a RCC/NCC configuration are prone to develop valve disease in childhood, whereas BAV individuals with a RCC/LCC configuration tend to develop valve disease in adulthood (78). All together approximately 50% of individuals with BAV will have to undergo aortic valve surgery (12).

BAV individuals have an age-adjusted relative risk of aneurysm formation of approximately 86% in comparison with the general population, and about 25% of BAV individuals will develop indication for ascending aortic replacement (12).

Aortic dissection is caused by disruption of the intimal layer of the aortic wall, which results in bleeding between the aortic wall layers and creation of a dissection plane (88;89). The condition is associated with malperfusion of vital organs and predisposes for aortic rupture. The risk of aortic dissection is eight-fold higher in BAV individuals than in the general population (12).

b. Aortic valve stenosis

Aortic valve stenosis is the most frequent valvular dysfunction associated with BAV (80). The underlying mechanisms of aortic valve stenosis formation are believed to be similar to those of atherosclerosis (for review see (90)). A number of risk factors that are associated with aortic valve calcification have been identified and these are similar to those of atherosclerotic disease; however, a distinction between BAV and TAV has not been made (91;92). Even though BAV patients present with significant valve stenosis earlier in life than patients with TAV, the pathogenesis of the valve lesion is thought to be similar, however not identical (93-95). eNOS deficiency and signaling pathways such as NOTCH and Wnt/β- Catenin, which are implicated in BAV formation, have also been suggested to be important in valve calcification,
possibly providing a link between the malformation and its most frequent valvular pathology (60;96;97).
An association between aortic valve disease and BAV phenotype has been proposed; however there are conflicting results. Beppu et al. found a more rapid progression of valve stenosis in BAVs with a RCC/LCC BAV (82). Fernandes et al. found an association between valve disease (both stenosis and regurgitation) and RCC/NCC BAVs (98). Conversely, Tzemos et al concluded that BAV phenotype is not an independent predictor of cardiac events (including aortic valve disease) (80).

c. Aortic valve regurgitation
Aortic valve regurgitation is the second most common valvular lesion associated with BAV and is often secondary to valve calcification. BAV patients with isolated valve regurgitation are in general younger than those with combined valve stenosis/regurgitation. Further mechanisms of incompetence include incomplete closure of cusps, redundancy of the fused cusps leading to prolapse, infective endocarditis, dilation of the aortic root, and aortic dissection (5;86;99-102).

d. Aortic dilation: structural wall abnormalities or shear stress?
In patients with a normal TAV histological changes of the aortic wall related to age as well as aortic pathology (dilation/dissection) are associated with medial degeneration (focal loss of smooth muscle cell nuclei), regardless of aortic location (i.e., ascending, descending, or abdominal) (103;104). Histologic changes in BAV patients are controversial in the current literature. More severe histopathological features have been described in BAV patients as compared to TAV patients (105;106). Furthermore, elastic fragmentation is more pronounced in BAV patients than in TAV patients with isolated valve pathology (107) and an association of BAV phenotype and the severity of medial degeneration has been reported (108). Whereas others have shown that BAV exhibits less histopathologic features (109).
The pathogenesis of aortic dilation and aneurysm formation of the ascending, descending, and abdominal aorta differs (110;111). Experimental studies have shown that the embryonic origin of the cells populating the aorta in mammals differs with the aortic location. Neural crest cells give rise to the ascending aorta, aortic arch, pulmonary trunk, and ductus arteriosus. By contrast, cells originating from the mesoderm populate the descending
aorta and subclavian artery (112;113). Whether developmental differences have an impact on differences in the underlying pathogenesis of aortic dilation related to aortic location (i.e., ascending, descending, abdominal) is not known. Furthermore, ascending aortic dilation in TAV, but not BAV, is associated with inflammation and immune response (114).

In addition to differences in the severity of medial degeneration and inflammatory profile between BAV and TAV patients with aortic dilation, several other factors related to BAV-associated aortic dilation have been reported as follows: 1) dilation of the ascending aorta is more progressive in BAV, as compared to TAV, even after aortic valve replacement (30;115); 2) children (and young adults) with BAV have larger dimensions of the aortic root/ascending aorta and impaired aortic elastic properties compared with children with TAV (116-118) 3) approximately one third of first degree relatives of BAV patients (with normally functioning TAVs) have dilated aortic roots and abnormal elastic properties of the aorta (10) 4) there are differences in signaling pathways involved in extracellular matrix homeostasis between BAV and TAV patients with dilated aortas (119-125) 5) eNOS expression is lower in BAV patients than in TAV patients and is inversely correlated with aortic diameter (126) and 6) mutations in genes encoding components of the extracellular matrix are linked to aortic dilation and BAV (FBN-1(124;127), TGFBR-2 (127), ACTA-2 (128)).

Many aspects of what causes BAV formation and subsequent BAV disease are not known, but there are two predominating theories, i.e., genetics and haemodynamics. The description above entails the genetic theory; haemodynamics will now further be discussed.

