

From the Cardiothoracic Surgery Unit, Department of Molecular
Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

THE BICUSPID AORTIC VALVE

Studies on Valve Morphology and Pathology in
Relation to Ascending Aortic Dilatation and
Coronary Artery Disease

Veronica Jackson



**Karolinska
Institutet**

Stockholm 2013

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet.

Printed by Larserics Digital Print AB, Sundbyberg, Sweden

© Veronica Jackson, 2013

ISBN 978-91-7549-080-9

ABSTRACT

The prevalence of bicuspid aortic valve (BAV) is 1-2% and is thereby the most common cardiac malformation. BAV is highly associated with valvular dysfunction and aortic conditions such as ascending aortic aneurysm and aortic dissection. Of BAV individuals, 25-50% will develop indications for surgical intervention. The underlying molecular mechanisms of BAV formation and the reason for the high prevalence of ascending aortic aneurysm in these patients are unknown.

The overall aim of this thesis was to characterise morphological, molecular and clinical aspects of BAV disease in adult patients undergoing cardiac surgery due to aortic valve and/or ascending aortic pathology. In the study population of this thesis more than 50% of the patients had a BAV. BAV patients were approximately 10 years younger than patients with tricuspid aortic valves (TAV) at the time of surgery. Patients that had additional coronary artery disease were older than patients that did not, regardless of whether they had a BAV or a TAV. Ascending aortic aneurysm was substantially more common in BAV patients than in TAV patients while aortic ectasia was equally common regardless of valve morphology. In patients with ascending aortic dilatation, aortic valve stenosis was almost exclusively associated with BAV whereas aortic valve regurgitation was associated with either BAV or TAV.

Study I assessed the morphology of the aortic root and ascending aorta in relation to valve morphology and BAV phenotype (n = 300). BAV patients had larger dimensions of the left ventricular outflow tract and annulus than TAV patients regardless of aortic morphology. The relative distribution of aortic aneurysm or ectasia was not related to BAV phenotype.

Study II investigated a possible association between severity of valve pathology and morphology of the aortic root and ascending aorta (n = 500). The combination of aortic valve stenosis and ascending aortic aneurysm was common in BAV patients but was virtually non-existent in TAV patients. Increasing severity of valve pathology was associated with smaller aortic dimensions. The distribution of valve pathology did not differ with the various BAV phenotypes.

Study III evaluated a possible correlation between ascending aortic dilatation and dilatation of the distal aorta (n = 97). BAV patients with ascending aortic aneurysms had smaller dimensions of the distal aorta than the corresponding group of TAV patients. Concomitant dilatation of the descending aorta was predominantly found in TAV patients.

Study IV analysed the occurrence of matrix degrading proteases in the media of the aortic wall (n = 109). Expression of matrix metalloproteinase 14 and 19 was associated with ascending aortic dilatation in TAV patients, but not BAV patients.

Study V evaluated patient characteristics in relation to valve morphology, valve pathology, aortic morphology and coronary artery disease (n = 702). BAV patients with aortic valve pathology and/or ascending aortic dilatation rarely had concomitant coronary artery disease. Ascending aortic dilatation and coronary artery disease seldom co-existed regardless of valve morphology.

SAMMANFATTNING

Bikuspid aortaklaff (BAV) har en prevalens på 1-2% och är därmed den vanligaste medfödda hjärmissbildningen. BAV är i hög grad associerad med klaffdysfunktion och aortapatologi såsom aortaaneurysm och aortadissektion. 25-50% av individer med BAV kommer att uppfylla indikation för hjärtkirurgisk intervention. De molekylära mekanismerna som ligger till grund för bildingen av BAV samt orsaken till den höga prevalensen av ascendensaneurysm som ses hos dessa patienter är inte kända.

Det övergripande syftet med denna avhandling var att karakterisera morfologiska, molekylära och kliniska aspekter av BAV-sjukdom hos vuxna patienter som genomgår hjärtkirurgi till följd av aortaklaff- och/eller aorta ascendenspatologi. I den aktuella studiepopulationen hade mer än 50% av patienterna en BAV. BAV-patienter var cirka 10 år yngre än patienter med en normal trikuspid aortaklaff (TAV) vid tiden för kirurgisk intervention. Patienter med samtidig kränkylssjukdom var äldre än de utan, oavsett om de hade en BAV eller en TAV. Aorta ascendensaneurysm var betydligt vanligare hos BAV-patienter till skillnad från ektasi av aortan som var lika vanligt hos BAV-patienter och TAV-patienter. Hos patienter med aorta ascendensdilatation var aortaklaffstenos nästan uteslutande associerat med BAV till skillnad från aortaklaffläckage som var associerat med BAV eller TAV.

Arbete I utvärderade aortarots- och aorta ascendensmorfologi i relation till klaffmorfologi och BAV-fenotyp (n = 300). BAV-patienter hade större dimension på vänster kammars utflödestrakt och aortaannulus oavsett aortans morfologi. Den relativa distributionen av ascendensaneurysm eller ektasi av aortan var inte relaterad till BAV-fenotyp.

Arbete II utforskade en möjlig association mellan grad av klaffpatologi och aortarots- och aorta ascendensmorfologi (n = 500). Kombinationen aortaklaffstenos och ascendensaneurysm var vanlig hos BAV-patienter men förekom mycket sällan hos TAV-patienter. Ökande grad av aortaklaffpatologi var associerat med mindre aortadimensioner. Distributionen av klaffpatologi var inte relaterad till BAV-fenotyp.

Arbete III sökte fastställa om en korrelation mellan dilatation av aorta ascendens och dilatation av distala aorta förelåg (n = 97). Dimensionerna av distala aorta var mindre hos BAV-patienter med ascendensaneurysm jämfört med TAV-patienter med ascendensaneurysm. Samtidig dilatation av aorta descendens sågs i huvudsak hos TAV-patienter.

Arbete IV analyserade förekomsten av matrixnedbrytande proteaser i aortaväggens media (n = 109). Genexpression av matrixmetalloprotein 14 och -19 var associerat med aortadilatation hos TAV-patienter men inte hos BAV-patienter.

Arbete V utvärderade patientkarakteristika i relation till klaffmorfologi, klaffpatologi, aortamorfologi och kränkylssjukdom (n = 702). BAV-patienter med klaff- och/eller aortapatologi hade mycket sällan samtidig kränkylssjukdom. Aorta ascendensdilatation och kränkylssjukdom samexisterar sällan oavsett klaffmorfologi.

LIST OF PUBLICATIONS

This thesis is based on the following original articles which will be referred to in the text by their Roman numerals.

- I. **Jackson V**, Petrini J, Caidahl K, Eriksson MJ, Liska J, Eriksson P, Franco-Cereceda A
Bicuspid aortic valve leaflet morphology in relation to aortic root morphology: a study of 300 patients undergoing open-heart surgery
Eur J Cardiothorac Surg 2011;40:e118-e124
- II. **Jackson V**, Petrini J, Eriksson M, Caidahl K, Eriksson P, Franco-Cereceda A
Impact of valve pathology on aortic dimensions in patients with tricuspid- or bicuspid aortic valves
Submitted
- III. **Jackson V**, Olsson C, Eriksson P, Franco-Cereceda A
Aortic dimensions in patients with bicuspid and tricuspid aortic valves
J Thorac Cardiovasc Surg [2012 Sep 13. pii: S0022-5223(12)00882-3. doi: 10.1016/j.jtcvs.2012.07.039. Epub ahead of print]
- IV. **Jackson V**, Olsson T, Kurtovic S, Folkersen L, Paloschi V, Wågsäter D, Franco-Cereceda A, Eriksson P
Matrix metalloproteinase 14 and 19 expression is associated with thoracic aortic aneurysms
J Thorac Cardiovasc Surg 2012;144:459-66
- V. **Jackson V**, Eriksson M, Caidahl K, Eriksson P, Franco-Cereceda A
Ascending aortic dilatation is rarely associated with coronary artery disease regardless of aortic valve morphology
Manuscript

CONTENTS

1	Introduction.....	11
2	Background.....	13
2.1	History.....	13
2.2	Valve formation.....	15
2.2.1	Embryology.....	15
2.2.2	Genetics.....	17
2.3	BAV disease.....	19
2.3.1	Aortic valve stenosis.....	21
2.3.2	Aortic valve regurgitation.....	21
2.3.1	Aortic pathology.....	22
3	Aims.....	27
4	Methods.....	28
4.1	Advanced Study of Aortic Pathology (ASAP).....	28
4.2	Echocardiography.....	29
4.2.1	Transthoracic echocardiography.....	29
4.2.2	Transesophageal echocardiography.....	29
4.3	Computed tomography scanning.....	29
4.4	Gene expression and immunohistochemistry.....	30
4.5	Definitions.....	31
4.5.1	Anatomic structures.....	31
4.5.2	BAV phenotype.....	32
4.5.3	Valve pathology.....	32
4.5.4	Aortic morphology.....	32
4.6	Statistical analyses.....	33
4.7	Ethical considerations.....	33
5	Results.....	34
5.1	BAV phenotype and aortic morphology.....	35
5.1.1	General findings.....	35
5.1.2	Morphology of the aortic root and ascending aorta.....	37
5.2	Valve pathology and aortic morphology.....	38
5.2.1	General findings.....	38
5.2.2	Aortic dimensions in relation to valve pathology.....	39
5.2.3	Severity of valve pathology and non-dilated aorta.....	39
5.2.4	Severity valve pathology and dilated aorta.....	39
5.3	Dimensions of the distal aorta.....	41
5.3.1	General findings.....	41
5.3.2	Arch, descending, and abdominal aortic dimensions.....	41
5.3.3	BAV phenotype, valve pathology, and the distal aorta.....	41
5.3.4	Concomitant dilatation of the distal aorta.....	41
5.4	Proteases in the aortic wall.....	42
5.4.1	MMP and TIMP expression in the aortic wall.....	42
5.4.2	Protease expression and aortic morphology.....	42
5.4.1	MMP14 and MMP19 protein expression in TAV patients.....	44
5.5	Clinical characteristics and coronary artery disease.....	45
5.5.1	Patient characteristics.....	45

5.5.2	Echocardiographic findings	47
5.5.3	Medical history.....	47
5.5.4	Medical treatment.....	47
5.5.5	Laboratory findings.....	47
6	Discussion.....	48
6.1	Clinical characteristics	48
6.2	BAV phenotype and aortic morphology.....	49
6.3	Valve pathology and aortic morphology	50
6.4	Dimensions of the distal aorta.....	52
6.5	Aortic wall degeneration	53
6.6	BAV-associated aortopathy	54
6.7	Clinical implications.....	57
6.8	Study limitations.....	58
7	Conclusions	59
8	Acknowledgements	60
9	References	62

LIST OF ABBREVIATIONS

ASAP	Advanced Study of Aortic Pathology
BAV	Bicuspid aortic valve
LN	BAV phenotype: fusion of the left- and non-coronary leaflet
MMP	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
PCR	Polymerase chain reaction
RL	BAV phenotype: fusion of the right- and left coronary leaflet
RN	BAV phenotype: fusion of the right- and non-coronary leaflet
RNA	Ribonucleic acid
TAV	Tricuspid aortic valve
TB	BAV phenotype: “true” bicuspid aortic valve
TIMP	Tissue inhibitor of metalloproteinase
UAV	Unicuspid aortic valve

1 INTRODUCTION

A bicuspid aortic valve (BAV) is the most common cardiac malformation with a prevalence of 1-2 % and is frequently associated with valvular dysfunction such as aortic valve stenosis and/or regurgitation (for review see ¹). In addition, BAV is associated with potentially lethal aortic conditions such as ascending aortic aneurysm and aortic dissection. In Sweden about 100 000-200 000 individuals have a BAV. Approximately 50% of BAV individuals will have to undergo aortic valve surgery and about 25% will require ascending aortic replacement due to dilatation of the ascending aorta. Furthermore, having a BAV carries an eight-fold increase in the risk of aortic dissection.² BAV-associated conditions often reach clinical significance in relatively young individuals and therefore not only have personal but also socioeconomic consequences.

In view of the high number of individuals with BAV and the conditions associated with this malformation, surprisingly little is known about the underlying molecular mechanisms of BAV formation or the reason for the high prevalence of ascending aortic aneurysm in these patients. Both BAV formation and the associated ascending aortic dilatation have been linked to genetic aberrations and theories of an inherent weakness of the aortic wall have been proposed. In addition, rheological studies have shown that flow and shear stress are important factors in the development of BAV disease. These two main theories (i.e., genetics and hemodynamics) have generated several clues as to what causes BAV disease although many questions remain unanswered.

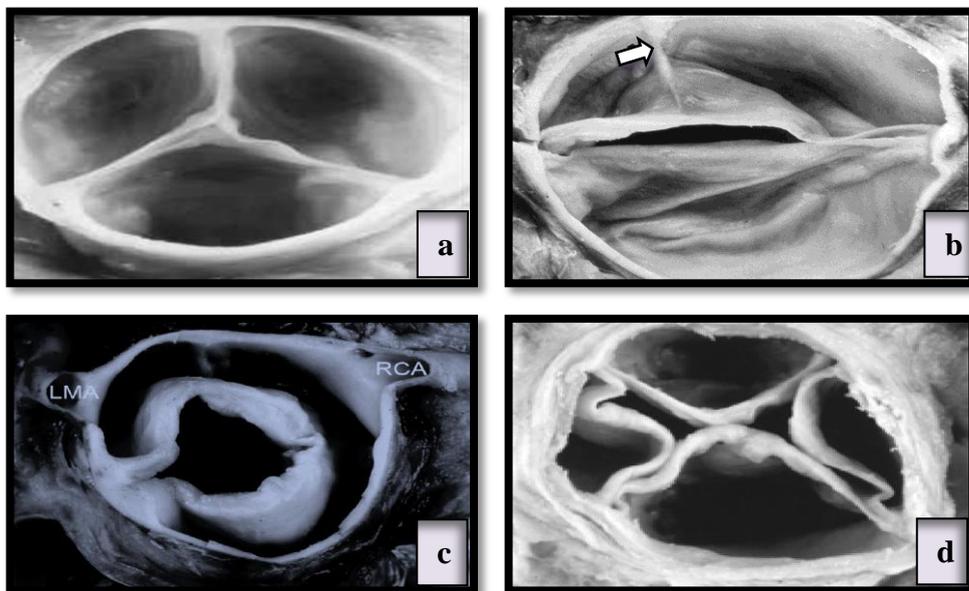
This thesis addresses morphological, molecular and clinical aspects of BAV disease in adult patients undergoing cardiac surgery. The thesis aims to provide insights into the relation between valve morphology and aortic morphology, and possible mechanisms that are important in BAV-associated aneurysm formation.

2 BACKGROUND

2.1 HISTORY

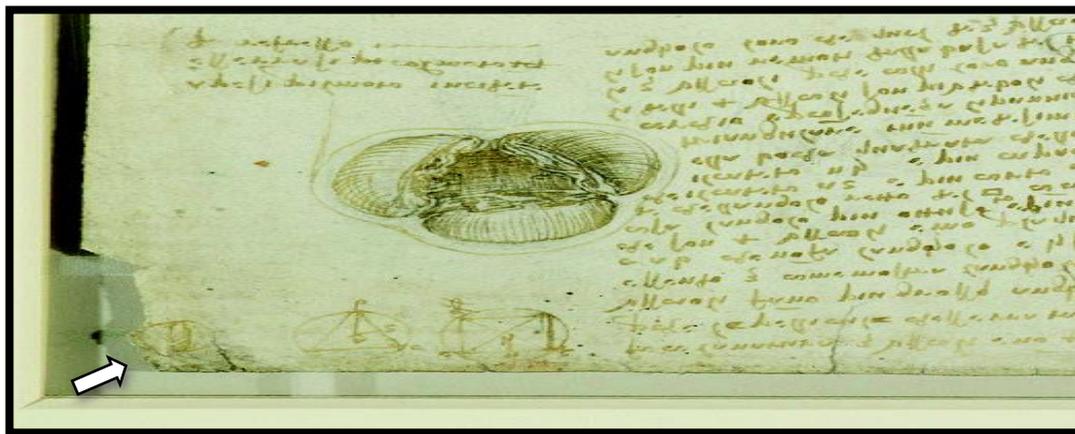
A normal aortic valve is tricuspid and consists of three valvular leaflets (i.e., cusps) and three commissures (Fig. 1). The first recognition of a BAV, with two valvular leaflets and two commissures, is usually ascribed to Leonardo da Vinci (1452-1519) who drew a sketch of a quadricuspid, a tricuspid and a bicuspid aortic valve about 500 years ago (Fig. 2). Since then, the knowledge of the prevalence, clinical significance, and associated malformations and pathologies of BAV has grown substantially, with the largest contribution in recent years. The time line of historical discoveries regarding BAV disease is presented in Table 1. In the middle of the 19th century, Paget noted from necropsy studies that bicuspid valves seemed to have a predilection for disease. Twenty years later, Peacock added to the understanding of BAV disease by reporting a tendency of BAVs to initially develop stenosis and, as a later consequence valvular incompetence. However, it was not until the end of the 19th century that the clinical importance of a BAV was put forward by Osler who also pointed out an association of BAV and infective endocarditis. In the early 20th century, BAV was recognised as the most common cardiac malformation and during the 1950s it was confirmed that valve stenosis in BAV disease is primarily caused by calcification and is not mainly the consequence of rheumatic disease. As early as 1927, Abbot commented on an association of BAV and aortic dissection. Sixty years later, Larson and Edwards used necropsy studies to report that the risk of aortic dissection is nine-fold higher in patients with BAV than in patients with tricuspid aortic valves (TAV).

Figure 1. Aortic valve morphology



a) Tricuspid aortic valve [www.78steps.com]; b) bicuspid aortic valve with a raphe (arrow);³ c) unicuspid aortic valve (LMA – left main coronary artery; RCA – right coronary artery);⁴ d) quadricuspid aortic valve.³ Panels b, c, and d are reprinted with permission from Elsevier.

Figure 2. Drawing by Leonardo da Vinci



According to historians the text describes the optimal geometric properties of a tricuspid aortic valve in comparison with a quadricuspid valve and the sketch at the bottom left is thought to represent a bicuspid aortic valve (arrow).¹ Reproduced with permission from Royal Collection Trust/© Her Majesty Queen Elisabeth II 2013.

Table 1. Historical notes on bicuspid aortic valve disease

Year	Author	Statement
1512-13	Leonardo da Vinci ¹	Drawing of a BAV
1844	Paget ⁵	States that bicuspid valves are liable to disease
1866	Peacock ⁶	Points out that BAVs tend to initially develop stenotic lesions and subsequently incompetence
1886	Osler ⁷	Stresses the clinical importance of BAV and points out the malformation as a substrate for infective endocarditis
1927	Abbot ⁸	Comments on an association between BAV and aortic dissection
1928	Wauchope ⁹	States that BAV is the most common congenital anomaly of the heart
1947	Karsner and Koletsky ¹⁰	Hypothesises that valve stenosis in BAV is mainly rheumatic in origin
1953	Campbell and Kauntze ¹¹	Mounting evidence that BAV stenosis is mainly calcific and not rheumatic in origin
1955	Smith and Matthews ¹²	
1959	Bacon and Matthews ¹³	
1978	Leech <i>et al.</i> ¹⁴	Concludes that in the absence of other signs of aortic valve stenosis, a non-diseased BAV can be identified by an isolated aortic ejection sound and then confirmed with echocardiography
1984	Larson and Edwards ¹⁵	Shows that a BAV is a risk factor for aortic dissection

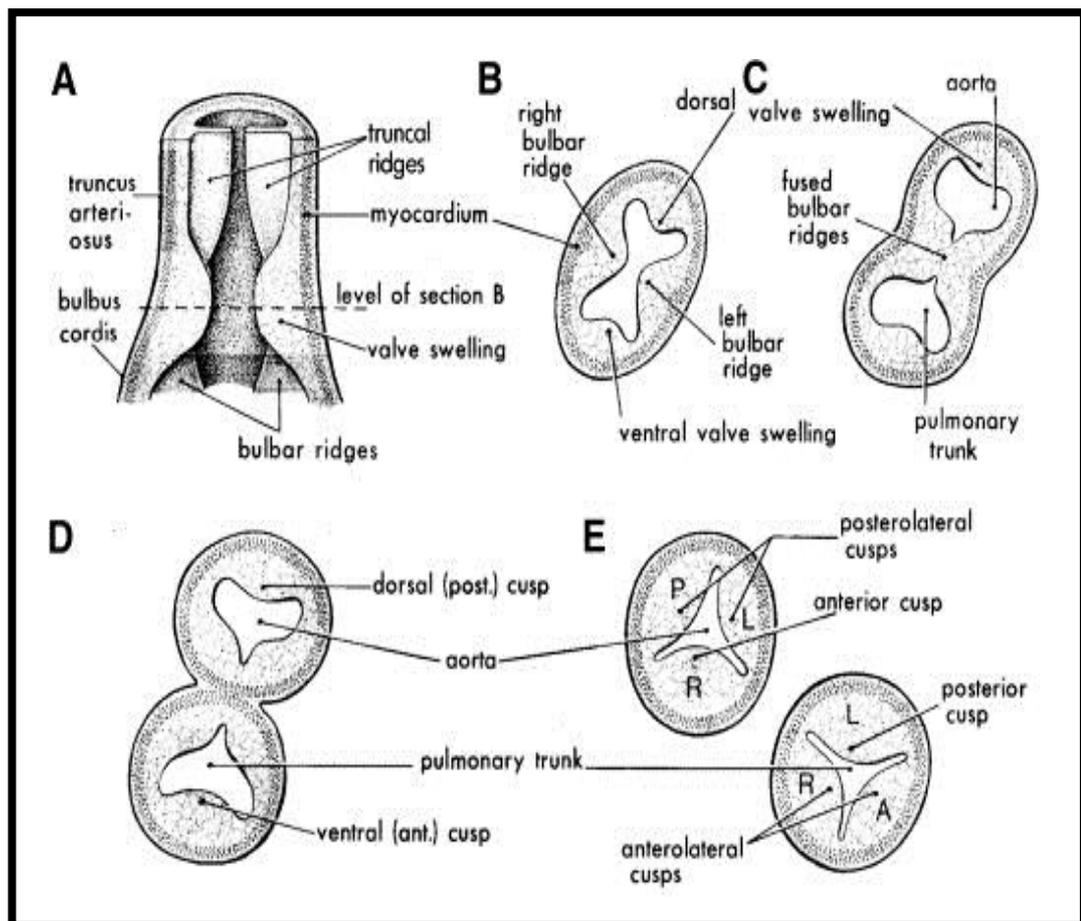
2.2 VALVE FORMATION

In the developing human embryo, the primitive heart tube is formed in the fourth week of gestation and already has the ability to beat and pump the blood forward by day 22. The formation of the heart valves also starts around the fourth week of gestation.

2.2.1 Embryology

The division of the common outflow tract (truncus arteriosus) of the primitive heart is accomplished by the growth of two swellings (Fig. 3 A-B). These swellings are located laterally in the wall of the truncus arteriosus and grow and further fuse into a septum that separates the two ventricular outflow tracts (Fig. 3 B-C). Moreover, two small tubercles located on the tips of the truncus swellings at the inferior part of the truncus arteriosus are formed. These tubercles split into halves by fusion of the truncus swellings and give rise to the right and left valvular leaflet of the aortic and pulmonary valve, respectively. During fusion of the truncus swellings, an additional pair of tubercles are formed at the anterior and posterior part of the truncus arteriosus wall and these give rise to the third leaflet of the aortic- (posterior tubercle) and pulmonary (anterior tubercle) valve (Fig. 3 D). The valvular leaflets are formed by excavation of the truncal tissue (Fig. 3 E).¹⁶

Figure 3. Fusion of the truncus swelling



Reprinted with permission from Elsevier.¹

The truncus swellings undergo maturation into endocardial cushions via a complex process. The initial swelling of the truncal tissue is caused by increased deposition of extracellular matrix, which is also referred to as “cardiac jelly”. The maturation involves not only remodelling of the extracellular matrix but also *epithelial-to-mesenchymal transformation*, a process that is essential for successful valve formation, and the cushions will hereby be populated with valve precursor cells. The cardiac cushions of the outflow tract are, through migration, also populated with neural crest cells that contribute to the formation of the septum that separates the ventricular outflow tracts. The mature leaflets 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.

The pathogenesis of BAV formation is not known, but it is evident that the extracellular matrix and *epithelial-to-mesenchymal transformation* are essential for valvulogenesis. A number of signalling pathways are thought to be involved in *epithelial-to-mesenchymal transformation* during valvulogenesis (Table 2) and disruptions in endocardial cushion formation are implicated in the pathogenesis of heart valve disease¹⁷ (for review see^{18,19}).

Table 2. Principal signalling pathways and transcription factors implicated in epithelial-to-mesenchymal transformation in semilunar valve formation

Gene	Description	Function
EGF superfamily	Epidermal growth factor	Signalling pathway
ErbB	V-erb-b2 erythroblastic leukemia viral oncogene homolog	Epidermal growth factor receptor signalling pathway/family of receptor tyrosine kinases
NF-1	Neurofibromin 1	Ras signalling pathway
NFATc1	Nuclear factor of T cells cytoplasmic 1	Transcription factor
NOTCH	Family of transmembrane receptors	Signalling pathway
Sox9	SRY (sex determining region Y)-box 9	Transcription factor
TGF-β superfamily	Transforming growth factor beta	Signalling pathway
Tbx20	T-box 20	Transcription factor
Twist-1	Twist homolog 1	Transcription factor
VEGF	Vascular endothelial growth factor	Signalling pathway
Wnt/β-catenin	Wingless type MMTV integration site family/cadherin-associated protein beta	Canonical Wnt signalling pathway. Wnt, growth factor; β-catenin, co-activator of transcription factors

2.2.2 Genetics

2.2.2.1 Inheritance of BAV and associated cardiovascular malformations

In 1866, Peacock suggested that BAV is congenital in origin based on findings of the malformation in still born fetuses and neonates with other cardiac malformations.⁶ It has since been confirmed that BAV is indeed related to other cardiac malformations. BAV is reportedly found in 25-85% of patients with aortic coarctation.²⁰ Hypoplastic left heart syndrome and BAV co-exist and are to some extent genetically linked.²¹ Shone's syndrome is characterised by supravulvar 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.²² William's syndrome is a neurodevelopmental disorder that is also associated with cardiovascular conditions such as supravulvar stenosis, peripheral pulmonary artery stenosis, aortic coarctation, and BAV.²³ 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.²⁴²⁵ BAV has been noted in conjunction with ventricular septal defects,²⁶ patent ductus arteriosus,^{27 28, 29} and atrial septal defects.²⁹ BAV is also associated with left coronary artery dominance.^{30, 31}

The genetic effect (heritability) on BAV formation is suggested to be as high as 89%.³² BAV has an inheritance consistent with an autosomal dominant pattern with reduced penetrance^{33, 34} and about 9% of first-degree relatives of BAV individuals will also have the malformation.^{32, 35} BAV formation has been linked to several different chromosomes and gene mutations, which suggests complex inheritance.^{36 34, 37, 38}

2.2.2.2 Human genes

Since BAV is a congenital malformation, it is reasonable to expect that mutations in genes encoding transcription factors, extracellular matrix components, and proteins of signalling pathways (Table 2) that are implicated in valvulogenesis are important in BAV formation.^{18, 19}

Linkage analyses revealed an association between BAV and chromosome 9q34-35 and subsequently *NOTCH1* in humans.³⁷ *NOTCH1* encodes a transmembrane receptor and its signalling pathway is important in developmental processes and organogenesis in a wide range of organisms including humans. Notch signalling is highly conserved in evolution and, regardless of what animal model is used, perturbations in the pathway inevitably lead to developmental abnormalities (for review see³⁹). *Notch1* is expressed in endocardial cells found in the common outflow tract in mouse embryos⁴⁰ and Notch signalling is suggested to have a role in BAV formation and valve calcification.³⁷ *SMAD6* (SMAD family member 6) encodes a protein that functions as a signal transducer and transcriptional modulator in BMP signalling (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.⁴¹ BAV has also been linked to chromosomes 18q22, 5q21 and 13q34; however which specific genes within these loci are associated with BAV is unknown.³⁶ The empirical association of BAV and hypoplastic left heart syndrome has also been confirmed genetically.²¹ 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.³⁸

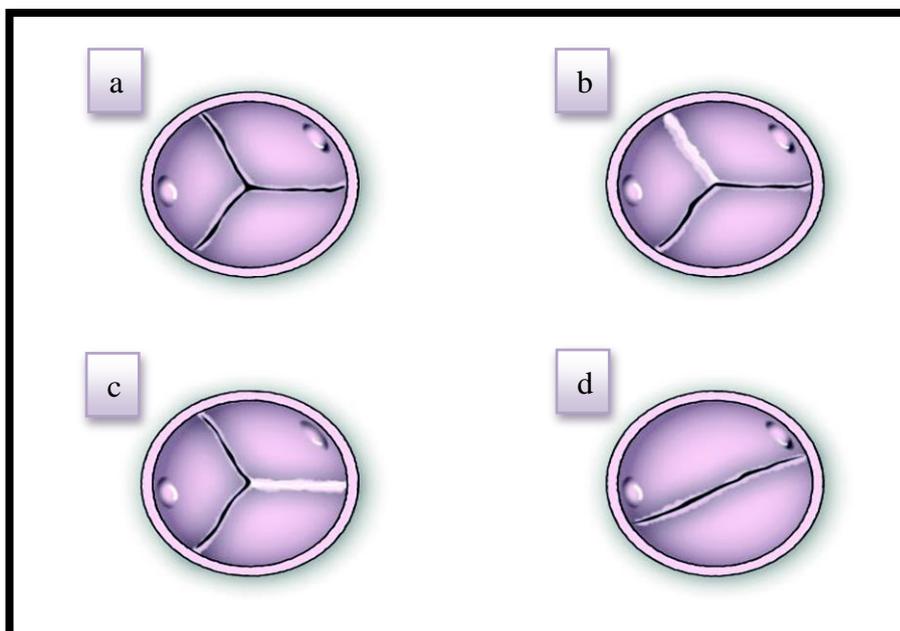
A number of other genes are implicated in BAV formation in mice but their relevance in humans remains to be shown. These include *Nkx2-5* (NK2 homeobox 5, transcription factor),⁴² *Nos3* (nitric oxide synthase 3 (endothelial cells), alias eNOS),⁴³ *Gata5* (GATA binding protein 5, transcription factor),⁴⁴ and *Acvr1* (activin A receptor, Type 1, alias Alk2, member of the TGF- β superfamily).⁴⁵

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.⁴⁶⁻⁴⁸ Thus, the genetic profiles of BAV individuals that form study populations in work on heritability, inheritance pattern, and gene mutations might represent a BAV population that is more susceptible to the associated malformations and conditions.^{34, 37}

2.2.2.3 BAV phenotype

BAV phenotype refers to how the leaflets of a BAV are fused and whether a commissural raphe is present, i.e., fusion of the right and left coronary leaflets with a raphe (RL), fusion of the right and non-coronary leaflets with a raphe (RN), fusion of the left and non-coronary leaflets with a raphe (LN), and a "true" BAV with two equally-sized leaflets without a raphe (TB) (Fig. 4).

Figure 4. BAV phenotype



a) Tricuspid aortic valve; b) bicuspid aortic valve (RL); c) bicuspid aortic valve (RN); d) true bicuspid aortic valve (TB). Reprinted with permission from Oxford University Press (Study I).

BAV phenotype is suggested to have moderate heritability,⁴⁹ meaning that the genetic basis of BAV phenotype is incomplete, i.e., families with several BAV individuals will not all have the same BAV phenotype. The aetiology of the different BAV phenotypes seems to differ. Mice deficient in eNOS or *Gata5* develop BAV with fusion of the right and non-coronary leaflets.^{43, 44, 50} The RN 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.⁵⁰ By contrast, the RL 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.⁵⁰

2.3 BAV DISEASE

The reported prevalence of BAV varies (Table 4); however BAV is commonly referred to as having a prevalence of 1-2%. When summarising reports of BAV prevalence, it seems that 2% might be an overestimation. However, the sensitivity of identifying a BAV in necropsy studies and echocardiographic studies is not 100%.^{51, 52} Roberts reported a BAV prevalence of 0.9% in normally functioning aortic valves but the BAV prevalence was notably higher when diseased aortic valves were included.⁵³ Of 1692 selected military recruits that were evaluated with echocardiography by Nistri *et al.*, 167 were found to have a BAV.⁵⁴ In addition, the prevalence of BAV depends on ethnicity and gender as BAV is more common in Caucasians and males, and these factors have not always been considered in analyses of BAV prevalence.^{51, 55} The male to female ratio of BAV is estimated to be approximately 2:1.^{1, 56}

BAV entails an increased risk of valvular dysfunction and aortic conditions such as aneurysm and dissection. BAV has an accelerated progression of valve thickening and calcification which is generally evident in the fourth decade of life.^{51, 57, 58} The valvular changes frequently progress into clinically significant valve stenosis⁵⁹ and it has been suggested that a BAV is subjected to abnormal stress even when it is clinically deemed to be of normal function.⁶⁰ 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 failure of a BAV. Furthermore, BAV individuals are predisposed to infective endocarditis. 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^{61 62, 63} (for review see⁶⁴). BAV phenotype has been proposed to affect the susceptibility for valve disease; BAV individuals with a RN configuration are prone to develop valve disease in childhood, whereas BAV individuals with a RL configuration tend to develop valve disease in adulthood.⁴⁹ Altogether approximately 50% of individuals with BAV will have to undergo aortic valve surgery.²

The BAV-associated aortic conditions (aneurysm/dissection) are potentially lethal if left untreated. 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.²

Table 4. Reported prevalence of BAV

Author, study type	Study pop. (n)	Age	Male: female	Ethnicity (%)	BAV (%)
Osler 1886, necropsy ⁷	> 800	8 m-60 y	3.5:1	–	2.3
Lewis <i>et al.</i> 1923, necropsy ⁶⁵	215	–	–	–	1.39
Wauchope 1928, necropsy ⁹	9 966	33.6 ± 20.1 y	4:1	–	0.5
Roberts 1970, necropsy ⁵³	1 440	15-79 y	3:1	–	0.9 (-2)
Larson <i>et al.</i> 1984, necropsy ¹⁵	21 417	–	–	–	1.37
Datta <i>et al.</i> 1988, necropsy ⁶⁶	8 800	8-70 y	11:1	–	0.59
Pauperio <i>et al.</i> 1999, necropsy ⁵¹	2 000	3 m-68 y	All male	White, 0.93; mulatto, 0.6 black 0	0.65
Basso <i>et al.</i> 2004, echocardiography ⁶⁷	817	10 y	3:1	–	0.5
Tutar <i>et al.</i> , echocardiography ⁶⁸	1 075	Neonates (27-42 w)	4:1	–	0.46
Nistri <i>et al.</i> , echocardiography ⁵⁴	20 946	18 ± 2 y	Only males included	–	0.8

m – months; *w* – weeks; *y* – years.

Aortic dissection is caused by disruption of the internal layer of the aortic wall, which results in bleeding between the aortic wall layers and creation of a dissection plane. The condition is associated with malperfusion of vital organs and predisposes for aortic rupture.⁶⁹ The risk of aortic dissection is eight-fold higher in BAV individuals than in the general population.²

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 and aortic conditions. Aortic valve stenosis is the dominating valve pathology in BAV patients followed by valve regurgitation (not secondary to infective endocarditis) and finally endocarditis.^{48,70} Ascending aortic dilatation 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.

2.3.1 Aortic valve stenosis

Aortic valve stenosis is the most frequent valvular dysfunction associated with BAV.⁷⁰ The underlying mechanisms of aortic valve stenosis formation are believed to be similar to those of atherosclerosis (for review see⁷¹). A number of risk factors that are associated with aortic valve calcification have been identified and these are similar to those of atherosclerotic disease (demographic: advanced age and male gender; clinical: cigarette smoking, diabetes, and hypertension; biochemical: total cholesterol, raised low-density lipoprotein cholesterol, raised triglycerides, low high-density lipoprotein cholesterol, raised lipoprotein (a), uraemia, raised serum creatinine, and raised serum calcium); however, the distinction between BAV and TAV has not been made.^{72,73} 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, but not identical.⁷⁴⁻⁷⁶ eNOS deficiency and signalling 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.^{37,77,78} However, valve pathology in BAV individuals is suggested to mainly be the result of non-genetic factors on the basis of human genetic studies.⁴⁹

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 an anterioposterior location (i.e., RL) in patients aged 15-76 years.⁵⁸ Moreover, Fernandes *et al.* found an association between valve disease (both stenosis and regurgitation) and BAVs with a RN configuration in patients aged 1 day-17.9 years.⁷⁹ By contrast, BAV phenotype was not found to be an independent predictor of cardiac events (including aortic valve disease) by Tzemos *et al.*⁷⁰

2.3.2 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 leaflets, redundancy of the fused leaflets leading to prolapse, infective endocarditis, dilatation of the aortic root, and aortic dissection.^{51,54,63,80-82}

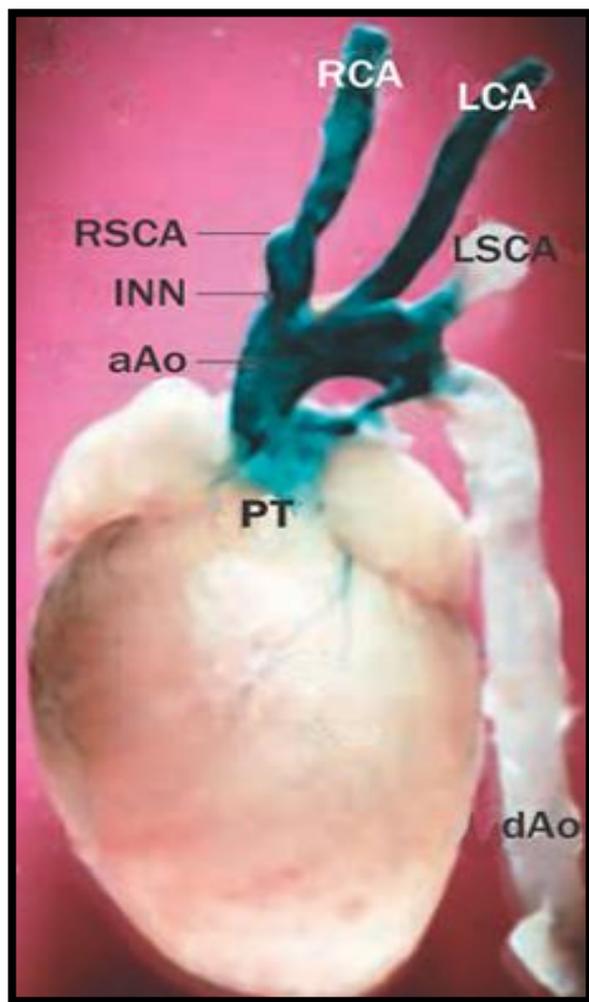
2.3.1 Aortic pathology

The wall of the aorta consists of three basic layers: the internal layer, *tunica intima*, which contains a single layer of endothelial cells and a thin layer of connective tissue; the middle layer, *tunica media*, which mainly contains vascular smooth muscle cells, elastic fibres and collagen; and the outer layer, *tunica adventitia*, which predominantly contains collagen. The intima and the media are separated by the *tunica elastica interna* and the media is separated from the adventitia by the *tunica elastic externa*.⁸³

The aortic media is arranged in lamellar units where two elastic lamellae enclose vascular smooth muscle cells. The smooth muscle cells are surrounded by extracellular matrix that contains microfibrils, small elastic fibres, collagen, and proteoglycans. The extracellular matrix interconnects the two elastic lamellae as well as connecting the elastic lamellae with the smooth muscle cells.⁸⁴⁻⁸⁶ Elastin fibres from the lamellae protrude between thick collagen fibres (containing collagen I, III, and V) and together with microfibrils of fibrillin-1 and collagen VI (also containing some fibronectin) facilitates the smooth muscle cell-elastin interaction.⁸⁷ Histological changes of the aortic wall related to age as well as aortic pathology (dilatation/dissection) are associated with medial degeneration, regardless of aortic location (i.e., ascending, descending, or abdominal).^{88, 89} Medial degeneration includes fragmentation of elastic fibres, rarefaction of smooth muscle cells and areas of mucoid degeneration (possibly constituted by intact or modified glycosaminoglycans).⁸⁵ Although there are no evident qualitative differences in medial degeneration related to age, type of aortic pathology, or aortic location, *quantitative* differences are evident and BAV patients have more severe histological changes than TAV patients.^{90, 91} Furthermore, elastic fragmentation is more pronounced in BAV patients than in TAV patients with isolated valve pathology⁹² and an association of BAV phenotype and the severity of medial degeneration has been reported.⁹³

The pathogenesis of aortic dilatation and aneurysm formation of the ascending, descending, and abdominal aorta differs.^{94, 95} 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^{96, 97} (Fig. 5). Whether developmental differences have an impact on differences in the underlying pathogenesis of aortic dilatation related to aortic location (i.e., ascending, descending, abdominal) is not known. However, aneurysm formation is on a progressive scale (ascending < descending < abdominal) associated with atherosclerosis and inflammation.^{98, 99} Furthermore, ascending aortic dilatation in TAV, but not BAV, is associated with inflammation and immune response.¹⁰⁰

Figure 5. Developmental origin of cells populating the ascending aorta



Experimental studies in mice. Green represents LacZ-positive neural crest cells. aAo – aortic root; INN – innominate artery; LCA – left carotid artery; LSCA – left subclavian artery; PT- pulmonary trunk; RCA – right carotid artery; RSCA – right subclavian artery. Reprinted with permission from Nature review and The Company of Biologists © Jiang et al. *Development* 127, 1607-1616 (2000).^{97, 101}

In addition to differences in the severity of medial degeneration and inflammatory profile between BAV and TAV patients with aortic dilatation, several other factors related to BAV-associated aortic dilatation have been reported as follows: I) dilatation of the ascending aorta is progressive in BAV, but not in TAV, regardless of aortic valve replacement;^{102, 103} II) 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;^{67, 104, 105} III) 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;¹⁰⁶ IV) there are differences in signalling pathways involved in extracellular matrix homeostasis between BAV and TAV patients with dilated aortas¹⁰⁷⁻¹¹⁴ (Study IV); V) eNOS expression is lower in BAV patients than in TAV patients and is inversely correlated with aortic diameter;¹¹⁵ and VI) mutations in genes encoding components of the extracellular matrix are linked to aortic dilatation and BAV (Table 5).

Table 5. Human genes encoding extracellular matrix components associated with aortic dilatation and BAV

Gene	Description	Function
FBN1 ^{113, 121}	Fibrillin 1	Extracellular matrix glycoprotein, forms microfibrils, structural component, provides force-bearing support
ACTA-2 ¹²²	Actin alpha-2	Member of the actin family of proteins, major constituent of contractile elements
TGFBR2 ¹²¹	Transforming growth factor, beta receptor II	Transmembrane receptor with a protein kinase domain, phosphorylates proteins

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 morphology of a BAV is evidently different from that of a normal TAV with the consequence of altered flow across the valve.¹¹⁶ The abnormal flow patterns are thought to predispose patients to aortic dilatation mediated by increased shear stress and differential gene expression in the aortic wall.^{109, 111, 117} An intrinsic “stenotic” state of a non-calcified BAV combined with accelerated progression of valve disease in BAVs is suggested to add to the hemodynamic stress that the aorta is subjected to.^{51, 57-60} However, findings such as progressive dilatation of the ascending aorta after aortic valve replacement in BAV patients but not in TAV patients, aortic dilatation out of proportion to existing valve lesions in both children and adults with a BAV, and a reduction of extracellular matrix components of the aortic media in BAV patients has led to the notion of an inborn weakness of the aorta in BAV individuals.^{56, 103, 113, 118, 119} Several molecular pathways involved in extracellular matrix integrity of the aortic media have been investigated and more recently defective repair mechanisms have been suggested to be important in BAV-associated aortic dilatation^{111, 112} (for review see ¹²⁰).

2.3.1.1 Matrix metalloproteinases

Matrix metalloproteinases (MMPs) constitute a family of enzymes that have the ability to degrade components of the extracellular matrix (for review see ¹²³). MMPs are expressed in several different cell types such as endothelial cells, leukocytes, macrophages, fibroblasts, and vascular smooth muscle cells. There are more than twenty different known human MMPs and four endogenous tissue inhibitors of metalloproteinases (TIMPs) (Table 6). The tissue distributions and functions of several of these enzymes are only known to a limited extent. However, MMPs are implicated as mediators of altered turnover of the extracellular matrix in the media of dilated or aneurysmatic aortas.

Table 6. Members of the MMP family in humans

MMP	Alias	Common substrates
MMP1	Interstitial collagenase, Collagenase 1	Collagen I, II, III, VII, VIII, X, aggrecan, gelatin, MMP2, -9
MMP2	Gelatinase A	Collagen I-V, VII, X, XI, aggrecan, elastin, fibronectin, gelatin, laminin, proteoglycan, MMP9, -13
MMP3	Stromelysin 1	Collagen II, III, IV, IX, X, XI, aggrecan, elastin, fibronectin, gelatin, laminin, proteoglycan, MMP7, -8, -13
MMP7	Matrilysin 1	Collagen IV, X, aggrecan, elastin, fibronectin, gelatin laminin, proteoglycan, MMP1, -2, -9
MMP8	Neutrophil collagenase, Collagenase 2	Collagen I, II, III, V, VII, VIII, X, aggrecan, elastin, fibronectin, gelatin, laminin
MMP9	Gelatinase B	Collagen IV, V, VII, X, IVX, aggrecan, elastin, fibronectin, gelatin
MMP10	Stromelysin 2	Collagen III-V, aggrecan, elastin, fibronectin, gelatin, laminin, MMP1, -8
MMP11	Stromelysin 3	Aggrecan, fibronectin, laminin
MMP12	Macrophage elastase	Collagen IV, elastin, fibronectin, gelatin, laminin
MMP13	Collagenase 3	Collagen I-IV, aggrecan, gelatin
MMP14 (MT1-MMP)	Membrane-type-1 matrix metalloproteinase	Collagen I, II,III, aggrecan, elastin, fibronectin, gelatin, laminin, MMP2, -13
MMP15 (MT2-MMP)	Membrane-type-2 matrix metalloproteinase	Collagen I, fibronectin, gelatin, laminin, MMP2
MMP16 (MT3-MMP)	Membrane-type-3 matrix metalloproteinase	Collagen I, MMP2
MMP17 (MT4-MMP)	Membrane-type-4 matrix metalloproteinase	Fibrin, gelatin
MMP19	RASI-1	Collagen IV, fibronectin, aggrecan, COMP, laminin, gelatin
MMP20	Enamelysin	Collagen V, aggrecan, amelogenin, cartilage oligomeric matrix protein

MMP21	–	α 1-Anti-trypsin
MMP23	–	–
MMP24 (MT5-MMP)	Membrane-type-5 matrix metalloproteinase	Fibrin, gelatin
MMP25 (MT6-MMP)	Membrane-type-6 matrix metalloproteinase	Collagen IV, gelatin, fibronectin, laminin, fibrin
MMP26	Matrilysin 2	Collagen IV, gelatin, fibronectin
MMP27	–	–
MMP28	Epilysin	Casein

*Reprinted with permission from Elsevier.*¹²⁴

3 AIMS

The overall aim of this thesis was to characterise morphological, molecular, and clinical aspects of BAV disease in adult patients undergoing cardiac surgery.

Specific aims:

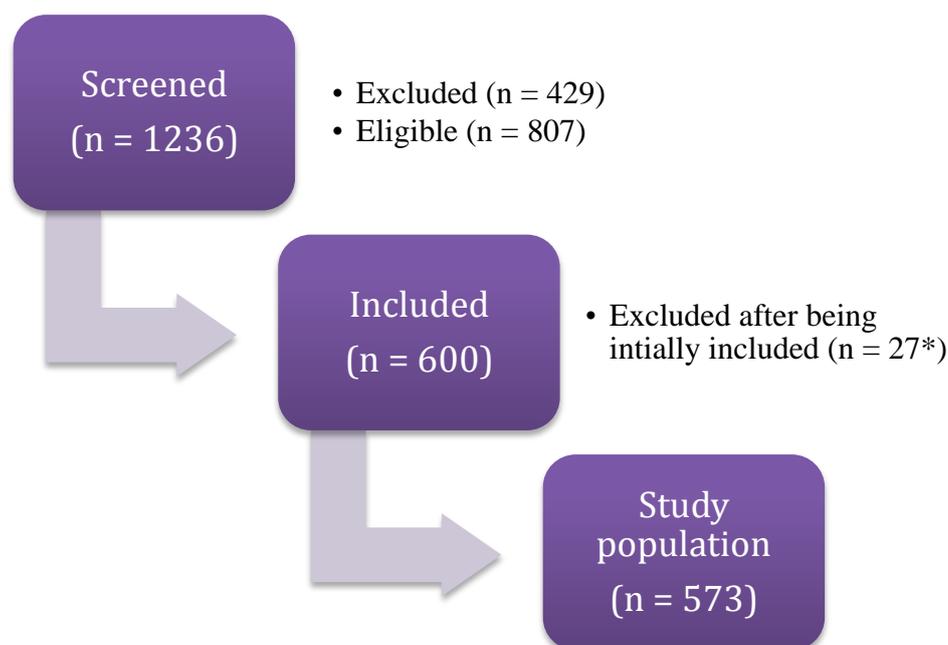
1. To assess the morphology of the aortic root and ascending aorta in relation to valve morphology and BAV phenotype (*Study I*)
2. To investigate a possible association between the severity of valve pathology and the morphology of the aortic root and ascending aorta (*Study II*)
3. To evaluate a possible correlation between ascending aortic dilatation and dilatation of the remaining aorta (*Study III*)
4. To analyse the occurrence of matrix-degrading proteases in the media of the aortic wall (*Study IV*)
5. To evaluate patients characteristics in relation to valve morphology, valve pathology, aortic morphology, and coronary artery disease (*Study V*)

4 METHODS

4.1 ADVANCED STUDY OF AORTIC PATHOLOGY (ASAP)

ASAP is a prospective study that enrolled 600 consecutive patients undergoing elective cardiac surgery due to aortic valve and/or pathology of the aortic root and/or ascending aorta at the Cardiothoracic Surgery Unit at Karolinska University Hospital (Fig. 6).