The morphology of a BAV is evidently different from that of a normal TAV with the consequence of altered flow across the valve (129). A bileaflet valve causes a turbulent flow compared to a normal tricuspid valve, due to asymmetric movement of valve cusps. A turbulent flow, along with other haemodynamic factors, as an increased stroke volume (i.e. aortic regurgitation) and aortic curvature, could play a facilitative role in developing aortic complications, such as dilation in BAV individuals (6;130-132). In regions of turbulent flow matrix degrading enzymes are expressed and smooth muscle cells (SMCs) go into apoptosis (133). In response to degradation of the extracellular matrix local fibroblasts and SMC are considered to synthesize new connective tissue components. This progressive remodeling presumably results in a new extracellular matrix of a different structure. The combination
of remodeling and loss of SMCs, due to apoptosis, weakens the integrity of the vessel wall, which could lead to subsequent aortic dilation (134). Though, on the other hand, several studies have confirmed that ascending aortic aneurysms can develop in the absence of valvular abnormality (7;106;135-142). Moreover, Yasuda et al. have reported development of aortic dilation after (an isolated) surgical repair of the diseased bicuspid aortic valve (115). These studies suggest that structural abnormalities occur at the cellular level. Haemodynamic factors alone seem therefore not sufficient to explain the pathogenesis of cardiovascular malformations associated with bicuspid aortic valves. Suggesting that genetically determined abnormalities of the aortic wall lead to a defect in the cellular microenvironment, causing, or at least contributing to the aortic pathology associated with BAV. Although, besides a strictly genetic or haemodynamic theory, a combination of both is plausible as an alternative hypothesis. We postulate that the structurally altered vascular wall, might be prone for secondary haemodynamic changes observed in BAV.
AIM OF THIS THESIS

The aim of this thesis is to determine clinical, histological, molecular biologic and morphological factors that may predict the clinical course and explain the increased susceptibility for aortic wall pathology seen in the majority of patients with BAV. We also intended to compare the pathobiology of aortic wall pathology in BAV with MFS.

In Chapter 2 we approach our hypothesis that aortic wall pathology associated with BAV could be the result of a shared development defect during embryogenesis. To address this hypothesis we provided an overview of embryonic cell lines involved in the normal development of the semilunar valves and the ascending aortic dilation, being neural crest cells, second heart field (SHF) progenitors and endocardial cushion derived cells. Genes involved in abnormal development of the aortic valve (BAV) are further discussed. To understand whether thoracic aortic dilation, not necessarily accompanied by BAV, is related to defects in neural crest signaling or SHF-derived cells. The genetic origin of syndromes associated with aortic dilation (including MFS, Ehlers-Danlos, Smad3 mutations and Loeys-Dietz) was reviewed in the view of a central role for TGFβ and linked to embryonic development.

In Chapter 3 we approach our hypothesis that the aortic wall is structurally different in BAV as compared to the TAV. By studying the dilated and also particularly the non-dilated aortic wall specimen, we investigated the expression of smooth muscle cells markers of diverse differentiation states. Further Lamin A/C, with a pivotal role in myoblasts differentiation and progerin, a marker for cardiovascular aging, were studied.

In Chapter 4 valve phenotype and aortic dimensions of 255 BAV patients were evaluated retrospectively. Patient characteristics, the clinical course and echocardiographic parameters including morphology of the valve were obtained. The aim of this study was to more definitively characterize whether an association exists between the morphology of the BAV and the degree of aortic dilation. And to provide a risk profile for clinical complications based patient characteristics and echocardiographic measurements.
In Chapter 5 we further investigated a panel of vascular wall markers that might distinguish within the non-dilated BAV group a susceptible and non-susceptible group for future aortic wall complications.

In Chapter 6 we investigated the aortic wall composition in patients with BAV, MFS and TAV. Recent studies suggest that in BAV patients the risk of aortic catastrophes, although higher than in the general population, remains low (12;80) as compared to the MFS. Controversies regarding aortic similarities and differences between various types of aortic wall pathology (BAV, MFS, TAV) in regard to aortic dilation and dissection of the aorta are as yet unresolved. Therefore the aim of this study was to shed light on the pathogenetic mechanism of aortic wall complications seen in both syndromes and compare those to the aortic wall pathology in dilated TAV patients. Findings were correlated to the effectiveness of clinical treatment modalities.

In Chapter 7 the activity of the arterial epicardium covering the ascending aorta is investigated in both non- and dilated aortic wall specimen of BAV, TAV and MFS. In chapter 5 a signaling cascade is presented consisting of markers which can aid in distinguishing BAV patients with an increased susceptibility for future aortic complications. However, the link between 2 markers in the cascade: eNOS and MMP9, could not be substantiated. In this paper we aimed to identify how these markers are associated with each other.

Chapter 8 provides a summary of this thesis and discusses future perspectives.
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