Figure 6. Flowchart of patients included in ASAP



Exclusion criteria: < 18 years old; unable to give informed consent; significant coronary artery disease (i.e., significant stenosis at coronary angiogram); other concomitant valve surgery indicated; acute intervention indicated; previous cardiac surgery; blood-borne infection.) These patients were excluded due to logistic reasons (n = 4), discovery of existing exclusion criteria (n = 7), other medical reasons (n = 6), and patient withdrawal (n = 10).*

The preoperative screening included cardiovascular risk factor profiling, medical history, medication, laboratory findings and echocardiographic evaluation. Patients who needed further evaluation of the aorta due to clinical presentation and/or echocardiographic findings also underwent a preoperative computed tomography scan. Intraoperatively, the aortic valve was determined to be unicuspid (a single leaflet and commissure), bicuspid (two leaflets and two commissures), or tricuspid (three leaflets and three commissures) based on the surgeon's inspection of the valve (Fig. 1). Furthermore, BAV phenotype was determined (i.e., fusion of the right and left leaflets with a commissural raphe, fusion of the right and non-coronary leaflets with a commissural raphe, fusion of the left and non-coronary leaflets with a commissural raphe or a true BAV without a raphe; Fig. 4). Tissue samples from the anterior part of the ascending aorta were collected and prepared for ribonucleic acid (RNA) isolation, gene array analyses, and immunohistochemistry.

The study population of this thesis consisted of the first 500 consecutive patients operated on within the setting of ASAP between February 2007 and February 2012 (Table 7). In addition, 202 patients who were undergoing cardiac surgery (within the same time frame) due to aortic valve and/or ascending aortic pathology and concurrent coronary artery bypass grafting were included and studied retrospectively. Patients who were operated on between 2007 and 2009 and from whom RNA was extracted from the ascending aorta that was of sufficient quantity and quality for gene array analyses were included in *Study IV* (n= 109).

Table 7. Study population of the thesis

	Study population (n)	ASAP
Study I	300	1-300
Study II	500	1-500
Study III	97	1-500
Study IV	109	Operated in 2007-2009
Study V	702	1-500 (+202)

4.2 ECHOCARDIOGRAPHY

4.2.1 Transthoracic echocardiography

Transthoracic echocardiography was used to preoperatively evaluate the presence and grade of valve pathology as well as cardiac dimensions and function in all patients. To assess the occurrence and grade of aortic valve stenosis, valve area, peak gradient and mean gradient across the valve were calculated according to the continuity- and Bernoulli equation. The occurrence and grade of aortic valve regurgitation was determined based on colour flow jet area, vena contracta, pressure half-time, jet-density, and diastolic flow reversal in the descending aorta.

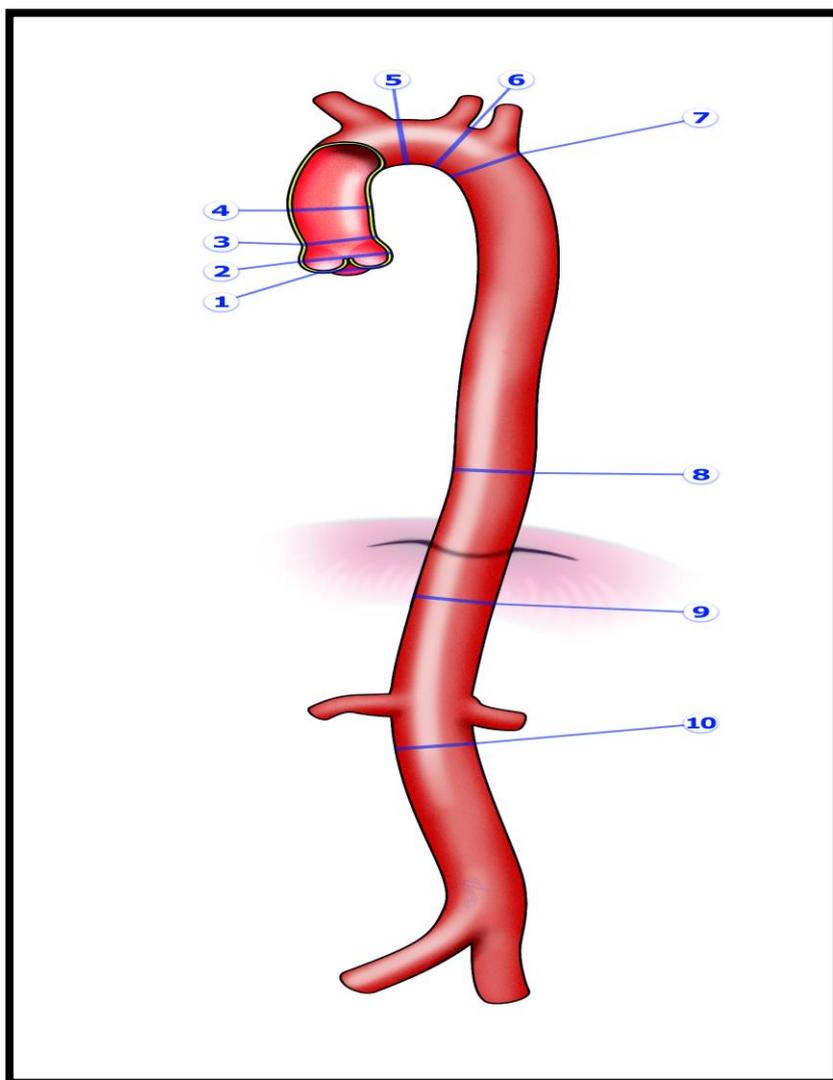
4.2.2 Transesophageal echocardiography

Perioperatively all patients were examined with transesophageal echocardiography when fully anaesthetised. The dimensions of the left ventricular outflow tract, annulus, sinus of Valsalva, sinotubular junction, ascending aorta and the root height (i.e., the distance between the annulus and the sinotubular junction) were measured. All measurements were obtained in diastole except for the diameter of the left ventricular outflow tract which was measured in systole.

4.3 COMPUTED TOMOGRAPHY SCANNING

Patients with suspected or confirmed dilatation of the aorta and/or aortic root were examined by computed tomography scanning. Maximal outer wall measurements were obtained in the axial plane at the annulus, sinus of Valsalva, sinotubular junction, ascending aorta, proximal arch, distal arch, aortic isthmus, descending aorta, suprarenal aorta, and infrarenal aorta (Fig. 7).

Figure 7. Aortic measurement points in computed tomography scanning



1) Annulus; 2) sinus of Valsalva; 3) sinotubular junction; 4) ascending aorta; 5) proximal arch, 6) distal arch, 7) aortic isthmus; 8) descending aorta; 9) suprarenal aorta; 10) infrarenal aorta. Reprinted with permission from Elsevier (Study III).

4.4 GENE EXPRESSION AND IMMUNOHISTOCHEMISTRY

RNA was isolated from the aortic tissue that was collected intraoperatively, and RNA was then analysed using gene arrays (an automatized method to detect expression of all messenger RNAs (mRNA)). A subset of the mRNAs analysed with gene arrays was amplified by real-time polymerase chain reaction (PCR), and then analysed to validate the results of the gene arrays (Study IV). Immunohistochemistry was used to visualise and locate protein expression in the aortic wall of the corresponding gene of interest (Study IV).

4.5 DEFINITIONS

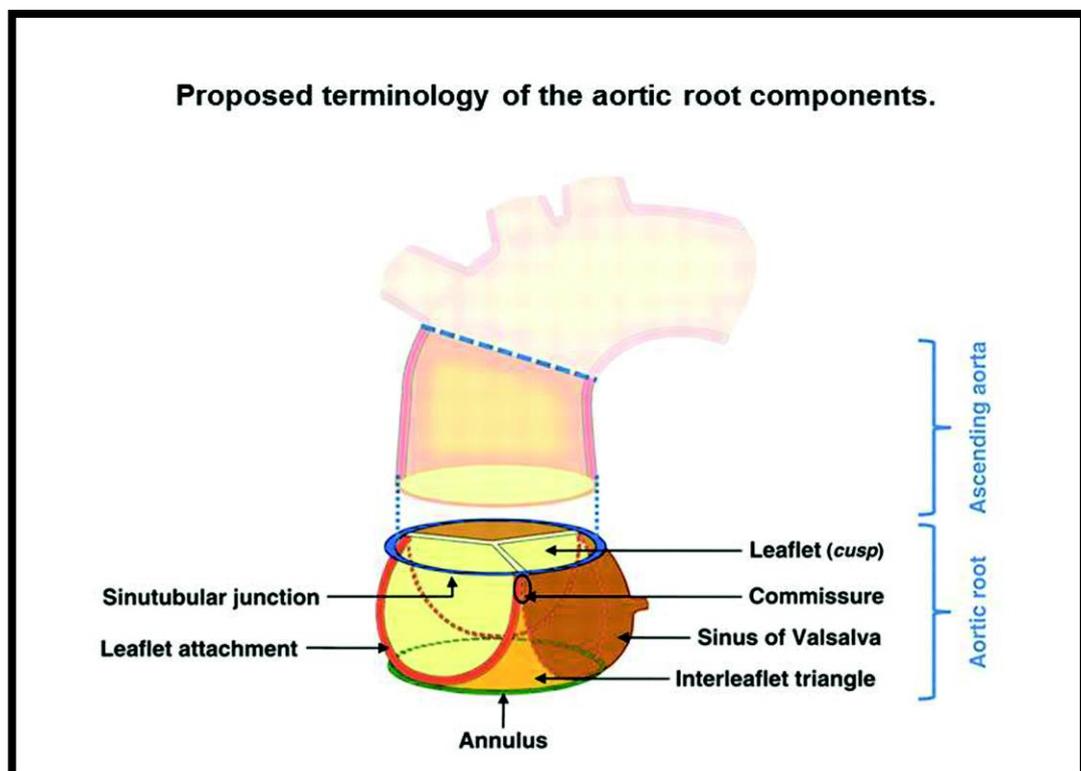
4.5.1 Anatomic structures

The “aortic valve” and the “aortic root” were defined as proposed by Sievers *et al.*¹²⁵ (Table 8, Fig. 8).

Table 8. Definition of the aortic valve and aortic root

Anatomic structure	Definition
Leaflets	The moving parts that separate the left ventricle and the aorta
Commissure	The most distal attachment of the leaflets to the aortic wall
Annulus	The virtual circle formed by the nadirs of the semi-lunar leaflet attachments
Interleaflet triangle	The area between the semi-lunar attachments of the leaflets from the nadir to the commissure
Aortic valve	The leaflets
Aortic root	The annulus, leaflets, leaflet attachments, interleaflet triangles, sinus of Valsalva and the sinotubular junction

Figure 8. Definition of the aortic root and ascending aorta



Reprinted with permission from Oxford University Press.¹²⁵

4.5.2 BAV phenotype

BAV phenotype was defined as a fusion of the right and left coronary leaflets with a commissural raphe (RL), a fusion of the right and non-coronary leaflets with a raphe (RN), a fusion of the left and non-coronary leaflets with a raphe (LN), or a true BAV without a raphe (TB) (Fig. 4).

Other classifications of the various types of BAV have been proposed and differ somewhat from the classification used in this thesis.¹²⁶ However, the major features of the most prevalent BAV phenotypes are the same: BAV-RL is identical to Sievers' BAV "type 1, L-R"; BAV-RN is identical to "type 1, R-N"; BAV-LN is identical to "type 1, N-L" and true BAV is identical to Sievers' "type 0". Sievers' BAV "type 2" corresponds to what we and others have classified as a unicuspid aortic valve (UAV).^{127, 128}

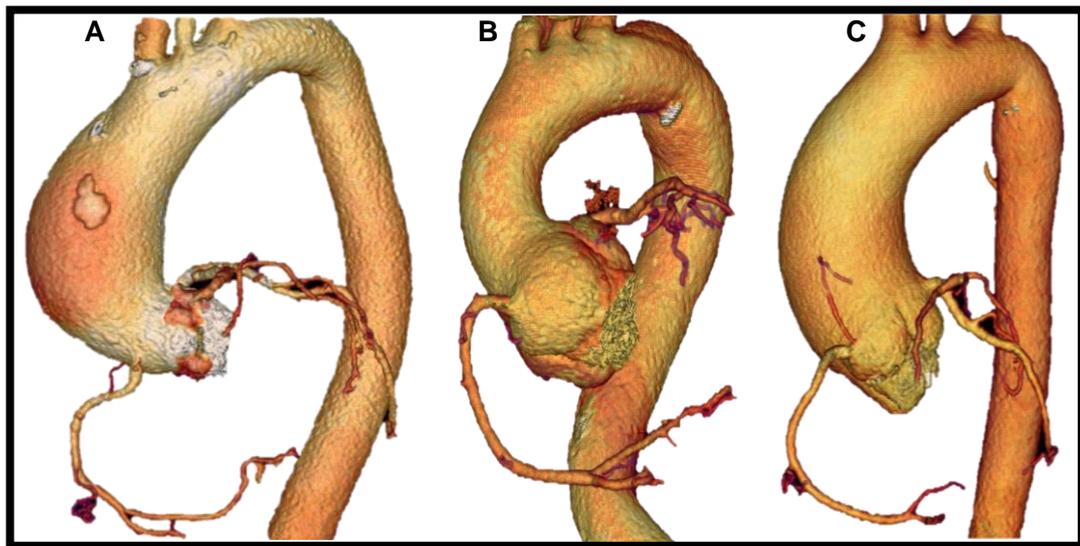
4.5.3 Valve pathology

Aortic valve stenosis was defined as when the peak gradient across the aortic valve exceeded 50 mm Hg and/or the mean gradient exceeded 40 mm Hg and/or the aortic valve area was less than 1.0 cm². If the criterion for valve stenosis was met, the valve was classified as stenotic regardless of the presence of concomitant valve regurgitation since regurgitation in these instances was a consequence of valve calcification. Grade 1 aortic valve stenosis was defined as a peak gradient across the valve of 51-64 mm Hg, grade 2 as 65-100 mm Hg and grade 3 as >100 mm Hg. Aortic valve regurgitation was graded as mild (grade 1), moderate (grade 2), moderate to severe (grade 3), and severe (grade 4).

4.5.4 Aortic morphology

A non-dilated aorta was defined as when the dimension of the sinus of Valsalva exceeded the dimension of the sinotubular junction, and the maximal aortic diameter did not exceed 40 mm (patients with BAV) or 45 mm (patients with TAV). An aneurysmatic aorta was defined as when the diameter of the sinus of Valsalva exceeded the diameter of the sinotubular junction, and the maximal aortic diameter exceeded 40 mm (patients with BAV) or 45 mm (patients with TAV). An ectatic aorta was defined as when the dimension of the sinotubular junction exceeded the dimension of the sinus of Valsalva, and the maximal aortic diameter exceeded 40 mm (patients with BAV) or 45 mm (patients with TAV). The different dilated aortic morphologies are depicted in Fig. 9. The descending and infrarenal aorta were deemed dilated according to the criteria proposed by Wanhainen *et al.* (i.e., the descending aorta exceeded 36 mm in males and 32 mm in females; the infrarenal aorta exceeded 29 mm in males and 26 mm in females).¹²⁹ To attain an adequate comparison of gene expression levels between non-dilated and dilated aorta as well as between BAV and TAV patients in *Study IV* the aorta was considered to be non-dilated at a maximal aortic diameter of 40 mm or less and dilated at 45 mm or more, regardless of BAV or TAV. Patients with aortic diameters between 41 and 44 mm were not included in the analyses.

Figure 9. Dilated ascending aortas with different aortic morphologies



a) Ascending aortic aneurysm with intact sinotubular junction and mid-ascending dilatation; b) aortic root aneurysm with intact sinotubular junction and no mid-ascending dilatation; c) aortic ectasia lacking defined sinotubular junction and with mid-ascending dilatation. Computed tomography reconstructions, courtesy of Anders Svensson, Radiology Department, Karolinska University Hospital, Huddinge.

4.6 STATISTICAL ANALYSES

Values were presented as the mean and standard deviation (*Study I-V*), median and interquartile range (*Study III-V*) frequency counts, and percentages (*Study I-V*) and 95% confidence interval (*Study II*). Mann-Whitney U test or Student's t test was used for comparisons of continuous variables. The Chi-square and Fisher's exact test was used for comparisons of categorical data (*Study I-V*). Spearman rank correlation test was used for correlation analyses (*Study IV*). Two-way factorial analysis of variance (ANOVA) (*Study I, II, V*) and Kruskal-Wallis one-way ANOVA (*Study III*) were used for continuous variables, and logistic regression analysis were used for dichotomous variables (*Study V*). If patient characteristics were associated with the outcome measures, they were included in the ANOVA model in a stepwise manner. In the case of a significant interaction between the factors in the ANOVA model, simple main effects tests were examined. In *Study III* analysis of covariance (ANCOVA) was used to adjust for patient characteristics and comorbidities in comparisons between BAV and TAV patients. Principal components analysis based on the gene expression found in the gene array analysis was performed (*Study IV*). A p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using the Statistica 10.0 software (StatSoft® Inc, Tulsa OK, USA) and the Statview® software (SAS Institute Inc, version 5.0.1).

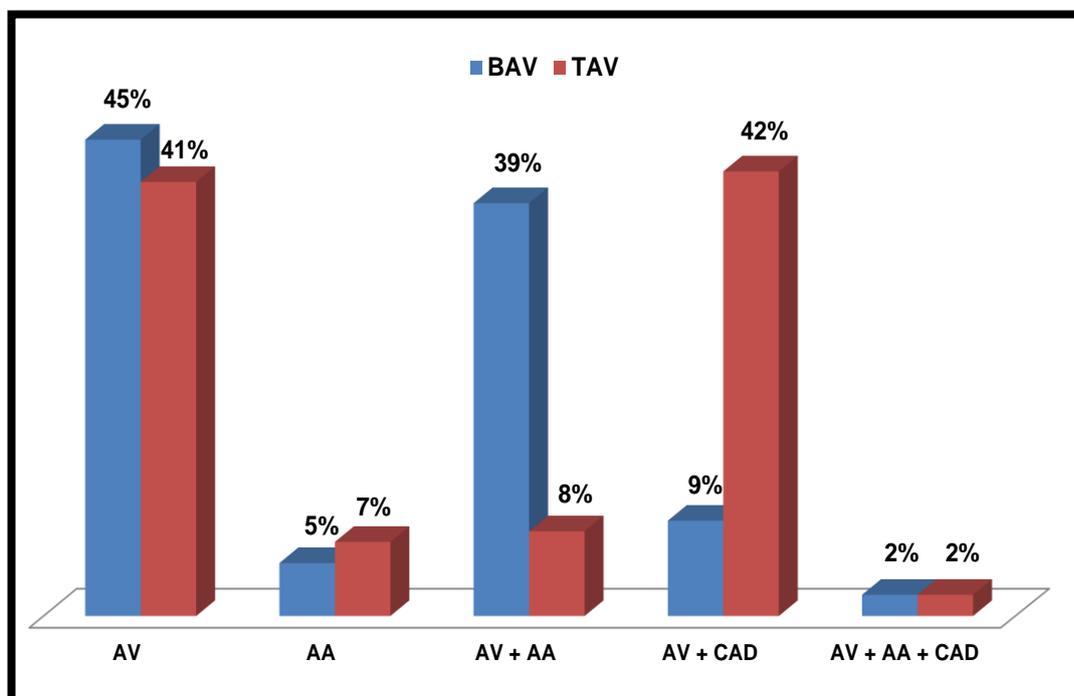
4.7 ETHICAL CONSIDERATIONS

All studies were approved by the Regional Ethical Review Board (in Stockholm) and informed consent was obtained from all patients.

5 RESULTS

Of 600 patients included in ASAP, 573 eventually underwent cardiac surgery within the setting of the study (Fig. 6). The first 500 consecutively operated patients in ASAP together with 202 patients with aortic valve and/or aortic pathology and in addition significant coronary artery disease constitute the study population of this thesis. The overall mean age of the patients was 66 ± 12 years (without coronary artery disease: 64 ± 12 years; with coronary artery disease: 72 ± 8 years) and the gender distribution was 2:1 males to females (without coronary artery disease: 2:1; with coronary artery disease: 4:1 ($p = 0.0007$); without coronary artery disease: BAV 2.4:1 and TAV 1.6:1 ($p = 0.03$); with coronary artery disease: BAV 7:1 and TAV 3:1 ($p = 0.24$)). In patients without coronary artery disease the surgical procedures included aortic valve replacement ($n = 319$), aortic valve plasty ($n = 17$) and replacement of the ascending aorta with a supracoronary graft with ($n = 53$) or without ($n = 26$) concurrent valve surgery. A composite graft was used in 60 patients and the David procedure was used in 25 patients. All patients with concomitant coronary artery disease underwent coronary artery bypass grafting in conjunction with aortic valve replacement ($n = 189$), aortic valve plasty ($n = 1$) or replacement of the ascending aorta with a supracoronary graft and concurrent valve surgery ($n = 7$). A composite graft was used in four patients and the David procedure in one patient. The distribution of valve morphology in relation to indication for surgery is depicted in Fig. 10.

Figure 10. Distribution of valve morphology in relation to indication for surgery



AV – aortic valve disease; AA – ascending aortic pathology; CAD – coronary artery disease.

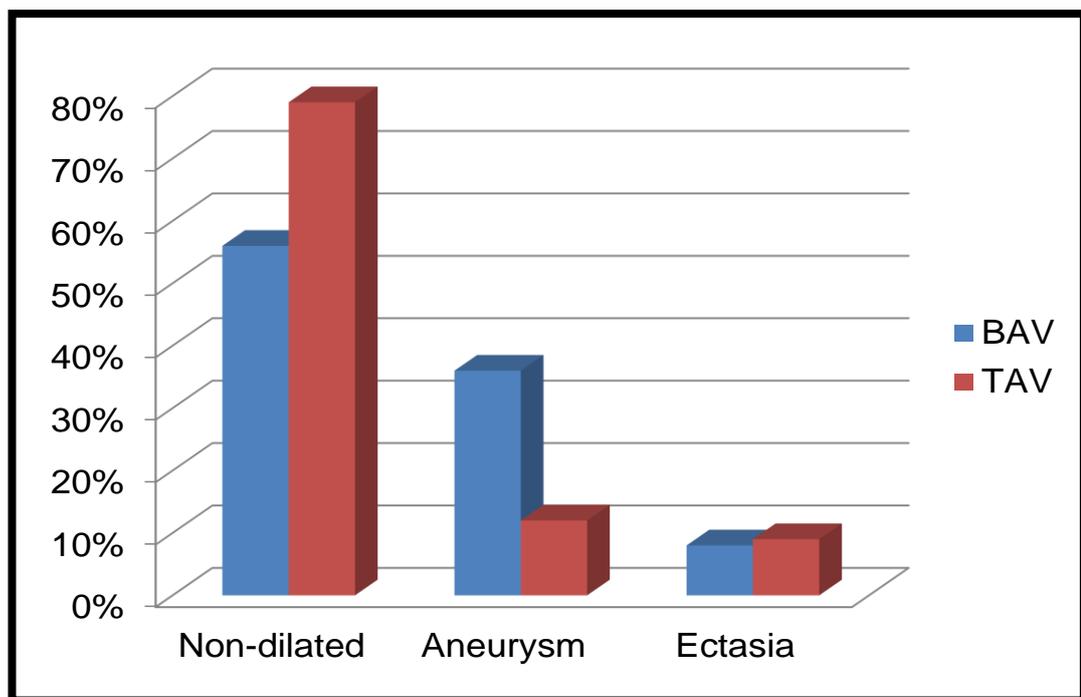
5.1 BAV PHENOTYPE AND AORTIC MORPHOLOGY

The aim of *Study I* was to assess the morphology of the aortic root and ascending aorta in relation to valve morphology and BAV phenotype in cardiac surgery patients (n = 300).

5.1.1 General findings

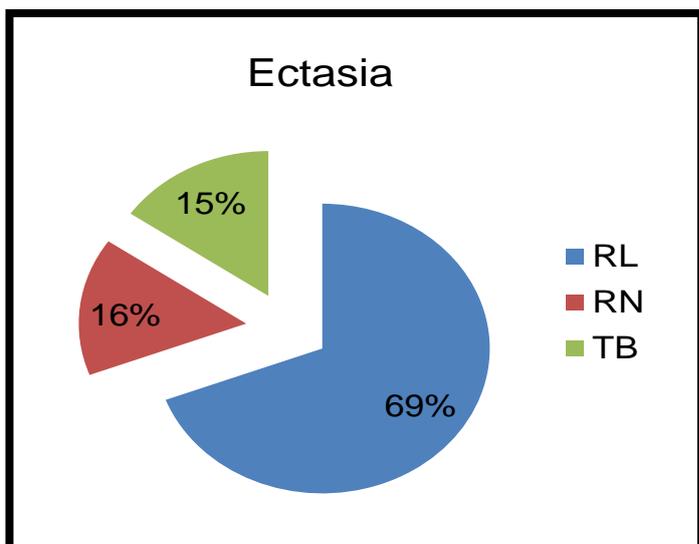
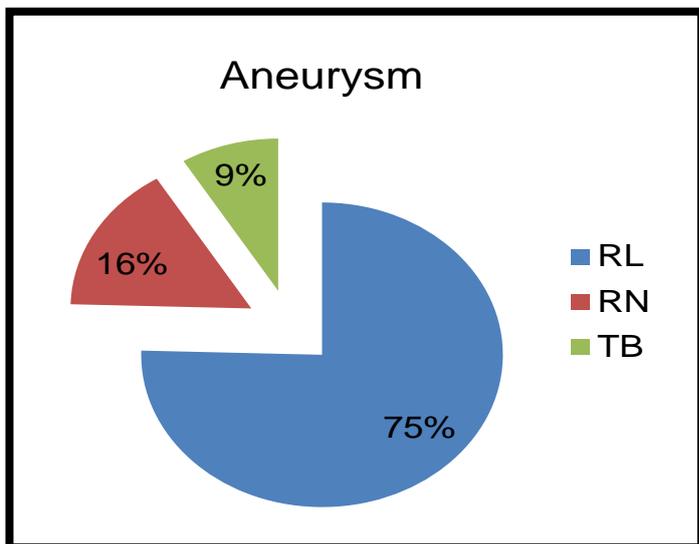
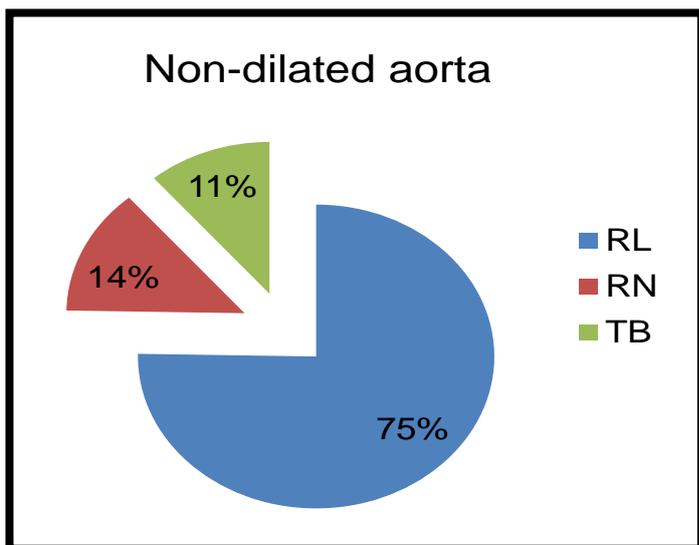
The majority of the study population had a BAV (53%) and BAV was more frequently associated with ascending aortic aneurysm than TAV (36% vs. 12%; $p < 0.001$), whereas ectasia was equally common in BAV and TAV patients (Fig. 11). At the time of surgery, BAV patients were approximately 10 years younger than TAV patients ($p < 0.0001$). Aortic valve pathology (valve stenosis or regurgitation) was equally distributed in BAV and TAV patients when the aorta was non-dilated ($p = 0.82$). When the aorta was dilated BAV patients predominantly had aortic valve stenosis (56% vs. 29%) while TAV patients predominantly had valve regurgitation (81% vs. 4%; $p < 0.001$ for BAV vs. TAV in both instances).

Figure 11. Distribution of valve morphology in relation to aortic morphology



In BAV patients, fusion of the right and left coronary leaflets was predominant (74%) followed by right and non-coronary leaflet fusion (14%), and true BAV (11%; $p < 0.001$). No patients had a fusion of the left and non-coronary leaflets. The distribution of BAV phenotypes was similar regardless of aortic morphology ($p = 0.95$; Fig. 12). A UAV was found in ten patients (3%), and four of these patients had dilatation of the ascending aorta.

Figure 12. Distribution of BAV phenotypes in relation to aortic morphology



RL – fusion of the right and left coronary leaflets; RN – fusion of the right and non-coronary leaflets; TB – true BAV.

5.1.2 Morphology of the aortic root and ascending aorta

Regardless of aortic morphology the annulus and left ventricular outflow tract were always larger in BAV patients than in TAV patients ($p < 0.001$; Table 9). BAV patients had smaller dimension of the ascending aorta when the aorta was ectatic than TAV patients (50 ± 7 vs. 56 ± 9 mm, $p = 0.005$). There were minor but statistically significant differences in aortic dimensions associated with BAV phenotype (Table 10).

Table 9. Aortic dimensions in relation to valve and aortic morphology

	BAV			TAV		
	N	A	E	N	A	E
Annulus	24 ± 4	25 ± 4	27 ± 2	23 ± 3	23 ± 5	23 ± 3
LVOT	25 ± 4	26 ± 4	27 ± 2	23 ± 3	24 ± 4	25 ± 3

A – aneurysm; E – ectasia; LVOT – left ventricular outflow tract; N – non-dilated aorta. Aortic dimensions are presented in millimetres and as mean \pm SD.

Table 10. Differences in aortic dimensions associated with BAV phenotype

Aortic morphology and measure points	Statistics
Aortic root height in non-dilated or aneurysmatic aorta	(RL and RN < TB); $p \leq 0.01$
Sinotubular junction and sinus of Valsalva in aneurysmatic aorta	(RL < TB); $p = 0.02$
Maximal aortic diameter in ectatic aorta	(RL and RN > TB); $p \leq 0.01$

RL – fusion of the right and left coronary leaflets; RN – fusion of the right and non-coronary leaflets; TB – true BAV.

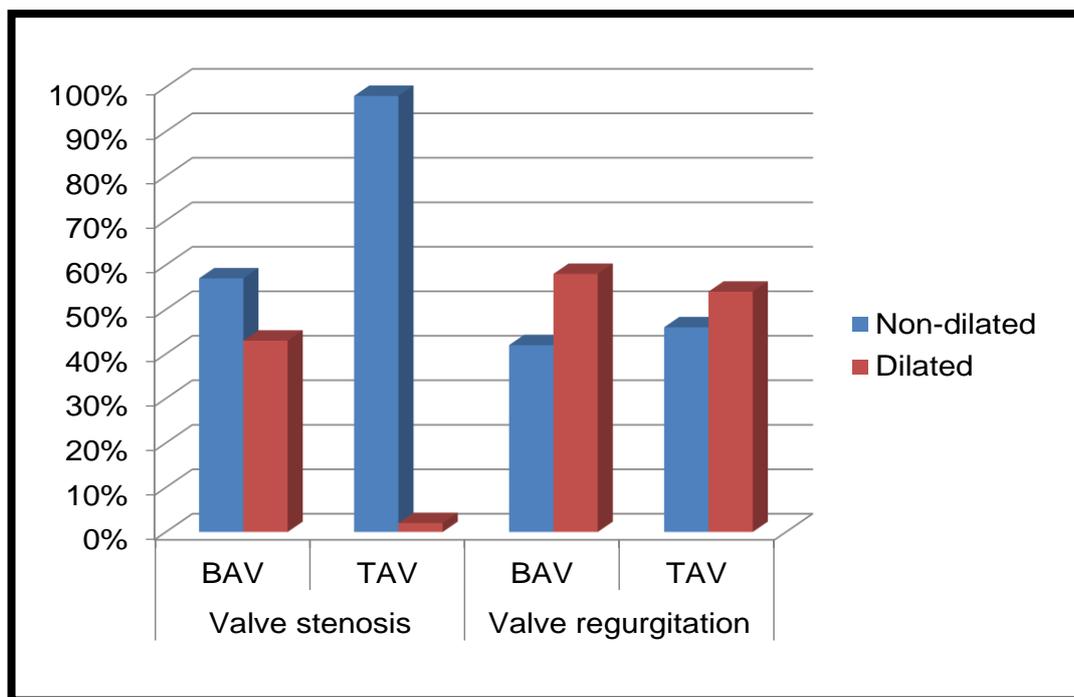
5.2 VALVE PATHOLOGY AND AORTIC MORPHOLOGY

The aim of *Study II* was to investigate a possible association between the severity of valve pathology and the morphology of the aortic root and ascending aorta (n = 500).

5.2.1 General findings

The majority of patients had a BAV (55%), and 42% of BAV patients and 15% of TAV patients had an ascending aortic aneurysm. Thus, an ascending aortic aneurysm was approximately three times more common in BAV patients than in TAV patients ($p < 0.0001$), whereas ectasia was equally common in BAV and TAV patients ($p = 0.40$) undergoing cardiac surgery. The distribution of valve pathology was similar in BAV and TAV patients when the aorta was non-dilated ($p \geq 0.42$), whereas aortic valve stenosis was more common in the BAV group than in the TAV group when the aorta was aneurysmatic or ectatic ($p \leq 0.04$). Conversely, valve regurgitation was more common in TAV patients than BAV patients when the aorta was dilated ($p \leq 0.008$; Fig. 13).

Figure 13. Distribution of valve pathology in relation to aortic morphology



The relative distribution of the different grades of valve pathology did not differ between TAV and BAV patients (Table 11) and there was no difference in left ventricular function (i.e., ejection fraction) between the groups ($p \geq 0.24$). BAV patients were younger than TAV patients when the aorta was non-dilated (~10 years, $p < 0.0001$) or aneurysmatic (~5 years, $p = 0.02$).

Table 11. Distribution of valve pathology in relation to valve morphology and BAV phenotype

	BAV (n = 249)				TAV (n = 192)
	All (n = 249)	RL (n = 184)	RN (n = 38)	TB (n = 25)	
AS1	15%	14%	18%	16%	8%
AS2	43%	46%	37%	32%	39%
AS3	12%	10%	13%	20%	10%
AR1-2	13%	13%	16%	12%	15%
AR3-4	17%	17%	16%	20%	28%

AS – aortic valve stenosis; AR – aortic valve regurgitation. For definition of grade of valve pathology see section 4.5.3.

5.2.2 Aortic dimensions in relation to valve pathology

In general, when the aorta was non-dilated, aortic dimensions were larger when valve regurgitation was present than when valve stenosis was present, and this was true of both BAV and TAV patients ($p \leq 0.03$). In BAV patients with an aneurysm, the sinotubular junction, annulus, and left ventricular outflow tract were larger when valve regurgitation was present than when valve stenosis was present ($p \leq 0.003$). There were no differences in the dimensions of ectatic aortas in relation to valve pathology in BAV patients. Notably only two TAV patients had a combination of aortic aneurysm or ectasia and valve stenosis, while this was seen in 76 BAV patients (Fig. 13).

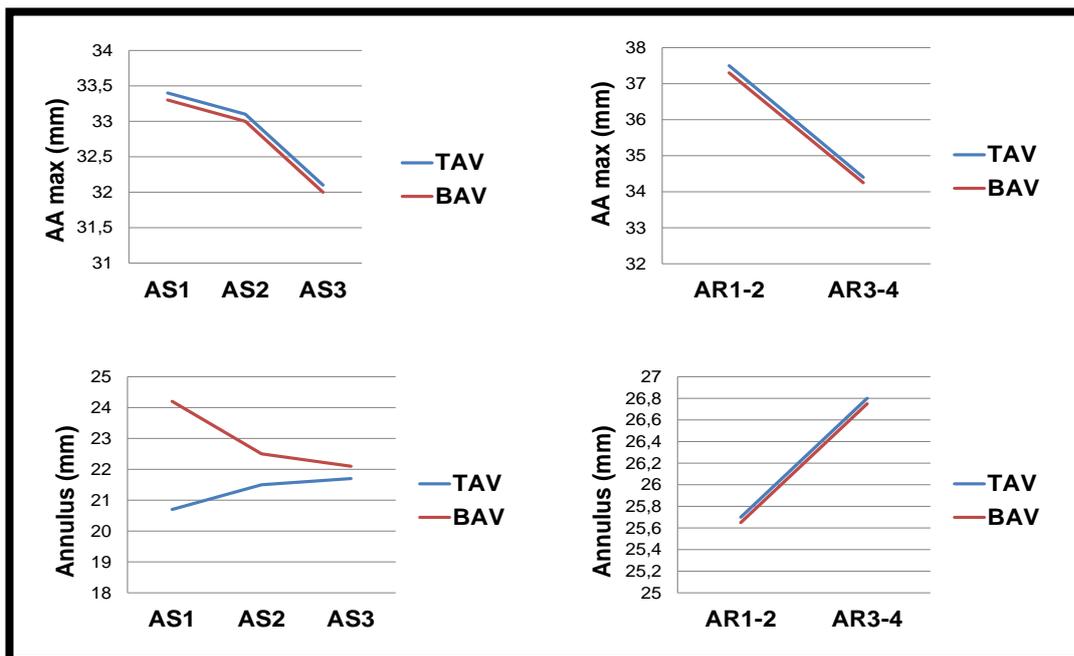
5.2.3 Severity of valve pathology and non-dilated aorta

In both BAV and TAV patients with a non-dilated aorta, the general pattern was that increasing severity of valve pathology was associated with smaller dimensions of the aortic root and ascending aorta (Fig. 14). However, in both BAV and TAV patients with valve regurgitation and in TAV patients with valve stenosis, the annulus and left ventricular outflow tract were larger with increasing severity of valve pathology.

5.2.4 Severity valve pathology and dilated aorta

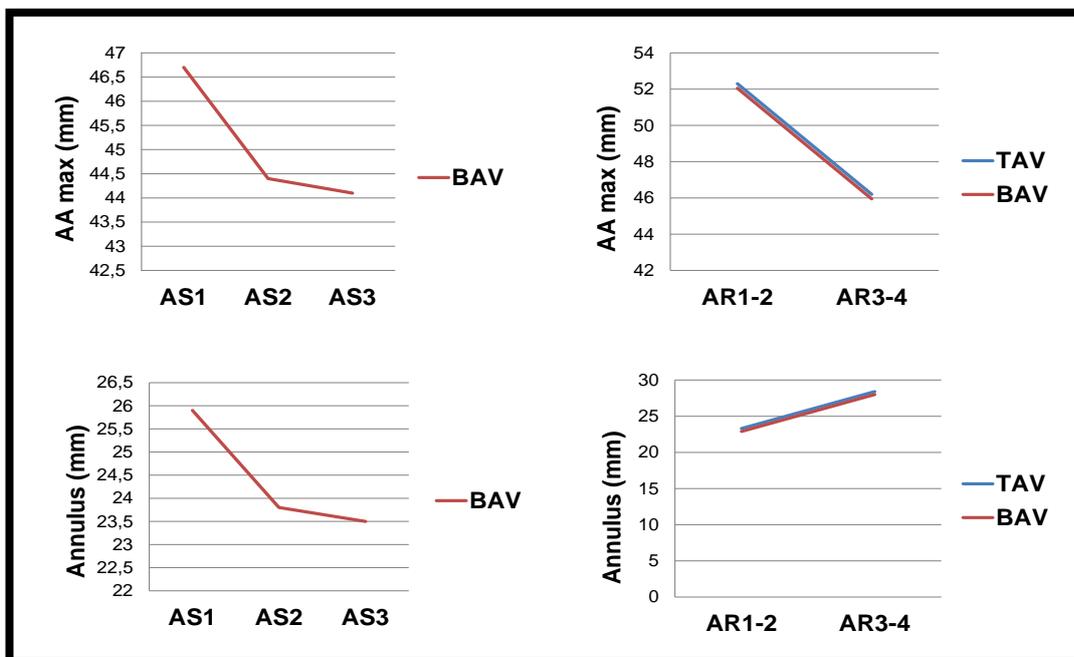
In BAV patients with valve stenosis and aortic aneurysm the general pattern of increasing grade of valve pathology being associated with smaller aortic dimensions was still evident (Fig. 15). This pattern was also found in both BAV and TAV patients with aortic aneurysm and valve regurgitation at the ascending aorta whereas the measurement points of the aortic root were larger with increasing severity of regurgitation.

Figure 14. Grade of valve pathology in relation to aortic dimensions in non-dilated aorta



AS – aortic valve stenosis; AR – aortic valve regurgitation. See section 4.5.3. for definition of grade of valve pathology. $p < 0.05$ for TAV patients with AS at the annulus and for both BAV and TAV patients with AR at the ascending aorta.

Figure 15. Grade of valve pathology in relation to aortic dimensions in aneurysmatic aorta



$p < 0.05$ for BAV patients with AS at the annulus and $p < 0.0001$ for both BAV and TAV patients with AR at the annulus and ascending aorta. See Fig. 14 for abbreviations.

5.3 DIMENSIONS OF THE DISTAL AORTA

The aim of *Study III* was to evaluate a possible correlation between ascending aortic dilatation and dilatation of the remaining aorta in patients with BAV or TAV that were undergoing surgery due to ascending aortic dilatation (n = 97).

5.3.1 General findings

In total, 64% of the patients had a BAV and 36% had a TAV. All patients underwent replacement of the ascending aorta due to aortic dilatation; the maximal aortic diameter was 52 (0.4) mm in BAV patients and 53 (0.8) mm in TAV patients (values are given as median and interquartile range). With the exception of the distribution of valve pathology, the characteristics of BAV and TAV patients did not differ. In the BAV group, 34% of patients had aortic valve stenosis and 45% of patients had valve regurgitation, whereas in the TAV group, no patients had valve stenosis and, 83% had valve regurgitation. Of the BAV patients, 77% had a RL configuration, 13% had a RN configuration, and 10% had a true BAV.

5.3.2 Arch, descending, and abdominal aortic dimensions

There were no differences between BAV and TAV patients in diameters at the annulus, sinus of Valsalva, sinotubular junction, or ascending aorta. By contrast, the dimensions of the proximal arch, distal arch, aortic isthmus, descending aorta, suprarenal aorta, and infrarenal aorta were consistently smaller in BAV patients than in TAV patients ($p < 0.001$).

5.3.3 BAV phenotype, valve pathology, and the distal aorta

In BAV patients, there were no differences in aortic dimensions related to functional status of the valve or BAV phenotype. TAV patients with valve regurgitation had larger aortic dimensions (indexed to body surface area) at the level of the sinus of Valsalva, sinotubular junction, and distal arch compared with TAV patients without valve pathology ($p \leq 0.03$).

5.3.4 Concomitant dilatation of the distal aorta

Concomitant dilatation of the descending aorta was more common in TAV patients than in BAV patients (37% vs. 5%; $p < 0.001$). Dilatation of the infrarenal aorta alone (five BAV patients, three TAV patients) or in combination with dilatation of the descending aorta (one BAV patient, two TAV patients) was equally common in both groups ($p > 0.2$).

5.4 PROTEASES IN THE AORTIC WALL

The aim of *Study IV* was to analyse the occurrence of MMPs and TIMPs in the aortic media of patients with BAV or TAV (n = 109).

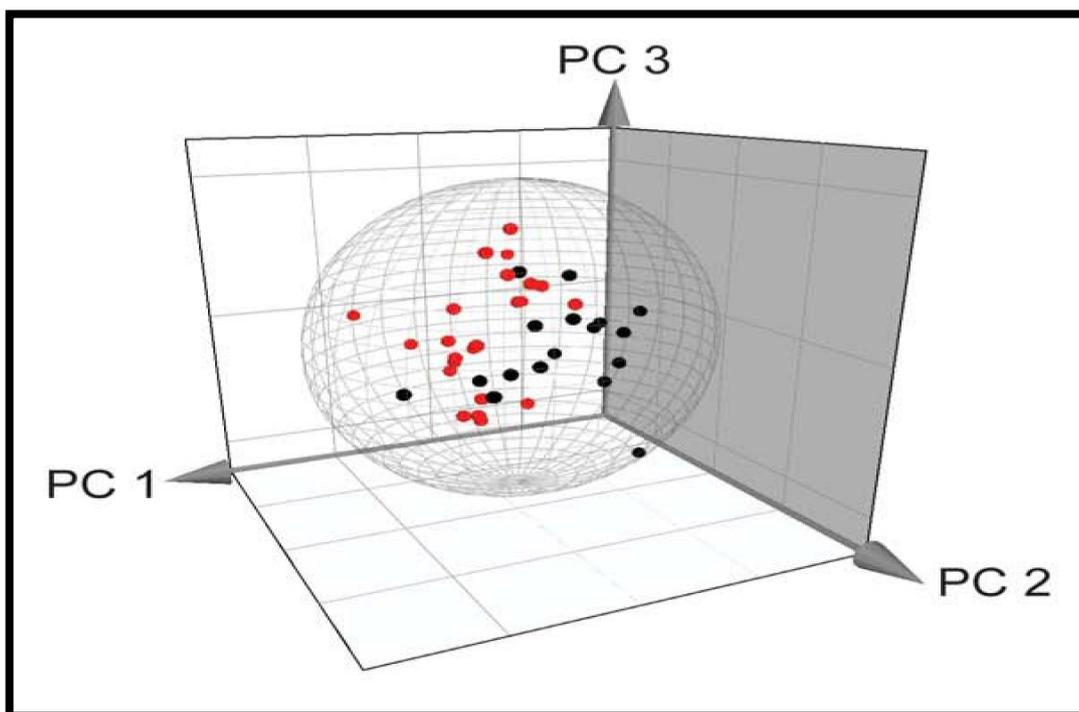
5.4.1 MMP and TIMP expression in the aortic wall

In general, mRNA expression of gelatinases (MMP2 and MMP9), stromelysin 3 (MMP11), all membrane-bound MMPs (MMP14, -15, -16, -17, -24, and -25), MMP19, MMP21, MMP28, and TIMP1-4 was detected in both the intima/media section and the adventitia section of the aortic wall. The expression pattern was seen in both non-dilated and dilated aortas and in both BAV and TAV patients. However, the collagenases (MMP1, -8, and -13) were not detected at levels above background expression.

5.4.2 Protease expression and aortic morphology

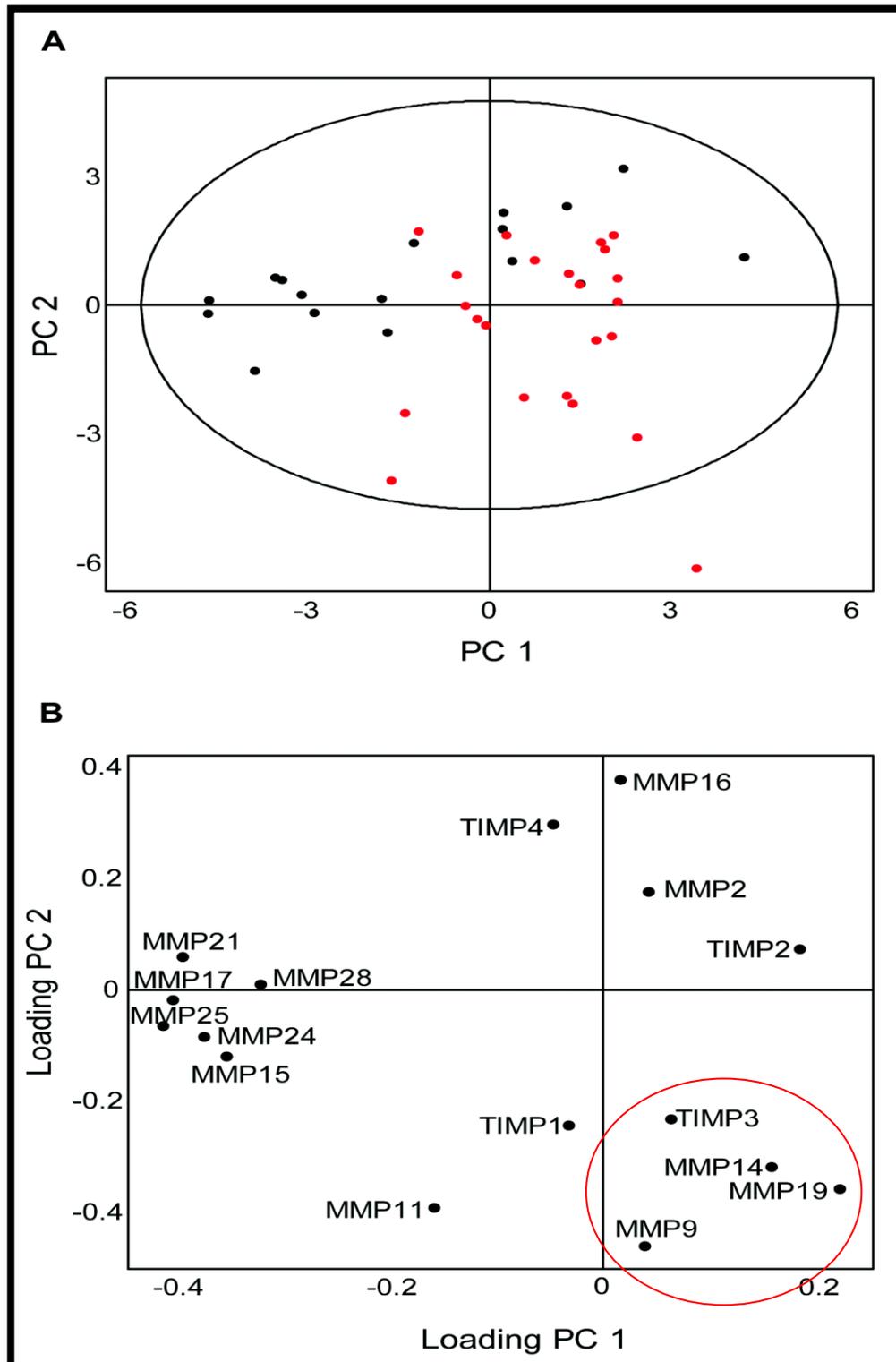
A principal component analysis of the MMPs and TIMPs expressed in the intima/media section of the ascending aorta revealed a separation of TAV patients with non-dilated aortas from TAV patients with dilated aortas (Fig. 16). By contrast, no separation was detected when analysing the BAV group separately or when analysing BAV and TAV patients together. Thus, differences in MMP and TIMP expression between non-dilated and dilated aortas seem to mainly be found in TAV patients. The MMPs and TIMPs responsible for the separation of non-dilated aortas from dilated aortas in TAV patients are in the lower right quadrant of the loading plot in Fig. 17. No differences between the groups were found when analysing the adventitia section of the aorta.

Figure 16. Principal component analysis of gene expression of MMPs and TIMPs in TAV patients with non-dilated (black) and dilated (red) aortas



Reprinted with permission from Elsevier (Study IV).

Figure 17. Score and loading plot of the principal component analysis of gene expression of MMPs and TIMPs in TAV patients with non-dilated (black) and dilated (red) aortas



Reprinted with permission from Elsevier (Study IV).

Univariate analyses showed no differences in the levels of mRNA expression between BAV and TAV patients with non-dilated aortas. In both BAV and TAV patients, expression levels of MMP14, MMP19, and TIMP2 were higher in the intima/media section of dilated aortas than in non-dilated aortas. In TAV patients, expression levels of MMP17, MMP21, MMP25, MMP28, and TIMP4 were all lower in dilated aortas than in non-dilated aortas. In BAV patients, the expression levels of none of the MMPs or TIMPs were lower in dilated vs. non-dilated aortas.

Correlation analyses of MMP expression levels in the intima/media section and the maximal ascending aortic diameter in TAV patients was performed. Of the MMPs that showed higher expression levels in TAV patients with dilated aortas, MMP19 correlated positively with maximal aortic diameter (Rho 0.61, $p = 0.004$ and Rho 0.57, $p = 0.008$ for raw values and values indexed to body surface area, respectively). MMP14 showed borderline significance for raw values (Rho 0.40, $p = 0.06$) but did not correlate with values indexed to body surface area (Rho 0.12, $p > 0.2$). Of the MMPs and TIMPs that showed lower expression levels in TAV patients with dilated aortas, TIMP4 correlated negatively with raw values (Rho -0.49, $p = 0.02$), whereas MMP25, MMP28, and TIMP4 correlated negatively with values indexed to body surface area (Rho -0.45, $p = 0.04$; Rho -0.43, $p < 0.05$; and Rho -0.55, $p = 0.01$, respectively). No other significant correlations between MMP/TIMP expression and maximal aortic diameter were found.

5.4.1 MMP14 and MMP19 protein expression in TAV patients

Immunohistochemistry analysis showed that protein expression of MMP19 was mainly seen in endothelial cells of TAV patients with non-dilated aortas. In dilated aortas endothelial expression MMP19 was still present but strong MMP19 expression was also evident in the medial layer. Similar to MMP19, MMP14 protein expression was upregulated in the media of dilated aortas.

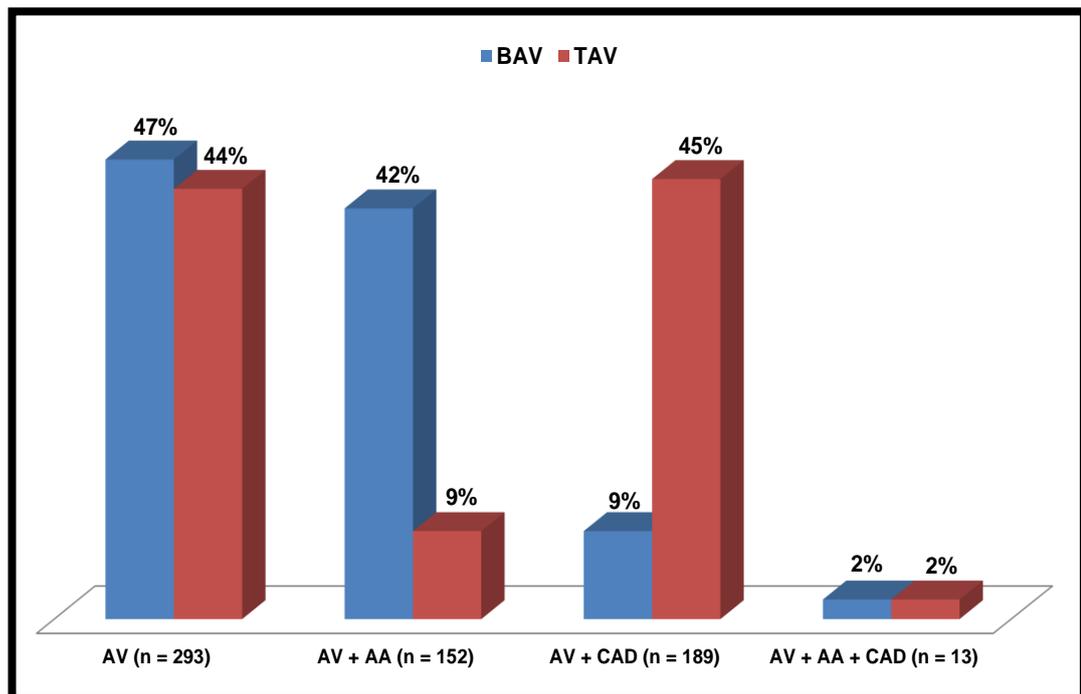
5.5 CLINICAL CHARACTERISTICS AND CORONARY ARTERY DISEASE

5.5.1 Patient characteristics

The aim of *Study V* was to evaluate patients characteristics in relation to valve morphology, valve pathology, aortic morphology and coronary artery disease (n = 702).

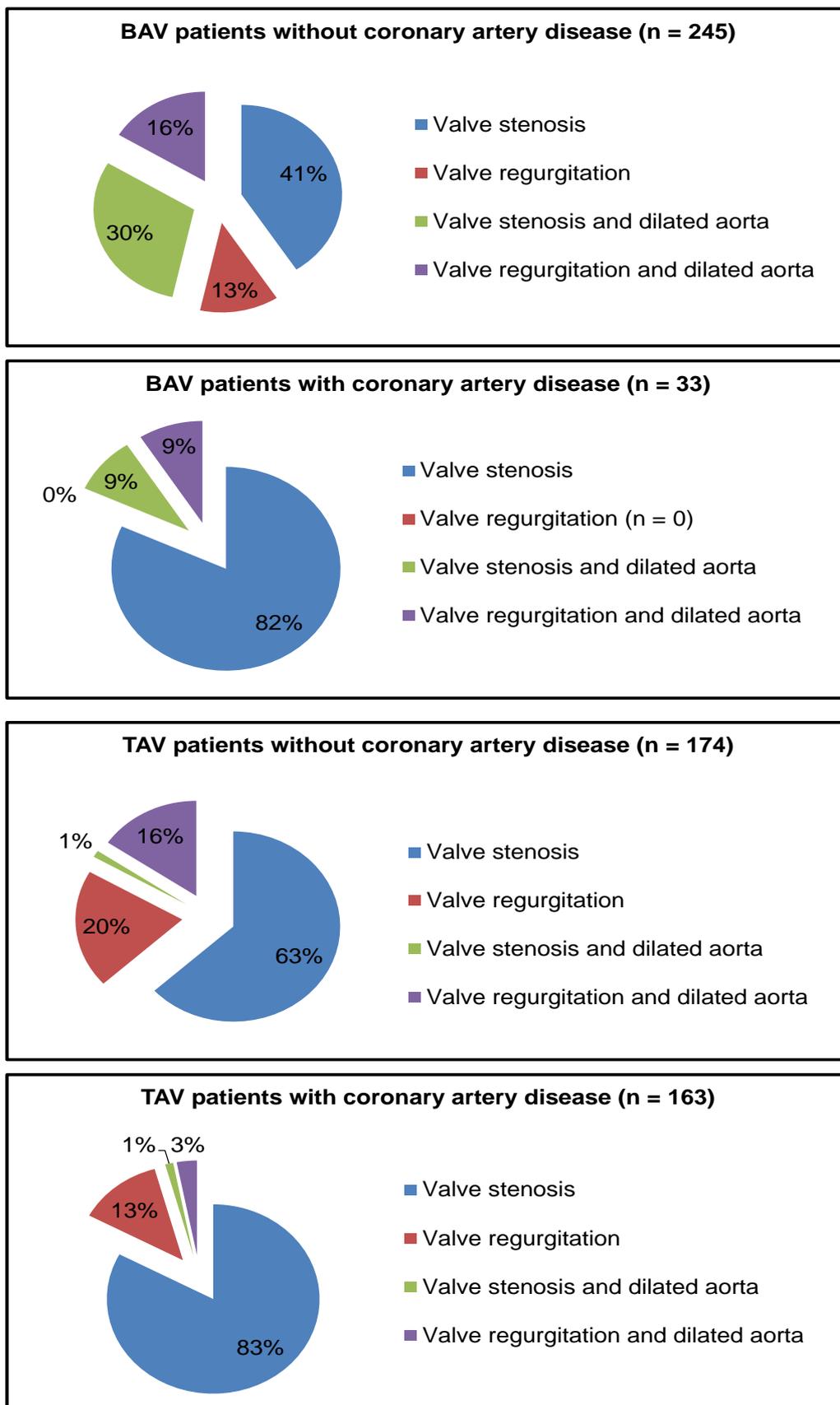
Distribution of valve morphology, valve pathology, and aortic morphology in patients without coronary disease (n = 500) is described in section 5.2.1 (*Study II*). In patients with coronary artery disease (n = 202), 16% had a BAV, 84% had a TAV, 94% had isolated aortic valve pathology, and 6% had concomitant ascending aortic dilatation. Distribution of valve, aortic, and coronary artery pathology in relation to valve morphology is depicted in Fig. 18. Aortic valve stenosis was the dominating valve pathology in both BAV and TAV patients when the aorta was non-dilated (Fig. 19; p = 0.13). Patients with coronary artery disease were older than those without, regardless of valve morphology. Without coronary artery disease, male gender was more common in TAV patients with valve and aortic pathology than in TAV patients with isolated valve pathology (p < 0.001). The male to female ratio in patients with coronary artery disease was 3.8:1 and was notably higher than in patients without coronary artery disease (2:1).

Figure 18. Distribution of valve, aortic and coronary artery pathology in relation to valve morphology



AV- aortic valve disease; AA – ascending aortic pathology; CAD – coronary artery disease.

Figure 19. Distribution of valve and aortic pathology in BAV and TAV patients with or without coronary artery disease



5.5.2 Echocardiographic findings

The aortic valve peak gradient was approximately 7 mm higher and the left ventricular end-systolic diameter was approximately 14 mm larger in TAV patients with coronary artery disease and isolated valve pathology than in patients without coronary artery disease ($p \leq 0.04$). In both BAV and TAV patients with isolated valve pathology, the ejection fraction was approximately 9% lower when coronary artery disease was present than when it was absent ($p \leq 0.003$). This was also observed in BAV patients with concomitant aortic dilatation ($p \leq 0.02$). Both BAV and TAV patients with coronary artery disease and isolated valve pathology had larger dimension of the ascending aorta than patients without coronary disease (~2-5 mm; $p < 0.0001$). This was also apparent in BAV patients with dilated aortas (~7 mm; $p = 0.02$).

5.5.3 Medical history

TAV patients without coronary artery disease and with isolated valve pathology were more likely to have diabetes (14% vs. 6%) and relatives with valve pathology (16% vs. 3%) than TAV patients with concomitant aortic dilatation ($p \leq 0.04$). TAV patients with coronary artery disease and valve pathology were less likely to have relatives with cerebrovascular lesions than TAV patients with aortic dilatation (13% vs. 57%; $p = 0.01$). Both BAV and TAV patients with coronary artery disease and valve pathology had a higher frequency of hypertension (BAV: 85% vs. 48%; TAV 88% vs. 65%) and more relatives who died suddenly (BAV: 32% vs. 14%; TAV 26% vs. 8%) than those without coronary artery disease ($p \leq 0.04$).

5.5.4 Medical treatment

TAV patients with isolated valve pathology but without coronary artery disease had a higher intake of lipid-lowering agents and calcium inhibitors than TAV patients with aortic dilatation (51% vs. 20% and 23% vs. 7%, respectively; $p \leq 0.03$). BAV patients with coronary artery disease and isolated valve pathology had a higher intake of lipid-lowering agents than patients with aortic dilatation (19% vs. 60%; $p = 0.02$). The corresponding group of TAV patients had a lower intake of angiotensin converting enzyme inhibitors (28% vs. 71%; $p = 0.03$). BAV patients with coronary artery disease and isolated valve pathology had a higher intake of beta blockers (73% vs. 41%), acetylsalicylic acid (81% vs. 31%), lipid-lowering agents (81% vs. 35%), thiazide diuretics (27% vs. 8%) and long-acting nitrates (73% vs. 41%) than BAV patients without coronary artery disease ($p \leq 0.02$). TAV patients with coronary artery disease had a higher intake of beta blockers (67% vs. 48%), acetylsalicylic acid (68% vs. 45%), lipid-lowering agents (78% vs. 51%), insulin (21% vs. 5%) and long-acting nitrates (67% vs. 48%; $p \leq 0.007$) than TAV patients without coronary artery disease.

5.5.5 Laboratory findings

BAV patients with coronary artery disease and isolated valve pathology had slightly higher levels of creatinine and C-reactive protein than BAV patients without coronary artery disease ($p \leq 0.03$).

6 DISCUSSION

6.1 CLINICAL CHARACTERISTICS

A consistent finding in the studies of this thesis was that more than 50% of patients who underwent cardiac surgery due to valve and/or aortic pathology had a BAV (*Study I-V*). Furthermore, in this population of cardiac surgery patients an ascending aortic aneurysm was approximately three times more common in BAV patients than in TAV patients. This finding confirms previous reports of aortic dilatation being substantially more common in BAV patients.² Thus, in both surgical and non-surgical patients, BAV is highly associated with ascending aortic dilatation. However, in patients with coronary artery disease, BAV was relatively rare (BAV, 16%; TAV, 84%) (*Study V*). Furthermore, in both BAV and TAV patients with the combination of valve pathology and coronary artery disease, ascending aortic dilatation was remarkably uncommon. The distribution of valve pathology was similar in BAV and TAV patients when the aorta was non-dilated. By contrast, in patients with an ascending aortic aneurysm, valve stenosis was the dominating valve pathology in BAV patients, whereas TAV patients rarely had a combination of valve stenosis and dilatation of the aortic root and/or ascending aorta. In both BAV and TAV patients with coronary artery disease, the predominant valve pathology was valve stenosis. BAV patients are usually referred to as being approximately 10 years younger than TAV patients at the time of cardiac surgery, which was consistent with our findings in patients with non-dilated aortas (*Study I, II, IV, V*).¹³⁰ In patients with ascending aortic aneurysm or coronary artery disease, this age difference was smaller but still significant (*Study II, III, V*). In general, a male to female ratio of 2:1 was found in both BAV and TAV patients, and this ratio increased in patients with ectasia of the aorta or coronary artery disease (*Study I, II, V*).

The finding that coronary artery disease was rare in BAV patients but was associated with TAV, advanced age, and male gender (*Study V*) is in agreement with previous work.^{130, 131} Risk factors of cardiovascular disease or the manifestations of cardiovascular disease were more common in both BAV and TAV patients with coronary artery disease (BAV and TAV patients: larger ascending aorta, higher frequency of hypertension, more relatives who died suddenly, and a higher intake of drugs used to treat cardiovascular disease; TAV patients: advanced age, male gender, more severe valve stenosis, larger ventricular dimensions, and reduced ejection fraction) than in patients without. Age, coronary artery disease, and concomitant coronary artery bypass grafting were suggested to be predictors of mortality in patients undergoing aortic valve surgery, although the distinction between BAV and TAV was not made.¹³² In this thesis, the combination of valve pathology and coronary artery disease was mainly found in TAV patients. This finding suggests that *valve morphology* might be associated with differences in outcome in patients undergoing isolated valve surgery in comparison with patients undergoing valve surgery and concomitant coronary artery bypass grafting. Interestingly, work by Roberts *et al.* found that patients with UAV or BAV have a greater probability of survival than TAV patients when undergoing aortic valve replacement with or without concurrent coronary artery bypass grafting. However, no difference in long-term survival related to surgical procedure was found. It is from these results possible to hypothesise that valve

morphology rather than surgical procedure (i.e., concomitant coronary artery bypass grafting) affects the probability of survival.¹³¹

The distribution of valve pathology, medical history, medication, and laboratory findings was similar in BAV and TAV patients with coronary artery disease. By contrast, in the group of patients without coronary artery disease and with isolated valve pathology, TAV patients were older, more frequently had hypertension and previous cerebrovascular lesions, had a higher intake of cardiovascular drugs, and had higher creatinine levels than BAV patients. This finding supports the notion that valve morphology is possibly linked to outcome in patients undergoing cardiac surgery; TAV patients seem to have higher cardiovascular risk than BAV patients, even without the presence of coronary artery disease. In accord, the relative distribution of BAV decreased substantially in patients with coronary artery disease (*Study V*).

Atherosclerosis and inflammation are important factors in the development of coronary artery disease and aortic valve stenosis.^{133, 134} To what extent inflammation and atherosclerosis contributes to aneurysm formation differs with aortic location and is thought to be less important in the ascending aorta than the descending and abdominal aorta.^{98, 99} Furthermore, coronary artery disease is a common finding in patients undergoing endovascular or surgical repair of descending, thoracoabdominal or abdominal aortic aneurysms.^{135, 136} Aneurysm formation of the ascending aorta is associated with inflammation and immune response in TAV patients, but not in BAV patients.¹⁰⁰ Interestingly, aortic valve stenosis was rarely found in TAV patients with dilated aortas (*Study I, II, III, V*). Furthermore, in both BAV and TAV patients, ascending aortic dilatation was uncommon when coronary artery disease was present which is in agreement with previous findings in BAV patients.¹³⁷ In spite of an association between inflammation and aneurysm formation in TAV patients, the combination of valve stenosis, coronary artery disease, and aortic dilatation was rare in TAV patients. BAV is frequently associated with ascending aortic dilatation, which in turn is rarely associated with coronary artery disease. Thus, valve morphology may influence the development of coronary artery disease.

6.2 BAV PHENOTYPE AND AORTIC MORPHOLOGY

BAV phenotype has been suggested to be related to aortic morphology and to several aspects of BAV disease. Novaro *et al.* reported a statistically significant correlation between the RL configuration and aortic valve regurgitation. In addition, a correlation was found between the RN configuration and larger dimensions of the ascending aorta and the presence of aortic stenosis, although none of these findings were statistically significant.¹³⁸ Fernandes *et al.* showed a relation between the severity of valve pathology (both stenosis and regurgitation) and BAV with a RN configuration.⁷⁹ Moreover, an association of BAV-RL and coarctation was demonstrated. A larger diameter of the sinus of Valsalva and a smaller diameter of the aortic arch has been associated with the RL configuration.¹³⁹ The authors also reported that the elastic properties of the aorta differed according to BAV phenotype where BAV-RL showed a higher stiffness index and a lower distensibility at the sinus of Valsalva. Russo *et al.* could not confirm a relation between BAV phenotype and the distribution of valve

pathology but found that patients with BAV-RL had larger dimensions of the sinus of Valsalva and more pronounced medial degeneration.⁹³ In patients with mild to moderate aortic valve stenosis, an association of BAV-RL with larger dimensions of the annulus and the sinus of Valsalva has been reported¹⁴⁰ which supports the results of Russo *et al.* and Schaefer *et al.* Ikonomidis *et al.* showed that BAV patients with dilated aortas have an increased global activity of MMPs in comparison with controls (i.e., normal valve and non-dilated aorta).¹⁴¹ Furthermore, the authors reported differences in MMP activity related to BAV phenotype.

The relative distribution of BAV phenotypes observed in this thesis is similar to those previously reported.^{104, 139, 142, 143} Thus, in general 60-85% of BAV patients have BAV-RL, while BAV-RN is less common and is found in approximately 15-40% of patients with BAV. A true BAV is found in about 10% of BAV patients, whereas fusion of the left and non-coronary leaflets is virtually non-existent. BAV patients had larger dimensions of the annulus and left ventricular outflow tract than TAV patients regardless of aortic morphology (i.e., non-dilated, aneurysm, and ectasia), valve pathology, and severity of valve pathology (*Study I, II*). In contrast to previous work, a relationship was not established between BAV phenotype and the frequency of aneurysm or ectasia.^{93, 139, 140} In addition, only minor differences in aortic dimensions related to BAV phenotype were shown and an association between BAV phenotype and the distribution of valve pathology could not be demonstrated (*Study I, II*), which is in contrast to the findings of Novaro *et al.*¹³⁸ The findings of *Study I* and *Study II* were further confirmed in *Study III* where neither BAV phenotype nor valve pathology influenced the aortic dimensions. Taken together, the relative influence of BAV phenotype on aortic morphology seems to be of minor importance in patients undergoing cardiac surgery, which is in agreement with the work of Cecconi *et al.*¹⁴⁴ This implies that differences in the blood flow pattern across the aortic valve related to BAV phenotype, aortic wall shear stress, and pressure distribution do not solely explain the increased prevalence of ascending aortic dilatation in BAV patients.

6.3 VALVE PATHOLOGY AND AORTIC MORPHOLOGY

Aortic valve stenosis and valve regurgitation were equally distributed when the aorta was non-dilated (*Study I*); however, when the aorta was dilated, valve stenosis was more common in the BAV group and valve regurgitation was more common in the TAV group. *Study II* confirmed these findings and further emphasised that the combination of a TAV, valve stenosis, and dilatation of the ascending aorta is rare (only 2 out of 212 TAV patients). As suggested by others^{1, 145, 146} and substantiated by our findings, the term “post-stenotic dilatation” seems to be appropriate only in BAV patients as aortic dilatation was rarely associated with aortic valve stenosis in TAV patients but was frequently seen in BAV patients with valve stenosis. It is also important to recognise that not all BAV patients with aortic valve stenosis have a dilated aorta, and that patients with dilated ascending aortas may have a well-functioning non-calcified BAV.^{46, 147, 148} In *Study I*, 6% of BAV patients and 4% of TAV patients had dilated aortas without any concomitant aortic valve pathology.

In general, and apparent in both BAV and TAV patients, aortic dimensions were larger in patients with valve regurgitation than in those with valve stenosis, and increasing severity of valve pathology was associated with *smaller* aortic dimensions. It has been suggested that a functionally normal BAV (i.e., not deemed calcified, stenotic, or regurgitant by standard echocardiographic definitions) is stenotic due to its inborn constitution and thereby causes turbulent flow across the valve to a greater extent than TAV.⁶⁰ Differences in aortic flow patterns related to BAV phenotype have been demonstrated.¹¹⁶ It has been proposed that aortic wall shear stress is more pronounced in BAV patients than in TAV patients and in addition related to aortic valve area.¹⁴⁹ BAV patients have accelerated progression of aortic valve calcification, which leads to hemodynamically and clinically significant valve stenosis earlier in life compared with TAV patients.^{51, 57-59} It has been suggested that the severity of valve pathology positively correlates with aortic root and ascending aortic dimensions.¹⁵⁰ However, the results of *Study II* demonstrated an inverse effect of the severity of valve pathology on aortic diameters, where increasing severity of valve pathology was associated with *smaller* aortic dimensions, which is in agreement with the work of Novaro *et al.*¹³⁸ This pattern was present in both BAV patients (both non-dilated and dilated aorta) and TAV patients (non-dilated aorta) with aortic valve stenosis.

Previous studies on the relationship between the severity of valve regurgitation and aortic dimensions have mainly shown that an association exists and that the aortic dilatation is predominantly located at the sinus of Valsalva.^{138, 150, 151} Our findings corroborate the majority of the previous results (*Study II*) and the few discrepancies noted might be related to differences in the study group composition such as whether stratification according to valve and aortic morphology was performed.

There are differences in the underlying pathological mechanisms of aneurysm formation that are related to aortic location (i.e., ascending, descending, and abdominal) and to valve morphology. Impaired elastic properties of the aortic wall have been shown to be present in BAV individuals.^{104, 152} Increased stiffness and reduced distensibility of the aortic wall has been found in both children and adults with BAV, although it is not causatively related to aortic dilatation.^{104, 152} Whether reduced elasticity and increased stiffness of the aorta explains the association between increasing grade of valve stenosis and smaller dimensions of the ascending aorta is not known.

In the absence of serial measurements of aortic dimensions, it may be hypothesised that patients with a small aortic annulus at baseline are prone to develop more severe aortic valve stenosis than patients with larger aortas. It could also be argued that this would be even more pronounced in BAV patients than TAV patients due to an increased turbulent flow across the valve, thereby explaining the findings of an association between the severity of valve stenosis and smaller aortic size. However, BAV patients have larger dimensions of the annulus and left ventricular outflow tract than TAV patients, regardless of aortic morphology (i.e., non-dilated or dilated aorta) (*Study I, II*). Furthermore, the hypothesis is contradicted by the finding that, in TAV patients with non-dilated aorta, increasing severity of valve stenosis was associated with larger diameters of the left ventricular outflow tract and annulus despite these measurement points being smaller in TAV patients than in BAV patients irrespective of the grade of

valve stenosis. Moreover, the association between increasing severity of valve pathology and smaller aortic dimensions was found in both non-dilated and dilated aortas. Thus, these findings do not support the suggestion that altered hemodynamics is the dominating factor responsible for the increased prevalence of ascending aortic dilatation in BAV patients. Based on the suggestion that a functionally normal BAV is in fact “stenotic” and therefore the cause of turbulent flow and increased wall shear stress in concert with the premature valve calcification seen in BAV, differences in aortic morphology between BAV and TAV patients related to the severity of valve pathology would be expected. However, the pattern of changes in aortic dimensions related to the severity of valve pathology is strikingly similar in the two groups (*Study II*). Furthermore, if higher grades of valve stenosis create a clinically significant increase in aortic wall shear stress, TAV patients with aortic valve stenosis should develop ascending aortic aneurysm; however this is, at least in a surgical population, virtually non-existent. It is however arguable that a BAV is subjected to a life-time of hemodynamic stress whereas a stenotic TAV develops valvular lesions responsible for aberrant flow patterns only later in life, still not *all* BAV patients develop aortic dilatation.

6.4 DIMENSIONS OF THE DISTAL AORTA

BAV patients with dilated ascending aortas consistently had smaller arch, descending, suprarenal, and infrarenal aortic dimensions than TAV patients (*Study III*). All patients studied underwent replacement of the ascending aorta, and BAV and TAV patients were comparable with regard to ascending aortic dimensions at the time of surgery. Differences in clinical characteristics and comorbidities were statistically corrected for, and neither valve pathology nor BAV phenotype influenced the aortic dimensions in BAV patients. Thus, the difference in dimensions of the distal aorta could not be attributed to these factors. Concomitant dilatation of the descending aorta was more common in TAV patients than in BAV patients, and this suggests that BAV-associated aortic dilatation is mainly confined to the aortic root and/or ascending aorta. By contrast, TAV-associated aortic dilatation can be present in all aortic segments.

The association between ascending aortic dilatation and smaller dimensions of the remaining aorta in BAV patients is in agreement with previous findings.¹⁴⁴ However, the results differ from those of Fazel *et al.* who reported that 28% of BAV patients with a dilated ascending aorta had concomitant dilatation of the transverse arch.¹⁵³ This discrepancy might be explained by differences in the definition of aortic measurement points and analytical methods between the studies. Alegret *et al.* reported an increased prevalence of concomitant dilatation of the abdominal aorta in TAV patients older than 60 years and who have dilatation of the sinus of Valsalva.¹⁵⁴ Conversely, patients with abdominal aortic aneurysm have been reported to have a high prevalence of concomitant dilatation of the descending aorta but the distinction between BAV and TAV was not made.¹⁵⁵

6.5 AORTIC WALL DEGENERATION

The mRNA expression of all known members of the MMP family and their endogenous inhibitors, TIMPs, in the ascending aortic wall was analysed with reference to valve and aortic morphology (*Study IV*). The results of principal components analysis based on the genes found to be expressed above the set threshold suggested that differences in gene expression between non-dilated and dilated aortas are predominately found in TAV patients. MMP19 expression was positively correlated with aortic diameter in TAV patients, but not in BAV patients, and correlation analysis of MMP14 expression and aortic diameter showed borderline significance. Thus, MMP14 and MMP19 were identified as proteolytic enzymes that are potentially involved in the pathogenesis of ascending aortic dilatation in TAV patients.

The results support previous findings that there are differences in molecular mechanisms responsible for aneurysm formation that are related to both aortic location (i.e., ascending, descending, abdominal)^{94,95} and valve morphology (BAV/TAV).^{156, 157} In both non-dilated and dilated ascending aortas, regardless of valve morphology, gene expression of gelatinases, MMP11, all membrane bound MMPs, MMP19, MMP21, and MMP28 was found. This MMP expression profile differs from that in the abdominal aorta, where MMP1, -2, -3, -8, -9, -12, -13, and -14 are suggested to affect aneurysm formation.¹⁵⁸

In the multivariate analysis, there were no differences in mRNA expression between BAV and TAV patients with non-dilated aortas. Furthermore, analysing BAV and TAV patients separately, differences related to aortic morphology was mainly found in the TAV group. Univariate analyses of gene expression in relation to aortic morphology revealed that MMP14, MMP19, and TIMP2 were upregulated in dilated aortas compared with non-dilated aortas in both BAV and TAV patients. A downregulation of gene expression was only found in the TAV group (MMP17, MMP21, MMP25, MMP28, and TIMP4). Correlation analysis of MMP14, MMP19, and TIMP2 gene expression with maximal aortic diameter showed a strong positive correlation of MMP19 with maximal aortic diameter; however this was only in TAV patients. The principal components analysis showed a separation of non-dilated aortas from dilated aortas only in the TAV group, which corroborates the results of the univariate analysis and the correlation analysis. Taken together, these findings suggest that MMP and TIMP gene expression profiles are likely to affect aneurysm formation in TAV patients, but not in BAV patients.

MMP19 can hydrolyse several matrix components such as collagen IV, laminin, nidogen, tenascin, fibronectin and gelatin¹⁵⁹ and MMP19 is expressed in various non-diseased tissues but not found in leukocytes.¹⁶⁰ Previous reports of MMP19 expression in the endothelium, medial layer, and smooth muscle cells of blood vessels are in accord with our findings.^{161, 162}

MMP14 is membrane bound and in its substrate specificity similar to the MMPs implicated in abdominal aortic aneurysm formation (i.e., the collagenases, matrilysins and stromelysins), and is in this context considered to promote the invasion of inflammatory cells into the aortic wall.^{158, 163} However, similar to MMP19 the MMP14

protein expression was mainly upregulated in the medial layer of the dilated aorta as shown by the immunohistochemical analysis. This region consists mainly of smooth muscle cells and myofibroblasts but only a few inflammatory cells. MMP14 has been suggested to be involved in the activation of proMMP2 via complex binding to TIMP2.¹⁶⁴ TIMP2 was found to have higher expression levels in dilated aortas in both BAV and TAV patients. However, TIMP2 expression did not contribute to the separation of non-dilated from dilated aortas in the principal components analysis and further did not correlate to maximal aortic diameter.

There are previous reports of MMP1, -8, -13 (collagenases) and MMP7 protein expression in aneurysms of the ascending aorta.¹⁰⁸ Moreover, MMP3 and MMP7 have been shown to be retained in areas of mucoid degeneration in the ascending aorta of patients with various aortic pathologies and aetiologies.⁸⁵ Increased levels of MMP1 and MMP9 protein expression in patients with thoracic aortic aneurysm have also been demonstrated.¹⁶⁵ However, the distinction between the ascending and the descending aorta was not made and the aortic pathologies included aneurysms, intramural hematomas, and aortic dissections.

There are several possible explanations for the diverging results. Differences in study population composition (i.e., aetiology, aortic pathology, and aortic section studied) make a direct comparison between the studies difficult. It is possible that the exon array used in this thesis was not sensitive enough, however the results from the exon array analysis were confirmed by real-time PCR. The medial and adventitial layer were analysed separately based on the hypothesis that the inflammatory profiles of the layers differ. However, the same MMPs were expressed above background in both layers. It is important to remember that gene expression does not always mirror protein expression or enzymatic activity, and that local expression cannot be excluded when analysing large tissue samples (i.e., dilated aortic specimens). It is also possible that proteins found in the aortic wall are not synthesised locally and the finding a result of entrapment of circulating proteases within the aortic wall.

The relevance of the decreased MMP17, -21, -25, and -28 expression in TAV patients with dilated aortas is not known. The downregulation of these MMPs may represent a marker of de-differentiation of smooth muscle cells and fibroblasts and/or smooth muscle cell or myofibroblast apoptosis. MMP21 and MMP25 are structurally similar and found in various tissues and in cells such as fibroblasts and inflammatory cells.^{166, 167} Decreased MMP21 expression has been suggested to be a marker of de-differentiated tumour cells¹⁶⁸ and MMP28 is downregulated in malignant tumours.¹⁶⁹ It is also plausible that these proteases have a role in apoptosis that is highly associated with aneurysm formation.¹⁷⁰ MMP21 expression is not associated with apoptosis in tumour cells,¹⁷¹ and the potential role of MMP21, -25 and -28 in ascending aortic aneurysm formation needs to be analysed further.

6.6 BAV-ASSOCIATED AORTOPATHY

In view of the apparent differences in morphology and opening shape between a non-diseased BAV and a non-diseased TAV⁶⁰ and how gravely valve stenosis can alter the

morphology and function of an aortic valve it is reasonable to adhere to the hemodynamic theory of BAV-associated aortic dilatation. Indeed, differences in transvalvular flow have been demonstrated between BAV and TAV patients.¹⁷² Aortic wall shear stress, i.e., the force of friction between the blood and the endothelial cells of the aortic intima, also differs between BAV and TAV patients and is related to aortic size.^{109, 172, 173} Hope *et al.* reported an association between BAV and elevated shear stress and proposed that elevated and asymmetrical shear stress constitutes an increased risk of aortic dilatation. Interestingly, Cheng *et al.* showed in a mouse model that “lowered shear stress and vortices” are associated with larger and more vulnerable atherosclerotic lesions whereas regions of increased shear stress are protected.¹⁷⁴ Furthermore, lowered shear stress/vortices were associated with loss of smooth muscle cells, increased expression of proatherogenic inflammatory mediators, and MMP activity. These molecular findings are similar to those of studies investigating aneurysm formation in TAV patients^{100, 157} (*Study IV*). Abnormal flow patterns are not restricted to BAV patients but are also seen in TAV patients with aortic valve stenosis and are suggested to be the cause of post-stenotic aortic dilatation.¹⁷² However, in the present cohort of cardiac surgery patients, the combination of a TAV, aortic valve stenosis and dilatation of the ascending aorta was extremely rare (*Study I, II*). It is possible that abnormal flow patterns and elevated shear stress caused by a BAV (non-calcified or stenotic) or a stenotic TAV induces aneurysm formation in BAV patients but not TAV patients. Whether this phenomenon is explained by an inherent wall weakness and/or by long-standing hemodynamic stress on the aortic wall caused by the malformed valve is not known.

Della Corte *et al.* showed that the “cusp opening angle” of the fused leaflet in BAV patients with the RL configuration is narrower than the corresponding TAV leaflets and that this causes an altered flow across the BAV. The authors propose measurement of the “cusp opening angle” as a novel method for quantifying the abnormal opening of a BAV.¹⁷⁵ It was also shown that the flow jet stream in BAV patients is skewed and that the “cusp opening angle” is inversely correlated to ascending aortic diameter, and age, and also correlated with the annual rate of aortic enlargement. It is important to remember that an enlarged aorta at baseline is a predictor of further dilatation of the aorta and aneurysm formation^{2, 176} and as frequently shown, BAV patients, even if comparable in other parameters, consistently have larger aortas than their TAV counterparts.^{56, 119, 147} This was also true in the study by Della Corte *et al.* in which not only the “cusp opening angle” but also the diameter of the sinus of Valsalva and body surface area (but not BAV or TAV) were predictors of ascending aortic dimensions in multivariable regression analyses. It has not been clarified whether a dilated aorta causes abnormal flow or vice versa.^{109, 173, 177}

“Nested helical flow”, with different directions according to BAV phenotype (RL and RN), is present in BAV patients but not in TAV patients.¹¹⁶ This finding led to the hypothesis that flow differences related to BAV phenotype might explain differences in aortic morphology.¹⁷⁸ Nathan *et al.*, in accord with other investigators,^{172, 173} showed that wall stress differs between BAV and TAV; however an association between BAV phenotype and differences in wall stress could not be established.¹⁴² The present finding that, in spite of differences in the prevalence of various BAV phenotypes, the distribution of BAV phenotypes was similar regardless of aortic morphology (i.e., non-

dilated, aneurysm, and ectasia) in patients scheduled for cardiac surgery is in agreement with the experimental findings of Nathan *et al.* (*Study I, II*).

Histological differences related to ascending aortic location with reference to valve morphology, valve pathology, and aortic morphology were not investigated in this thesis but constitute an interesting aspect of BAV disease. Russo *et al.* reported a greater prevalence of more severe medial degeneration in BAV patients with RL than those with RN leaflet fusion,⁹³ and it has been suggested that these histological findings might be related to the orientation of the flow jet across the valve.¹⁷⁸ Cotrufo *et al.* demonstrated differences in the expression of extracellular matrix proteins related to valve pathology, aortic morphology, and aortic location (convex or concave portion of the ascending aorta) but not to valve morphology (i.e., BAV/TAV).¹⁷⁹ The finding that smooth muscle cell apoptosis in BAV patients with aortic valve stenosis differs with aortic location (concavity/convexity) even when the aorta is non-dilated corroborated the work of Cotrufo *et al.*¹⁵⁷ Based on their findings, Cotrufo *et al.* proposed that the aortic wall in BAV patients may have an impaired ability to adapt to the increased hemodynamic stress.¹⁷⁹ Interestingly, in an analysis of flow-mediated gene expression in the ascending aorta Maleki *et al.* found evident differences between BAV and TAV patients.¹¹⁷ Further experimental analyses revealed that a large portion of the genes of interest was related to angiogenesis and/or wound healing, and the expression pattern was indicative of defective wound healing in BAV patients. The quality, stability, and thereby strength of the aortic media collagen has been suggested to be abnormal in BAV patients in comparison with TAV patients, and this is likely due to impaired synthesis and post-translational modification of collagen.¹⁸⁰ In the work by Cotrufo *et al.*, differences in collagen expression related to valve pathology were found; levels of both collagen I and collagen I mRNA were reduced in BAV patients with valve regurgitation, whereas BAV patients with valve stenosis had a decreased amount of collagen protein but similar amounts of mRNA as controls.¹⁷⁹ The authors speculated that BAV patients with valve stenosis may have an impaired ability to adapt to increased stress, possibly due to an abnormal turnover of collagen. Thus, the two dominating theories of what causes BAV-associated aortic dilatation (i.e., genetics and hemodynamics) seem to intertwine.

Le Canna *et al.* reported no differences in the progression of aortic dimensions between BAV and TAV patients with dilated aorta but without significant valve pathology.¹⁸¹ It has been argued that the lack of differences between these groups indicate that BAV patients do not have an inherent wall weakness.¹⁸² Beroukhim *et al.* investigated baseline aortic dimensions and progression rates in children without valve pathology and, in contrast to La Canna *et al.*, found significant differences between BAV and TAV individuals.¹¹⁹ In view of recent findings of differences in flow¹⁰⁹ and shear stress¹⁷³ related to valve morphology and the notion that a non-calcified BAV is inherently stenotic⁶⁰ the result of La Canna *et al.* may indicate that there is an intrinsic (but individual) genetic susceptibility for aortic dilatation in the presence of hemodynamic stress in BAV patients since not *all* BAV individuals develop ascending aortic dilatation. In agreement with previous work,¹²⁶ in this thesis, patients with UAV were younger than BAV patients who in turn were younger than TAV patients (TAV, 69 ± 11; BAV, 60 ± 12; UAV, 48 ± 9 years respectively, $p < 0.0001$). Patients with ascending aortic aneurysm and a true BAV or BAV-RN were younger than those with BAV-RL (RL 64

± 10 ; RN 59 ± 7 ; TB 54 ± 12 years respectively, $p \leq 0.03$), and patients with UAV were younger than BAV-RN (UAV 50 ± 6 ; RN 59 ± 7 years respectively, $p = 0.02$). Moreover, the dimensions of the sinus of Valsalva, sinotubular junction, and aortic root height were larger in patients with aneurysm and true BAV than in those with BAV-RL or BAV-RN (*Study I*). It is likely that the different valve morphologies (BAV, BAV phenotype, and UAV) constitute a spectrum of the same malformation, and the differences in age and aortic dimensions suggest a heterogeneous genetic influence that is related to valve morphology and BAV phenotype. It has been proposed that dilatation of the aortic root in BAV patients is a more genetically influenced phenotype than other BAV-associated aortic morphologies based on the “root type” being fairly uncommon and mainly found in males with aortic valve regurgitation.^{150, 178} Nistri *et al.* has showed that valve regurgitation is the predominant valve pathology in young males with BAV and that the associated aortic dilatation in these patients is related to the presence of valve regurgitation but not to the severity of valve pathology.⁵⁴ In accord, in this thesis, valve regurgitation was rare in females regardless of valve morphology and aortic morphology (male to female ratio in patients without coronary artery disease: with valve regurgitation, 4.4:1 vs. without 1.4:1, $p < 0.0001$; with valve stenosis 1.4:1 vs. without 3.6:1, $p < 0.0001$). Roberts *et al.* has further found that BAV patients with aneurysmatic aortas and valve regurgitation have a more pronounced loss of elastic fibres than those with valve stenosis.¹⁸³

The idea of impaired wound healing and a reduced ability to adapt to stress in BAV patients conjoins the two dominating theories of what causes BAV-associated aortic dilatation. An aorta with an inborn weakness, an insufficient capacity to adapt to hemodynamic stress, and an impaired regenerative ability is likely susceptible to dilatation when subjected to abnormal hemodynamic stress.

6.7 CLINICAL IMPLICATIONS

With a prevalence of 1-2% in the population and an estimated need for surgical intervention in 25-50% of cases, the malformation has major medical implications. Many aspects of the natural history of BAV and BAV disease remain uncertain and this topic is difficult to address due to the heterogeneous character of both healthy and diseased BAV individuals.^{56, 70, 102, 103, 118, 137, 181, 184-191} Not all BAV individuals will develop valve and/or aortic pathology and to prospectively follow children with BAV or TAV into adulthood, taking BAV phenotype, severity of valve pathology, aortic morphology, aortic pathology, other comorbidities (e.g., coronary artery disease), and effects of possible interventions into account, is probably neither feasible nor ethical.

The finding of this thesis that ascending aortic dilatation is rarely associated with aortic valve stenosis in TAV patients but is frequently seen in BAV patients with valve stenosis may, from a clinical standpoint, help guide selection of the appropriate surgical treatment strategy for these patients. The fate of a non-replaced aorta following aortic valve replacement is uncertain and there is an on-going debate concerning the appropriate timing and extent of aortic replacement in BAV patients.^{102, 103, 153, 186, 189, 190, 192-197} The results of the present work do not support the proposal that BAV phenotype is a predictor of preoperative aortic dilatation in BAV patients scheduled for

cardiac surgery. The finding that there is a small likelihood of concomitant dilatation of the descending and/or abdominal aorta in BAV patients with dilated ascending aorta could be used to reassure patients and to guide the surveillance strategy. As coronary artery disease is rarely associated with ascending aortic dilatation, this finding may be grounds for a less invasive preoperative evaluation of the coronary arteries than a coronary angiogram (i.e., computed tomography angiogram) in patients with ascending aortic dilatation, especially in patients with a BAV.

Current guidelines state that aortic surgery is recommended when the dimension of the aortic root and/or the ascending aorta exceeds 45 mm in BAV patients scheduled for aortic valve surgery.^{63, 198, 199} With a normally functioning BAV aortic replacement is recommended when aortic dimensions exceed 50 mm or when the growth rate exceeds 2-5 mm per year. However, the recommendations of the guidelines are also debated.^{145, 182, 188, 191, 200} Regardless, it is important to recognise the heterogeneity of BAV disease and thus evaluate each patient individually. In addition to conventional preoperative and perioperative considerations in cardiac surgery patients, factors that have a major impact in BAV disease should be recognised and considered, and thus an appropriate treatment and surveillance strategy can be tailored for each BAV individual.

6.8 STUDY LIMITATIONS

The patients constituting the study population of this thesis are all cardiac surgery patients and therefore the results not applicable to a non-diseased BAV population. Furthermore, the single centre setup might reduce the generalisability of the results. Moreover, the studies of this thesis were all cross-sectional as opposed to longitudinal and are therefore not designed to answer questions concerning the progression of pathology. The multifaceted character of BAV disease entails a statistical challenge where outcome measures are possibly dependent on several co-factors. Set cut-off values for aortic dilatation differed between BAV and TAV patients based on surgical guidelines. It should also be emphasised that in the echocardiographic evaluation of the study participants, the transthoracic examinations were performed on awake patients whereas the transesophageal examinations were performed on anaesthetised patients, and blood pressure and loading conditions differs between these two conditions.

7 CONCLUSIONS

More than 50% of patients scheduled for aortic valve and/or ascending aortic surgery have a BAV. BAV patients are approximately 10 years younger than TAV patients at the time of surgery. Patients with additional coronary artery disease are older than patients without regardless of BAV or TAV. Ascending aortic aneurysm is substantially more common in BAV patients than in TAV patients while ectasia is equally common regardless of valve morphology. With ascending aortic dilatation, aortic valve stenosis is almost exclusively associated with BAV whereas aortic valve regurgitation is associated with either BAV or TAV.

The dimensions of the left ventricular outflow tract and annulus are larger in BAV patients than in TAV patients, regardless of aortic morphology. The relative distributions of aortic aneurysm and ectasia are not related to BAV phenotype.

The combination of aortic valve stenosis and ascending aortic aneurysm is common in BAV patients but virtually non-existent in TAV patients. Increasing severity of valve pathology is associated with smaller aortic dimensions. The distribution of valve pathology does not differ with the various BAV phenotypes.

BAV patients with ascending aortic aneurysm have smaller dimensions of the distal aorta than the corresponding group of TAV patients. Concomitant dilatation of the descending aorta is predominantly found in TAV patients.

Expression of matrix metalloproteinase 14 and 19 is associated with ascending aortic aneurysm formation in TAV patients, but not BAV patients.

BAV patients with aortic valve pathology and/or ascending aortic dilatation rarely have concomitant coronary artery disease. Ascending aortic dilatation and coronary artery disease seldom co-exist regardless of valve morphology.

8 ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to:

Professor Anders Franco-Cereceda – supervisor and mentor, for even considering engaging a ... years old mother of two in this project and thereby giving me such a fantastic opportunity. It has been a true pleasure and I will always be indebted to you.

Professor Per Eriksson – co-supervisor and mentor, for, by being immensely skilled but yet completely prestigeless, setting a wonderful example of what to strive for as a scientist and a person.

Ulf Lockowandt – head of department, for facilitation a research friendly environment and for providing invaluable insights on what I *really* was getting into and how to handle it.

Jan Liska, Christian Olsson, Carl-Gustav Hillebrant, Ulrik Sartipy – co-authors and fellow surgeons, for performing astonishing work and thereby providing me with invaluable data and for tirelessly answering my endless questions.

Kenneth Caidahl, Maria Eriksson Johan Petrini – co-authors and collaborators, for numerous echocardiographic evaluations of the study participants and valuable input on the interpretation and presentation of our results. A special thanks to Johan Petrini for your kindness and perseverance through this.

Therese Olsson, Sanela Kjellqvist, Lasse Folkersen, Dick Wågsäter, Valentina Paloschi, Shoreh Maleki – co-authors and collaborators, for patiently leading me through kindergarten of molecular science. It is painfully clear that I have much more to learn but also wonderfully stimulating to work with you and I am hoping for future encounters.

Susanne Hylander – colleague, for always pleasing my sudden whims such as running off to the library two minutes after your shift finished when I desperately needed an old book from the last century but importantly for offering me friendship and laughter when most needed, I adore you.

Elisabeth Berg – statistician, for being thorough and precise and thereby making sure that I could be confident in my results and for answering all my not always intelligent questions without ever letting on just that.

Mats Arnhög – external mentor, for repeatedly reassuring me that I can do it if I really put my mind to it.

Family and friends, for being there, I love you.

Special thanks are directed **to all the study participants** who selflessly contributed to this work. Without you none of this would have been possible and several important medical findings would still have been unknown.

This work was supported by:

A donation from Mr Fredrik Lundberg and The European Commission (FAD – Fighting Aneurysmal Disease) Health 2008-200647.

9 REFERENCES

1. Braverman AC, Guven H, Beardslee MA, Makan M, Kates AM, Moon MR. The bicuspid aortic valve. *Curr Probl Cardiol.* 2005;30:470-522
2. Michelena HI, Khanna AD, Mahoney D, Margaryan E, Topilsky Y, Suri RM, Eidem B, Edwards WD, Sundt TM, 3rd, Enriquez-Sarano M. Incidence of aortic complications in patients with bicuspid aortic valves. *JAMA.* 2011;306:1104-1112
3. van Son JA, Reddy VM, Black MD, Rajasinghe H, Haas GS, Hanley FL. Morphologic determinants favoring surgical aortic valvuloplasty versus pulmonary autograft aortic valve replacement in children. *J Thorac Cardiovasc Surg.* 1996;111:1149-1156; discussion 1156-1147
4. Fealey ME, Edwards WD, Miller DV, Maleszewski JJ. Unicommissural aortic valves: Gross, histological, and immunohistochemical analysis of 52 cases (1978-2008). *Cardiovasc Pathol.* 2012;21:324-333
5. Paget J. On obstructions of the branches of the pulmonary artery. *Med Chir Trans.* 1844;27:162-494 164
6. Peacock T. Malformations occurring the later periods of foetal life. *On malformations of the human heart.* London: Churchill; 1866:133.
7. Osler W. The bicuspid condition of the aortic valves. *Trans Assos Am Physicians.* 1886;1:185-192
8. Acierno L. Arterial lesions. *History of cardiology.* Pearl River, New York: Pathenon Publishing Group Ltd; 1994:96.
9. Wauchope G. The clinical importance of variations in the number of cusps forming the aortic and pulmonary valves. *Q J Med.* 1928;21:383-399
10. Karsner H. *Calcific disease of the aortic valve.* Philadelphia: Lippincott; 1947.
11. Campbell M, Kauntze R. Congenital aortic valvular stenosis. *Br Heart J.* 1953;15:179-194
12. Smith DE, Matthews MB. Aortic valvular stenosis with coarctation of the aorta, with special reference to the development of aortic stenosis upon congenital bicuspid valves. *Br Heart J.* 1955;17:198-206
13. Bacon AP, Matthews MB. Congenital bicuspid aortic valves and the aetiology of isolated aortic valvular stenosis. *Q J Med.* 1959;28:545-560
14. Leech G, Mills P, Leatham A. The diagnosis of a non-stenotic bicuspid aortic valve. *Br Heart J.* 1978;40:941-950
15. Larson EW, Edwards WD. Risk factors for aortic dissection: A necropsy study of 161 cases. *Am J Cardiol.* 1984;53:849-855
16. Larsen W. Development of the heart. In: M Strauss, D Terry, ed. *Essentials of human embryology.* Singapore: Churchill Livingstone Inc; 1998:97-121.
17. Hinton RB, Jr., Lincoln J, Deutsch GH, Osinska H, Manning PB, Benson DW, Yutzey KE. Extracellular matrix remodeling and organization in developing and diseased aortic valves. *Circ Res.* 2006;98:1431-1438
18. Hinton RB, Yutzey KE. Heart valve structure and function in development and disease. *Annu Rev Physiol.* 2011;73:29-46
19. Person AD, Klewer SE, Runyan RB. Cell biology of cardiac cushion development. *Int Rev Cytol.* 2005;243:287-335
20. Roos-Hesselink JW, Scholzel BE, Heijdra RJ, Spitaels SE, Meijboom FJ, Boersma E, Bogers AJ, Simoons ML. Aortic valve and aortic arch pathology after coarctation repair. *Heart.* 2003;89:1074-1077

21. Hinton RB, Martin LJ, Rame-Gowda S, Tabangin ME, Cripe LH, Benson DW. Hypoplastic left heart syndrome links to chromosomes 10q and 6q and is genetically related to bicuspid aortic valve. *J Am Coll Cardiol*. 2009;53:1065-1071
22. Bolling SF, Iannettoni MD, Dick M, 2nd, Rosenthal A, Bove EL. Shone's anomaly: Operative results and late outcome. *Ann Thorac Surg*. 1990;49:887-893
23. Collins RT, 2nd, Kaplan P, Somes GW, Rome JJ. Cardiovascular abnormalities, interventions, and long-term outcomes in infantile williams syndrome. *J Pediatr*. 2010;156:253-258 e251
24. Lopez L, Arheart KL, Colan SD, Stein NS, Lopez-Mitnik G, Lin AE, Reller MD, Ventura R, Silberbach M. Turner syndrome is an independent risk factor for aortic dilation in the young. *Pediatrics*. 2008;121:e1622-1627
25. Carlson M, Airhart N, Lopez L, Silberbach M. Moderate aortic enlargement and bicuspid aortic valve are associated with aortic dissection in turner syndrome: Report of the international turner syndrome aortic dissection registry. *Circulation*. 2012;126:2220-2226
26. Ciotti GR, Vlahos AP, Silverman NH. Morphology and function of the bicuspid aortic valve with and without coarctation of the aorta in the young. *Am J Cardiol*. 2006;98:1096-1102
27. Deshpande J, Kinare SG. The bicuspid aortic valve--an autopsy study. *Indian J Pathol Microbiol*. 1991;34:112-118
28. Glower DD, Bashore TM, Spritzer CE. Congenital aortic stenosis and patent ductus arteriosus in the adult. *Ann Thorac Surg*. 1992;54:368-370
29. Karp N, Grosse-Wortmann L, Bowdin S. Severe aortic stenosis, bicuspid aortic valve and atrial septal defect in a child with joubert syndrome and related disorders (jsrd) - a case report and review of congenital heart defects reported in the human ciliopathies. *Eur J Med Genet*. 2012;55:605-610
30. Higgins CB, Wexler L. Reversal of dominance of the coronary arterial system in isolated aortic stenosis and bicuspid aortic valve. *Circulation*. 1975;52:292-296
31. Hutchins GM, Nazarian IH, Bulkley BH. Association of left dominant coronary arterial system with congenital bicuspid aortic valve. *Am J Cardiol*. 1978;42:57-59
32. Cripe L, Andelfinger G, Martin LJ, Shooner K, Benson DW. Bicuspid aortic valve is heritable. *J Am Coll Cardiol*. 2004;44:138-143
33. Loscalzo ML, Goh DL, Loeys B, Kent KC, Spevak PJ, Dietz HC. Familial thoracic aortic dilation and bicommissural aortic valve: A prospective analysis of natural history and inheritance. *Am J Med Genet A*. 2007;143A:1960-1967
34. Ellison JW, Yagubyan M, Majumdar R, Sarkar G, Bolander ME, Atkinson EJ, Sarano ME, Sundt TM. Evidence of genetic locus heterogeneity for familial bicuspid aortic valve. *J Surg Res*. 2007;142:28-31
35. Huntington K, Hunter AG, Chan KL. A prospective study to assess the frequency of familial clustering of congenital bicuspid aortic valve. *J Am Coll Cardiol*. 1997;30:1809-1812
36. Martin LJ, Ramachandran V, Cripe LH, Hinton RB, Andelfinger G, Tabangin M, Shooner K, Keddache M, Benson DW. Evidence in favor of linkage to human chromosomal regions 18q, 5q and 13q for bicuspid aortic valve and associated cardiovascular malformations. *Hum Genet*. 2007;121:275-284
37. Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, King IN, Grossfeld PD, Srivastava D. Mutations in notch1 cause aortic valve disease. *Nature*. 2005;437:270-274

38. Andelfinger G, Tapper AR, Welch RC, Vanoye CG, George AL, Jr., Benson DW. Kcnj2 mutation results in andersen syndrome with sex-specific cardiac and skeletal muscle phenotypes. *Am J Hum Genet.* 2002;71:663-668
39. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: Cell fate control and signal integration in development. *Science.* 1999;284:770-776
40. Loomes KM, Taichman DB, Glover CL, Williams PT, Markowitz JE, Piccoli DA, Baldwin HS, Oakey RJ. Characterization of notch receptor expression in the developing mammalian heart and liver. *Am J Med Genet.* 2002;112:181-189
41. Tan HL, Glen E, Topf A, Hall D, O'Sullivan JJ, Sneddon L, Wren C, Avery P, Lewis RJ, ten Dijke P, Arthur HM, Goodship JA, Keavney BD. Nonsynonymous variants in the smad6 gene predispose to congenital cardiovascular malformation. *Hum Mutat.* 2012;33:720-727
42. Biben C, Weber R, Kesteven S, Stanley E, McDonald L, Elliott DA, Barnett L, Koentgen F, Robb L, Feneley M, Harvey RP. Cardiac septal and valvular dysmorphogenesis in mice heterozygous for mutations in the homeobox gene nkx2-5. *Circ Res.* 2000;87:888-895
43. Lee TC, Zhao YD, Courtman DW, Stewart DJ. Abnormal aortic valve development in mice lacking endothelial nitric oxide synthase. *Circulation.* 2000;101:2345-2348
44. Laforest B, Andelfinger G, Nemer M. Loss of gata5 in mice leads to bicuspid aortic valve. *J Clin Invest.* 2011;121:2876-2887
45. Thomas PS, Sridurongrit S, Ruiz-Lozano P, Kaartinen V. Deficient signaling via alk2 (acvr1) leads to bicuspid aortic valve development. *PLoS One.* 2012;7:e35539
46. Fenoglio JJ, Jr., McAllister HA, Jr., DeCastro CM, Davia JE, Cheitlin MD. Congenital bicuspid aortic valve after age 20. *Am J Cardiol.* 1977;39:164-169
47. Mills P, Leech G, Davies M, Leathan A. The natural history of a non-stenotic bicuspid aortic valve. *Br Heart J.* 1978;40:951-957
48. Michelena HI, Desjardins VA, Avierinos JF, Russo A, Nkomo VT, Sundt TM, Pellikka PA, Tajik AJ, Enriquez-Sarano M. Natural history of asymptomatic patients with normally functioning or minimally dysfunctional bicuspid aortic valve in the community. *Circulation.* 2008;117:2776-2784
49. Calloway TJ, Martin LJ, Zhang X, Tandon A, Benson DW, Hinton RB. Risk factors for aortic valve disease in bicuspid aortic valve: A family-based study. *Am J Med Genet A.* 2011;155A:1015-1020
50. Fernandez B, Duran AC, Fernandez-Gallego T, Fernandez MC, Such M, Arque JM, Sans-Coma V. Bicuspid aortic valves with different spatial orientations of the leaflets are distinct etiological entities. *J Am Coll Cardiol.* 2009;54:2312-2318
51. Pauperio HM, Azevedo AC, Ferreira CS. The aortic valve with two leaflets--a study in 2,000 autopsies. *Cardiol Young.* 1999;9:488-498
52. Ward C. Clinical significance of the bicuspid aortic valve. *Heart.* 2000;83:81-85
53. Roberts WC. The congenitally bicuspid aortic valve. A study of 85 autopsy cases. *Am J Cardiol.* 1970;26:72-83
54. Nistri S, Basso C, Marzari C, Mormino P, Thiene G. Frequency of bicuspid aortic valve in young male conscripts by echocardiogram. *Am J Cardiol.* 2005;96:718-721
55. Anabwani GM, Bonhoeffer P. Prevalence of heart disease in school children in rural kenya using colour-flow echocardiography. *East Afr Med J.* 1996;73:215-217

56. Keane MG, Wiegers SE, Plappert T, Pochettino A, Bavaria JE, Sutton MG. Bicuspid aortic valves are associated with aortic dilatation out of proportion to coexistent valvular lesions. *Circulation*. 2000;102:III35-39
57. Campbell M. Calcific aortic stenosis and congenital bicuspid aortic valves. *Br Heart J*. 1968;30:606-616
58. Beppu S, Suzuki S, Matsuda H, Ohmori F, Nagata S, Miyatake K. Rapidity of progression of aortic stenosis in patients with congenital bicuspid aortic valves. *Am J Cardiol*. 1993;71:322-327
59. Roberts WC, Ko JM. Frequency by decades of unicuspid, bicuspid, and tricuspid aortic valves in adults having isolated aortic valve replacement for aortic stenosis, with or without associated aortic regurgitation. *Circulation*. 2005;111:920-925
60. Robicsek F, Thubrikar MJ, Cook JW, Fowler B. The congenitally bicuspid aortic valve: How does it function? Why does it fail? *Ann Thorac Surg*. 2004;77:177-185
61. Angrist AA, Oka M. Pathogenesis of bacterial endocarditis. *JAMA*. 1963;183:249-252
62. Durack DT, Beeson PB. Experimental bacterial endocarditis. I. Colonization of a sterile vegetation. *Br J Exp Pathol*. 1972;53:44-49
63. Bonow RO, Carabello BA, Chatterjee K, de Leon AC, Jr., Faxon DP, Freed MD, Gaasch WH, Lytle BW, Nishimura RA, O'Gara PT, O'Rourke RA, Otto CM, Shah PM, Shanewise JS, Smith SC, Jr., Jacobs AK, Adams CD, Anderson JL, Antman EM, Fuster V, Halperin JL, Hiratzka LF, Hunt SA, Nishimura R, Page RL, Riegel B. Acc/aha 2006 guidelines for the management of patients with valvular heart disease: A report of the american college of cardiology/american heart association task force on practice guidelines (writing committee to revise the 1998 guidelines for the management of patients with valvular heart disease) developed in collaboration with the society of cardiovascular anesthesiologists endorsed by the society for cardiovascular angiography and interventions and the society of thoracic surgeons. *J Am Coll Cardiol*. 2006;48:e1-148
64. Bansal RC. Infective endocarditis. *Med Clin North Am*. 1995;79:1205-1240
65. T Lewis RG. Observations relating to subacute infective endocarditis. 1. Notes on the normal structure of the aortic valve. 2. Bicuspid aortic valves of congenital origin. 3. Bicuspid aortic valves in subacute infective endocarditis. *Heart*. 1923:21-99.
66. Datta BN, Bhusnurmath B, Khattri HN, Sapru RP, Bidwai PS, Wahi PL. Anatomically isolated aortic valve disease. Morphologic study of 100 cases at autopsy. *Jpn Heart J*. 1988;29:661-670
67. Basso C, Boschello M, Perrone C, Mecenero A, Cera A, Bicego D, Thiene G, De Dominicis E. An echocardiographic survey of primary school children for bicuspid aortic valve. *Am J Cardiol*. 2004;93:661-663
68. Tutar E, Ekici F, Atalay S, Nacar N. The prevalence of bicuspid aortic valve in newborns by echocardiographic screening. *Am Heart J*. 2005;150:513-515
69. Olsson C. Thoracic aortic surgery: Epidemiology, outcomes, and prevention of cerebral complications. *Department of surgical sciences, Thoracic surgery*. 2006;PhD
70. Tzemos N, Therrien J, Yip J, Thanassoulis G, Tremblay S, Jamorski MT, Webb GD, Siu SC. Outcomes in adults with bicuspid aortic valves. *JAMA*. 2008;300:1317-1325
71. Freeman RV, Otto CM. Spectrum of calcific aortic valve disease: Pathogenesis, disease progression, and treatment strategies. *Circulation*. 2005;111:3316-3326

72. Mohler ER, 3rd. Are atherosclerotic processes involved in aortic-valve calcification? *Lancet*. 2000;356:524-525
73. Stewart BF, Siscovick D, Lind BK, Gardin JM, Gottdiener JS, Smith VE, Kitzman DW, Otto CM. Clinical factors associated with calcific aortic valve disease. Cardiovascular health study. *J Am Coll Cardiol*. 1997;29:630-634
74. Wallby L, Janerot-Sjoberg B, Steffensen T, Broqvist M. T lymphocyte infiltration in non-rheumatic aortic stenosis: A comparative descriptive study between tricuspid and bicuspid aortic valves. *Heart*. 2002;88:348-351
75. Carabello BA, Paulus WJ. Aortic stenosis. *Lancet*. 2009;373:956-966
76. Nagy E, Andersson DC, Caidahl K, Eriksson MJ, Eriksson P, Franco-Cereceda A, Hansson GK, Back M. Upregulation of the 5-lipoxygenase pathway in human aortic valves correlates with severity of stenosis and leads to leukotriene-induced effects on valvular myofibroblasts. *Circulation*. 2011;123:1316-1325
77. Rajamannan NM, Subramaniam M, Caira F, Stock SR, Spelsberg TC. Atorvastatin inhibits hypercholesterolemia-induced calcification in the aortic valves via the Irf5 receptor pathway. *Circulation*. 2005;112:1229-234
78. Rajamannan NM, Subramaniam M, Stock SR, Stone NJ, Springett M, Ignatiev KI, McConnell JP, Singh RJ, Bonow RO, Spelsberg TC. Atorvastatin inhibits calcification and enhances nitric oxide synthase production in the hypercholesterolaemic aortic valve. *Heart*. 2005;91:806-810
79. Fernandes SM, Sanders SP, Khairy P, Jenkins KJ, Gauvreau K, Lang P, Simonds H, Colan SD. Morphology of bicuspid aortic valve in children and adolescents. *J Am Coll Cardiol*. 2004;44:1648-1651
80. Olson LJ, Subramanian R, Edwards WD. Surgical pathology of pure aortic insufficiency: A study of 225 cases. *Mayo Clin Proc*. 1984;59:835-841
81. Sadee AS, Becker AE, Verheul HA, Bouma B, Hoedemaker G. Aortic valve regurgitation and the congenitally bicuspid aortic valve: A clinico-pathological correlation. *Br Heart J*. 1992;67:439-441
82. Lewin MB, Otto CM. The bicuspid aortic valve: Adverse outcomes from infancy to old age. *Circulation*. 2005;111:832-834
83. Kinlay S. Vascular biology. In: Rooke T, ed. *Vascular medicine and endovascular interventions*. Columbia: Blackwell Futura; 2007.
84. Wolinsky H, Glagov S. A lamellar unit of aortic medial structure and function in mammals. *Circ Res*. 1967;20:99-111
85. Borges LF, Touat Z, Leclercq A, Zen AA, Jondeau G, Franc B, Philippe M, Meilhac O, Gutierrez PS, Michel JB. Tissue diffusion and retention of metalloproteinases in ascending aortic aneurysms and dissections. *Hum Pathol*. 2009;40:306-313
86. Bunton TE, Biery NJ, Myers L, Gayraud B, Ramirez F, Dietz HC. Phenotypic alteration of vascular smooth muscle cells precedes elastolysis in a mouse model of marfan syndrome. *Circ Res*. 2001;88:37-43
87. Dingemans KP, Teeling P, Lagendijk JH, Becker AE. Extracellular matrix of the human aortic media: An ultrastructural histochemical and immunohistochemical study of the adult aortic media. *Anat Rec*. 2000;258:1-14
88. Schlatmann TJ, Becker AE. Pathogenesis of dissecting aneurysm of aorta. Comparative histopathologic study of significance of medial changes. *Am J Cardiol*. 1977;39:21-26
89. Schlatmann TJ, Becker AE. Histologic changes in the normal aging aorta: Implications for dissecting aortic aneurysm. *Am J Cardiol*. 1977;39:13-20
90. de Sa M, Moshkovitz Y, Butany J, David TE. Histologic abnormalities of the ascending aorta and pulmonary trunk in patients with bicuspid aortic valve

- disease: Clinical relevance to the Ross procedure. *J Thorac Cardiovasc Surg.* 1999;118:588-594
91. Niwa K, Perloff JK, Bhuta SM, Laks H, Drinkwater DC, Child JS, Miner PD. Structural abnormalities of great arterial walls in congenital heart disease: Light and electron microscopic analyses. *Circulation.* 2001;103:393-400
 92. Bauer M, Pasic M, Meyer R, Goetze N, Bauer U, Siniawski H, Hetzer R. Morphometric analysis of aortic media in patients with bicuspid and tricuspid aortic valve. *Ann Thorac Surg.* 2002;74:58-62
 93. Russo CF, Cannata A, Lanfrancioni M, Vitali E, Garatti A, Bonacina E. Is aortic wall degeneration related to bicuspid aortic valve anatomy in patients with valvular disease? *J Thorac Cardiovasc Surg.* 2008;136:937-942
 94. Jondeau G, Boileau C. Genetics of thoracic aortic aneurysms. *Curr Atheroscler Rep.* 2012;14:219-226
 95. Nordon I, Brar R, Taylor J, Hinchliffe R, Loftus IM, Thompson MM. Evidence from cross-sectional imaging indicates abdominal but not thoracic aortic aneurysms are local manifestations of a systemic dilating diathesis. *J Vasc Surg.* 2009;50:171-176 e171
 96. Gittenberger-de Groot AC, DeRuiter MC, Bergwerff M, Poelmann RE. Smooth muscle cell origin and its relation to heterogeneity in development and disease. *Arterioscler Thromb Vasc Biol.* 1999;19:1589-1594
 97. Jiang X, Rowitch DH, Soriano P, McMahan AP, Sucov HM. Fate of the mammalian cardiac neural crest. *Development.* 2000;127:1607-1616
 98. Montgomery DH, Ververis JJ, McGorisk G, Frohwein S, Martin RP, Taylor WR. Natural history of severe atheromatous disease of the thoracic aorta: A transesophageal echocardiographic study. *J Am Coll Cardiol.* 1996;27:95-101
 99. Isselbacher EM. Thoracic and abdominal aortic aneurysms. *Circulation.* 2005;111:816-828
 100. Folkersen L, Wagsater D, Paloschi V, Jackson V, Petrini J, Kurtovic S, Maleki S, Eriksson MJ, Caidahl K, Hamsten A, Michel JB, Liska J, Gabrielsen A, Franco-Cereceda A, Eriksson P. Unraveling divergent gene expression profiles in bicuspid and tricuspid aortic valve patients with thoracic aortic dilatation: The asap study. *Mol Med.* 2011;17:1365-1373
 101. El-Hamamsy I, Yacoub MH. Cellular and molecular mechanisms of thoracic aortic aneurysms. *Nat Rev Cardiol.* 2009;6:771-786
 102. Russo CF, Mazzetti S, Garatti A, Ribera E, Milazzo A, Bruschi G, Lanfrancioni M, Colombo T, Vitali E. Aortic complications after bicuspid aortic valve replacement: Long-term results. *Ann Thorac Surg.* 2002;74:S1773-1776; discussion S1792-1779
 103. Yasuda H, Nakatani S, Stugaard M, Tsujita-Kuroda Y, Bando K, Kobayashi J, Yamagishi M, Kitakaze M, Kitamura S, Miyatake K. Failure to prevent progressive dilation of ascending aorta by aortic valve replacement in patients with bicuspid aortic valve: Comparison with tricuspid aortic valve. *Circulation.* 2003;108 Suppl 1:II291-294
 104. Oulego-Erroz I, Alonso-Quintela P, Mora-Matilla M, Gautreaux Minaya S, Lapena-Lopez de Armentia S. Ascending aorta elasticity in children with isolated bicuspid aortic valve. *Int J Cardiol.* 2012. <http://dx.doi.org/10.1016/j.ijcard.2012.11.080>
 105. Nistri S, Sorbo MD, Basso C, Thiene G. Bicuspid aortic valve: Abnormal aortic elastic properties. *J Heart Valve Dis.* 2002;11:369-373; discussion 373-364
 106. Biner S, Rafique AM, Ray I, Cuk O, Siegel RJ, Tolstrup K. Aortopathy is prevalent in relatives of bicuspid aortic valve patients. *J Am Coll Cardiol.* 2009;53:2288-2295

107. Boyum J, Fellingner EK, Schmoker JD, Trombley L, McPartland K, Ittleman FP, Howard AB. Matrix metalloproteinase activity in thoracic aortic aneurysms associated with bicuspid and tricuspid aortic valves. *J Thorac Cardiovasc Surg.* 2004;127:686-691
108. Ikonomidis JS, Jones JA, Barbour JR, Stroud RE, Clark LL, Kaplan BS, Zeeshan A, Bavaria JE, Gorman JH, 3rd, Spinale FG, Gorman RC. Expression of matrix metalloproteinases and endogenous inhibitors within ascending aortic aneurysms of patients with bicuspid or tricuspid aortic valves. *J Thorac Cardiovasc Surg.* 2007;133:1028-1036
109. den Reijer PM, Sallee D, 3rd, van der Velden P, Zaaijer ER, Parks WJ, Ramamurthy S, Robbie TQ, Donati G, Lamphier C, Beekman RP, Brummer ME. Hemodynamic predictors of aortic dilatation in bicuspid aortic valve by velocity-encoded cardiovascular magnetic resonance. *J Cardiovasc Magn Reson.* 2010;12:4
110. Kurtovic S, Paloschi V, Folkersen L, Gottfries J, Franco-Cereceda A, Eriksson P. Diverging alternative splicing fingerprints in the transforming growth factor-beta signaling pathway identified in thoracic aortic aneurysms. *Mol Med.* 2011;17:665-675
111. Paloschi V, Kurtovic S, Folkersen L, Gomez D, Wagsater D, Roy J, Petrini J, Eriksson MJ, Caidahl K, Hamsten A, Liska J, Michel JB, Franco-Cereceda A, Eriksson P. Impaired splicing of fibronectin is associated with thoracic aortic aneurysm formation in patients with bicuspid aortic valve. *Arterioscler Thromb Vasc Biol.* 2011;31:691-697
112. Kjellqvist S, Maleki S, Olsson T, Chwastyniak M, Branca RM, Lehtio J, Pinet F, Franco-Cereceda A, Eriksson P. A combined proteomic and transcriptomic approach shows diverging molecular mechanisms in thoracic aortic aneurysm development in patients with tricuspid- and bicuspid aortic valve. *Mol Cell Proteomics.* 2013;12:407-425
113. Fedak PW, de Sa MP, Verma S, Nili N, Kazemian P, Butany J, Strauss BH, Weisel RD, David TE. Vascular matrix remodeling in patients with bicuspid aortic valve malformations: Implications for aortic dilatation. *J Thorac Cardiovasc Surg.* 2003;126:797-806
114. Forte A, Della Corte A, Grossi M, Bancone C, Provenzano R, Finicelli M, De Feo M, De Santo LS, Nappi G, Cotrufo M, Galderisi U, Cipollaro M. Early cell changes and tgfbeta pathway alterations in the aortopathy associated with bicuspid aortic valve stenosis. *Clinical science.* 2013;124:97-108
115. Aicher D, Urbich C, Zeiher A, Dimmeler S, Schafers HJ. Endothelial nitric oxide synthase in bicuspid aortic valve disease. *Ann Thorac Surg.* 2007;83:1290-1294
116. Hope MD, Hope TA, Meadows AK, Ordovas KG, Urbania TH, Alley MT, Higgins CB. Bicuspid aortic valve: Four-dimensional mr evaluation of ascending aortic systolic flow patterns. *Radiology.* 2010;255:53-61
117. Maleki S, Bjorck HM, Folkersen L, Nilsson R, Renner J, Caidahl K, Franco-Cereceda A, Lanne T, Eriksson P. Identification of a novel flow-mediated gene expression signature in patients with bicuspid aortic valve. *J Mol Med (Berl).* 2013;91:129-139
118. Borger MA, Preston M, Ivanov J, Fedak PW, Davierwala P, Armstrong S, David TE. Should the ascending aorta be replaced more frequently in patients with bicuspid aortic valve disease? *J Thorac Cardiovasc Surg.* 2004;128:677-683

119. Beroukhim RS, Kruzick TL, Taylor AL, Gao D, Yetman AT. Progression of aortic dilation in children with a functionally normal bicuspid aortic valve. *Am J Cardiol.* 2006;98:828-830
120. Maleki S, Björck HM, Paloschi V, Kjellqvist S, Folkersen L, Jackson V, Franco-Cereceda A, Eriksson P. Aneurysm development in patients with bicuspid aortic valve (bav): Possible connection to repair deficiency? *Aorta.* 2013;1:1-10
121. Attias D, Stheneur C, Roy C, Collod-Beroud G, Detaint D, Faivre L, Delrue MA, Cohen L, Francannet C, Beroud C, Claustres M, Iserin F, Khau Van Kien P, Lacombe D, Le Merrer M, Lyonnet S, Odent S, Plauchu H, Rio M, Rossi A, Sidi D, Steg PG, Ravaud P, Boileau C, Jondeau G. Comparison of clinical presentations and outcomes between patients with *tgfr2* and *fbn1* mutations in marfan syndrome and related disorders. *Circulation.* 2009;120:2541-2549
122. Guo DC, Pannu H, Tran-Fadulu V, Papke CL, Yu RK, Avidan N, Bourgeois S, Estrera AL, Safi HJ, Sparks E, Amor D, Ades L, McConnell V, Willoughby CE, Abuelo D, Willing M, Lewis RA, Kim DH, Scherer S, Tung PP, Ahn C, Buja LM, Raman CS, Shete SS, Milewicz DM. Mutations in smooth muscle alpha-actin (*acta2*) lead to thoracic aortic aneurysms and dissections. *Nat Genet.* 2007;39:1488-1493
123. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. *Circ Res.* 2003;92:827-839
124. Raffetto JD, Khalil RA. Matrix metalloproteinases and their inhibitors in vascular remodeling and vascular disease. *Biochem Pharmacol.* 2008;75:346-359
125. Sievers HH, Hemmer W, Beyersdorf F, Moritz A, Moosdorf R, Lichtenberg A, Misfeld M, Charitos EI. The everyday used nomenclature of the aortic root components: The tower of babel? *Eur J Cardiothorac Surg.* 2012;41:478-482
126. Sievers HH, Schmidtke C. A classification system for the bicuspid aortic valve from 304 surgical specimens. *J Thorac Cardiovasc Surg.* 2007;133:1226-1233
127. Edwards JE. Pathology of left ventricular outflow tract obstruction. *Circulation.* 1965;31:586-599
128. Falcone MW, Roberts WC, Morrow AG, Perloff JK. Congenital aortic stenosis resulting from a unicommissural valve. Clinical and anatomic features in twenty-one adult patients. *Circulation.* 1971;44:272-280
129. Wanhainen A, Themudo R, Ahlstrom H, Lind L, Johansson L. Thoracic and abdominal aortic dimension in 70-year-old men and women - a population-based whole-body magnetic resonance imaging (mri) study. *J Vasc Surg.* 2008;47:504-512
130. Bauer M, Bauer U, Siniawski H, Hetzer R. Differences in clinical manifestations in patients with bicuspid and tricuspid aortic valves undergoing surgery of the aortic valve and/or ascending aorta. *Thorac Cardiovasc Surg.* 2007;55:485-490
131. Roberts WC, Roberts CC, Vowels TJ, Ko JM, Filardo G, Hamman BL, Matter GJ, Henry AC, 3rd, Hebel RF, Jr. Effect of coronary bypass and valve structure on outcome in isolated valve replacement for aortic stenosis. *Am J Cardiol.* 2012;109:1334-1340
132. Tjang YS, van Hees Y, Korfer R, Grobbee DE, van der Heijden GJ. Predictors of mortality after aortic valve replacement. *Eur J Cardiothorac Surg.* 2007;32:469-474
133. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med.* 2005;352:1685-1695

134. Ngo DT, Sverdlov AL, Horowitz JD. Prevention of aortic valve stenosis: A realistic therapeutic target? *Pharmacol Ther.* 2012;135:78-93
135. Kieffer E, Chiche L, Baron JF, Godet G, Koskas F, Bahnini A. Coronary and carotid artery disease in patients with degenerative aneurysm of the descending thoracic or thoracoabdominal aorta: Prevalence and impact on operative mortality. *Ann Vasc Surg.* 2002;16:679-684
136. Marin ML, Hollier LH, Ellozy SH, Spielvogel D, Mitty H, Griep R, Lookstein RA, Carroccio A, Morrissey NJ, Teodorescu VJ, Jacobs TS, Minor ME, Sheahan CM, Chae K, Oak J, Cha A. Endovascular stent graft repair of abdominal and thoracic aortic aneurysms: A ten-year experience with 817 patients. *Ann Surg.* 2003;238:586-593; discussion 593-585
137. Goland S, Czer LS, De Robertis MA, Mirocha J, Kass RM, Fontana GP, Chang W, Trento A. Risk factors associated with reoperation and mortality in 252 patients after aortic valve replacement for congenitally bicuspid aortic valve disease. *Ann Thorac Surg.* 2007;83:931-937
138. Novaro GM, Tiong IY, Pearce GL, Grimm RA, Smedira N, Griffin BP. Features and predictors of ascending aortic dilatation in association with a congenital bicuspid aortic valve. *Am J Cardiol.* 2003;92:99-101
139. Schaefer BM, Lewin MB, Stout KK, Byers PH, Otto CM. Usefulness of bicuspid aortic valve phenotype to predict elastic properties of the ascending aorta. *Am J Cardiol.* 2007;99:686-690
140. Jassal DS, Bhagirath KM, Tam JW, Sochowski RA, Dumesnil JG, Giannoccaro PJ, Jue J, Pandey AS, Joyner CD, Teo KK, Chan KL. Association of bicuspid aortic valve morphology and aortic root dimensions: A substudy of the aortic stenosis progression observation measuring effects of rosuvastatin (astronomer) study. *Echocardiography.* 2010;27:174-179
141. Ikonomidis JS, Ruddy JM, Benton SM, Jr., Arroyo J, Brinsa TA, Stroud RE, Zeeshan A, Bavaria JE, Gorman JH, 3rd, Gorman RC, Spinale FG, Jones JA. Aortic dilatation with bicuspid aortic valves: Cusp fusion correlates to matrix metalloproteinases and inhibitors. *Ann Thorac Surg.* 2012;93:457-463
142. Nathan DP, Xu C, Plappert T, Desjardins B, Gorman JH, 3rd, Bavaria JE, Gorman RC, Chandran KB, Jackson BM. Increased ascending aortic wall stress in patients with bicuspid aortic valves. *Ann Thorac Surg.* 2011;92:1384-1389
143. Fernandes SM, Khairy P, Sanders SP, Colan SD. Bicuspid aortic valve morphology and interventions in the young. *J Am Coll Cardiol.* 2007;49:2211-2214
144. Cecconi M, Manfrin M, Moraca A, Zanoli R, Colonna PL, Bettuzzi MG, Moretti S, Gabrielli D, Perna GP. Aortic dimensions in patients with bicuspid aortic valve without significant valve dysfunction. *Am J Cardiol.* 2005;95:292-294
145. Bonow RO. Bicuspid aortic valves and dilated aortas: A critical review of the acc/aha practice guidelines recommendations. *Am J Cardiol.* 2008;102:111-114
146. Morgan-Hughes GJ, Roobottom CA, Owens PE, Marshall AJ. Dilatation of the aorta in pure, severe, bicuspid aortic valve stenosis. *Am Heart J.* 2004;147:736-740
147. Hahn RT, Roman MJ, Mogtader AH, Devereux RB. Association of aortic dilation with regurgitant, stenotic and functionally normal bicuspid aortic valves. *J Am Coll Cardiol.* 1992;19:283-288
148. Nistri S, Sorbo MD, Marin M, Palisi M, Scognamiglio R, Thiene G. Aortic root dilatation in young men with normally functioning bicuspid aortic valves. *Heart.* 1999;82:19-22

149. Vergara C, Viscardi F, Antiga L, Luciani GB. Influence of bicuspid valve geometry on ascending aortic fluid dynamics: A parametric study. *Artif Organs*. 2012;36:368-378
150. Della Corte A, Bancone C, Quarto C, Dialetto G, Covino FE, Scardone M, Caianiello G, Cotrufo M. Predictors of ascending aortic dilatation with bicuspid aortic valve: A wide spectrum of disease expression. *Eur J Cardiothorac Surg*. 2007;31:397-404; discussion 404-395
151. Linhartova K, Beranek V, Sefrna F, Hanisova I, Sterbakova G, Peskova M. Aortic stenosis severity is not a risk factor for poststenotic dilatation of the ascending aorta. *Circ J*. 2007;71:84-88
152. Nistri S, Grande-Allen J, Noale M, Basso C, Siviero P, Maggi S, Crepaldi G, Thiene G. Aortic elasticity and size in bicuspid aortic valve syndrome. *Eur Heart J*. 2008;29:472-479
153. Fazel SS, Mallidi HR, Lee RS, Sheehan MP, Liang D, Fleischman D, Herfkens R, Mitchell RS, Miller DC. The aortopathy of bicuspid aortic valve disease has distinctive patterns and usually involves the transverse aortic arch. *J Thorac Cardiovasc Surg*. 2008;135:901-907, 907 e901-902
154. Alegret JM, Calvo N, Ligeró C, Palomares R, Milla L, Martín-Paredero V, Montero M, Joven J. Dilated aortic root is related to a global aortic dilating diathesis. *J Vasc Surg*. 2010;52:867-871
155. Larsson E, Vishnevskaya L, Kalin B, Granath F, Swedenborg J, Hultgren R. High frequency of thoracic aneurysms in patients with abdominal aortic aneurysms. *Ann Surg*. 2011;253:180-184
156. Jones JA, Stroud RE, Kaplan BS, Leone AM, Bavaria JE, Gorman JH, 3rd, Gorman RC, Ikonomidis JS. Differential protein kinase c isoform abundance in ascending aortic aneurysms from patients with bicuspid versus tricuspid aortic valves. *Circulation*. 2007;116:1144-149
157. Della Corte A, Quarto C, Bancone C, Castaldo C, Di Meglio F, Nurzynska D, De Santo LS, De Feo M, Scardone M, Montagnani S, Cotrufo M. Spatiotemporal patterns of smooth muscle cell changes in ascending aortic dilatation with bicuspid and tricuspid aortic valve stenosis: Focus on cell-matrix signaling. *J Thorac Cardiovasc Surg*. 2008;135:8-18, 18 e11-12
158. Rizas KD, Ippagunta N, Tilson MD, 3rd. Immune cells and molecular mediators in the pathogenesis of the abdominal aortic aneurysm. *Cardiol Rev*. 2009;17:201-210
159. Stracke JO, Hutton M, Stewart M, Pendas AM, Smith B, Lopez-Otin C, Murphy G, Knauper V. Biochemical characterization of the catalytic domain of human matrix metalloproteinase 19. Evidence for a role as a potent basement membrane degrading enzyme. *J Biol Chem*. 2000;275:14809-14816
160. Murphy G, Knauper V, Cowell S, Hembry R, Stanton H, Butler G, Freije J, Pendas AM, Lopez-Otin C. Evaluation of some newer matrix metalloproteinases. *Ann N Y Acad Sci*. 1999;878:25-39
161. Kolb C, Mauch S, Peter HH, Krawinkel U, Sedlacek R. The matrix metalloproteinase rasi-1 is expressed in synovial blood vessels of a rheumatoid arthritis patient. *Immunol Lett*. 1997;57:83-88
162. Djonov V, Hogger K, Sedlacek R, Laissue J, Draeger A. Mmp-19: Cellular localization of a novel metalloproteinase within normal breast tissue and mammary gland tumours. *J Pathol*. 2001;195:147-155
163. Takino T, Miyamori H, Watanabe Y, Yoshioka K, Seiki M, Sato H. Membrane type 1 matrix metalloproteinase regulates collagen-dependent mitogen-activated protein/extracellular signal-related kinase activation and cell migration. *Cancer Res*. 2004;64:1044-1049

164. Klein T, Bischoff R. Physiology and pathophysiology of matrix metalloproteases. *Amino Acids*. 2011;41:271-290
165. Koullias GJ, Ravichandran P, Korkolis DP, Rimm DL, Elefteriades JA. Increased tissue microarray matrix metalloproteinase expression favors proteolysis in thoracic aortic aneurysms and dissections. *Ann Thorac Surg*. 2004;78:2106-2110; discussion 2110-2101
166. Ahokas K, Lohi J, Lohi H, Elomaa O, Karjalainen-Lindsberg ML, Kere J, Saarialho-Kere U. Matrix metalloproteinase-21, the human orthologue for *xmmp*, is expressed during fetal development and in cancer. *Gene*. 2002;301:31-41
167. Skoog T, Ahokas K, Orsmark C, Jeskanen L, Isaka K, Saarialho-Kere U. Mmp-21 is expressed by macrophages and fibroblasts in vivo and in culture. *Exp Dermatol*. 2006;15:775-783
168. Kuivanen T, Ahokas K, Virolainen S, Jahkola T, Holtta E, Saksela O, Saarialho-Kere U. Mmp-21 is upregulated at early stages of melanoma progression but disappears with more aggressive phenotype. *Virchows Arch*. 2005;447:954-960
169. Bister VO, Salmela MT, Karjalainen-Lindsberg ML, Uria J, Lohi J, Puolakkainen P, Lopez-Otin C, Saarialho-Kere U. Differential expression of three matrix metalloproteinases, mmp-19, mmp-26, and mmp-28, in normal and inflamed intestine and colon cancer. *Dig Dis Sci*. 2004;49:653-661
170. Bonderman D, Gharehbaghi-Schnell E, Wollenek G, Maurer G, Baumgartner H, Lang IM. Mechanisms underlying aortic dilatation in congenital aortic valve malformation. *Circulation*. 1999;99:2138-2143
171. Ahokas K, Lohi J, Illman SA, Llano E, Elomaa O, Impola U, Karjalainen-Lindsberg ML, Saarialho-Kere U. Matrix metalloproteinase-21 is expressed epithelially during development and in cancer and is up-regulated by transforming growth factor-beta1 in keratinocytes. *Lab Invest*. 2003;83:1887-1899
172. Hope MD, Hope TA, Crook SE, Ordovas KG, Urbania TH, Alley MT, Higgins CB. 4d flow cmr in assessment of valve-related ascending aortic disease. *JACC Cardiovasc Imaging*. 2011;4:781-787
173. Barker AJ, Lanning C, Shandas R. Quantification of hemodynamic wall shear stress in patients with bicuspid aortic valve using phase-contrast mri. *Ann Biomed Eng*. 2010;38:788-800
174. Cheng C, Tempel D, van Haperen R, van der Baan A, Grosveld F, Daemen MJ, Krams R, de Crom R. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. *Circulation*. 2006;113:2744-2753
175. Della Corte A, Bancone C, Conti CA, Votta E, Redaelli A, Del Viscovo L, Cotrufo M. Restricted cusp motion in right-left type of bicuspid aortic valves: A new risk marker for aortopathy. *J Thorac Cardiovasc Surg*. 2012;144:360-369, 369 e361
176. Thanassoulis G, Yip JW, Filion K, Jamorski M, Webb G, Siu SC, Therrien J. Retrospective study to identify predictors of the presence and rapid progression of aortic dilatation in patients with bicuspid aortic valves. *Nat Clin Pract Cardiovasc Med*. 2008;5:821-828
177. Hope MD, Dyverfeldt P, Acevedo-Bolton G, Wrenn J, Foster E, Tseng E, Saloner D. Post-stenotic dilation: Evaluation of ascending aortic dilation with 4d flow mr imaging. *Int J Cardiol*. 2012;156:e40-42
178. Girdeuskas E, Disha K, Borger MA, Kuntze T. Relation of bicuspid aortic valve morphology to the dilatation pattern of the proximal aorta: Focus on the transvalvular flow. *Cardiol Res Pract*. 2012;2012:478259

179. Cotrufo M, Della Corte A, De Santo LS, Quarto C, De Feo M, Romano G, Amarelli C, Scardone M, Di Meglio F, Guerra G, Scarano M, Vitale S, Castaldo C, Montagnani S. Different patterns of extracellular matrix protein expression in the convexity and the concavity of the dilated aorta with bicuspid aortic valve: Preliminary results. *J Thorac Cardiovasc Surg.* 2005;130:504-511
180. Wagsater D, Paloschi V, Hanemaaijer R, Hultenby K, Bank RA, Franco-Cereceda A, Lindeman JH, Eriksson P. Impaired collagen biosynthesis and cross-linking in aorta of patients with bicuspid aortic valve. *J Am Heart Assoc.* 2013;2:e000034
181. La Canna G, Ficarra E, Tsagalau E, Nardi M, Morandini A, Chieffo A, Maisano F, Alfieri O. Progression rate of ascending aortic dilation in patients with normally functioning bicuspid and tricuspid aortic valves. *Am J Cardiol.* 2006;98:249-253
182. Guntheroth WG. A critical review of the american college of cardiology/american heart association practice guidelines on bicuspid aortic valve with dilated ascending aorta. *Am J Cardiol.* 2008;102:107-110
183. Roberts WC, Vowels TJ, Ko JM, Filardo G, Hebel RF, Jr., Henry AC, Matter GJ, Hamman BL. Comparison of the structure of the aortic valve and ascending aorta in adults having aortic valve replacement for aortic stenosis versus for pure aortic regurgitation and resection of the ascending aorta for aneurysm. *Circulation.* 2011;123:896-903
184. Nkomo VT, Enriquez-Sarano M, Ammash NM, Melton LJ, 3rd, Bailey KR, Desjardins V, Horn RA, Tajik AJ. Bicuspid aortic valve associated with aortic dilatation: A community-based study. *Arterioscler Thromb Vasc Biol.* 2003;23:351-356
185. Ferencik M, Pape LA. Changes in size of ascending aorta and aortic valve function with time in patients with congenitally bicuspid aortic valves. *Am J Cardiol.* 2003;92:43-46
186. Svensson LG, Kim KH, Lytle BW, Cosgrove DM. Relationship of aortic cross-sectional area to height ratio and the risk of aortic dissection in patients with bicuspid aortic valves. *J Thorac Cardiovasc Surg.* 2003;126:892-893
187. Davies RR, Kaple RK, Mandapati D, Gallo A, Botta DM, Jr., Elefteriades JA, Coady MA. Natural history of ascending aortic aneurysms in the setting of an unreplaced bicuspid aortic valve. *Ann Thorac Surg.* 2007;83:1338-1344
188. Debl K, Djavidani B, Buchner S, Poschenrieder F, Schmid FX, Kobuch R, Feuerbach S, Riegger G, Luchner A. Dilatation of the ascending aorta in bicuspid aortic valve disease: A magnetic resonance imaging study. *Clin Res Cardiol.* 2009;98:114-120
189. McKellar SH, Michelena HI, Li Z, Schaff HV, Sundt TM, 3rd. Long-term risk of aortic events following aortic valve replacement in patients with bicuspid aortic valves. *Am J Cardiol.* 2010;106:1626-1633
190. Park CB, Greason KL, Suri RM, Michelena HI, Schaff HV, Sundt TM, 3rd. Fate of nonreplaced sinuses of valsalva in bicuspid aortic valve disease. *J Thorac Cardiovasc Surg.* 2011;142:278-284
191. Girdauskas E, Disha K, Raisin HH, Secknus MA, Borger MA, Kuntze T. Risk of late aortic events after an isolated aortic valve replacement for bicuspid aortic valve stenosis with concomitant ascending aortic dilation. *Eur J Cardiothorac Surg.* 2012;42:832-837; discussion 837-838
192. Andrus BW, O'Rourke DJ, Dacey LJ, Palac RT. Stability of ascending aortic dilatation following aortic valve replacement. *Circulation.* 2003;108 Suppl 1:II295-299

193. Girdauskas E, Disha K, Borger MA, Kuntze T. Long-term prognosis of ascending aortic aneurysm after aortic valve replacement for bicuspid versus tricuspid aortic valve stenosis. *J Thorac Cardiovasc Surg.* 2012. 10.1016/j.jtcvs.2012.11.004
194. Etz CD, Homann TM, Silovitz D, Spielvogel D, Bodian CA, Luehr M, DiLuozzo G, Plestis KA, Griep RB. Long-term survival after the bentall procedure in 206 patients with bicuspid aortic valve. *Ann Thorac Surg.* 2007;84:1186-1193; discussion 1193-1184
195. Itoh A, Fischbein M, Arata K, Miller DC. "Peninsula-style" transverse aortic arch replacement in patients with bicuspid aortic valve. *Ann Thorac Surg.* 2010;90:1369-1371
196. Park CB, Greason KL, Suri RM, Michelena HI, Schaff HV, Sundt TM, 3rd. Should the proximal arch be routinely replaced in patients with bicuspid aortic valve disease and ascending aortic aneurysm? *J Thorac Cardiovasc Surg.* 2011;142:602-607
197. Svensson LG, Kim KH, Blackstone EH, Rajeswaran J, Gillinov AM, Mihaljevic T, Griffin BP, Grimm R, Stewart WJ, Hammer DF, Lytle BW. Bicuspid aortic valve surgery with proactive ascending aorta repair. *J Thorac Cardiovasc Surg.* 2011;142:622-629, 629 e621-623
198. Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE, Jr., Eagle KA, Hermann LK, Isselbacher EM, Kazerooni EA, Kouchoukos NT, Lytle BW, Milewicz DM, Reich DL, Sen S, Shinn JA, Svensson LG, Williams DM. 2010 accf/aha/aats/acr/asa/sca/scai/sir/sts/svm guidelines for the diagnosis and management of patients with thoracic aortic disease. A report of the american college of cardiology foundation/american heart association task force on practice guidelines, american association for thoracic surgery, american college of radiology, american stroke association, society of cardiovascular anesthesiologists, society for cardiovascular angiography and interventions, society of interventional radiology, society of thoracic surgeons, and society for vascular medicine. *J Am Coll Cardiol.* 2010;55:e27-e129
199. Vahanian A, Alfieri O, Andreotti F, Antunes MJ, Baron-Esquivias G, Baumgartner H, Borger MA, Carrel TP, De Bonis M, Evangelista A, Falk V, Lung B, Lancellotti P, Pierard L, Price S, Schafers HJ, Schuler G, Stepinska J, Swedberg K, Takkenberg J, Von Oppell UO, Windecker S, Zamorano JL, Zembala M. Guidelines on the management of valvular heart disease (version 2012): The joint task force on the management of valvular heart disease of the european society of cardiology (esc) and the european association for cardiothoracic surgery (eacts). *Eur J Cardiothorac Surg.* 2012;42:S1-44
200. Lee RS. Should the ascending aorta be replaced less frequently in patients with bicuspid aortic valve disease? *Eur J Cardiothorac Surg.* 2013. 10.1093/ejcts/ezt162