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Clinical and genetic aspects of bicuspid aortic valve: a proposed model for family screening based on a review of literature

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Abstract

Bicuspid aortic valve (BAV) is the most common congenital cardiac defect causing serious morbidity including valvular dysfunction and thoracic aortic aneurysms (TAA) in around 30% of BAV patients. Cardiological screening of first-degree relatives is advised in recent guidelines given the observed familial clustering of BAV. However, guidelines regarding screening of family members and DNA testing are not unequivocal. The aim of this review is to provide an overview of the literature on echocardiographic screening in first-degree relatives of BAV patients and to propose a model for family screening. In addition, we provide a flowchart for DNA testing. We performed a PubMed search and included studies providing data on echocardiographic screening in asymptomatic relatives of BAV patients. Nine studies were included. In 5.8-47.4% of the families BAV was shown to be familial. Of the screened first-degree relatives 1.8-11% was found to be affected with BAV. Results regarding a potential risk of TAA in first-degree relatives with a tricuspid aortic valve (TAV) were conflicting. The reported familial clustering of BAV underlines the importance of cardiological screening in relatives. After reviewing the available family history, patient characteristics and the results of cardiological screening in relatives, follow-up in relatives with a TAV and/or DNA testing may be advised in a subset of families. In this study we propose a model for the clinical and genetic work-up in BAV families, based on the most extensive literature review on family screening performed until now.

Introduction

With an estimated prevalence of 0.5-2% bicuspid aortic valve (BAV) (OMIM#109730) is the most common congenital cardiac defect associated with an increased risk of serious complications, including thoracic aortic aneurysms (TAA).[1-6] As BAV, either with or without associated TAA (+/-TAA), often is a familial condition, cardiological screening of first-degree relatives of BAV patients has been advised in recent guidelines of the American College of Cardiology and American Heart Association (ACC/AHA).[7-9] However, screening is currently largely dependent on local initiatives and several important questions remain to be addressed. For example, it remains unclear whether relatives with a tricuspid aortic valve (TAV) are at an increased risk for the development of TAA and if DNA testing is a useful tool in the identification of families with a high risk for TAA at young age. The aim of this study is to provide an overview of the literature on family screening in firs-degree relatives, to provide an overview of the results of DNA testing in BAV (+/-TAA) families and to propose a model for clinical and genetic work-up in BAV (+/-TAA) families. The results of the literature search and our proposed screening model are presented after a general overview of the clinical and genetic aspects of BAV (+/-TAA). The current understanding of the pathology, clinical aspects and management of BAV disease and genetic syndromes associated with BAV were recently reviewed and are outside the scope of our study.[10-13]

Bicuspid aortic valve: clinical and genetic aspects

The bicuspid aortic valve, in most cases, consists of two unequal sized leaflets. The larger leaflet typically has a central raphe or ridge resulting from a fusion of the commissures (in ~70% fusion of the right and left coronary cusp, the remainder mostly from fusion of the right and non coronary cusp, and rarely the left and non coronary cusp) resulting in a functionally bicuspid aortic valve.[10,14] A central raphe is absent in the less frequently occurring true bicuspid valve.[15-17] BAV can be an isolated congenital anomaly, but can also be associated with other abnormalities such as aortic coarctation, ventricular septal defects and hypoplastic left ventricle.[6,9,12]

In most cases, BAV can be diagnosed and hemodynamically assessed using transthoracic echocardiography. In a minority of patients, particularly in calcific valve disease, higher resolution imaging techniques such as cardiac magnetic resonance imaging, computed tomography or trans-esophageal echocardiography may be required.[16,18,19] Although BAV may retain normal function throughout adult life, around 30% of the people with BAV will develop clinical complications.[3-6] Therefore patients with BAV are advised to remain under regular surveillance by a cardiologist.[9] Taking into account the high incidence, BAV may be responsible for more morbidity and mortality than other congenital cardiac defects combined.[5,6] In patients with BAV aortic stenosis and/or insufficiency is more frequent. In addition, BAV patients are more prone to aortic dilatation.[16] Dilatation of the thoracic aorta, most commonly located in the ascending aorta, has been reported in 35-80% of adult BAV patients and has rarely been observed as early as childhood.[11-14,20-25] In adults TAA is defined as an aortic diameter with a z-score of ≥2, corresponding to an observed value >1.96 standard deviations above the predicted value for age, gender, and body surface area (BSA).[26] An aortic root >4.0 cm in adults is considered to be dilated irrespective of age, gender or BSA.[27-29] Aortic dilatation in BAV may be restricted to the ascending aorta, but can also include the aortic root.[25,30] In a retrospective study among 241 BAV patients referred for cardiac surgery,

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mean age 56 (range 16-85) years, aortic dilatation was seen in 97 patients (40%). The aortic root was involved in 9 (9.3%), the ascending aorta in 68 (70.1%), both the root and the ascending aorta in 14 (14.4%), the ascending aorta and the aortic arch in 5 (5.2%) and the root, ascending and aortic arch in 1 of the patients (1%).[25] The relatively rare dilatation restricted to the aortic root is most commonly observed in men below the age of 40 and is reported to be associated with an increased risk of aortic dissection. This *root* phenotype has been proposed to be the form of bicuspid aortopathy most likely to be associated with a genetic cause.[3,13,20,24,31-33]

Although rare, the most feared complication in BAV patients is thoracic aortic dissection, which has been reported at young age.[34] Whereas the lifetime risk of aortic dissection in BAV patients was initially reported around 5%, recent studies show a lifetime risk of aortic dissection of less than 1% in BAV patients and a normal life expectancy.[4,6,35,36] This difference can potentially be explained by increased surveillance and timely surgical intervention in recent years. Aortic dissection is, in the majority of cases, preceded by slowly progressive asymptomatic aortic dilatation. This allows screening and preventive surgery when indicated. Surgery is recommended in BAV patients with a diameter of the aortic sinuses or ascending aorta >5.5 cm, or >5.0 cm in the presence of an additional risk factor (growth ≥0.5 cm/year or a family history of aortic dissection).[37,38] Although the risk of aortic dissection is lower than initially estimated, cardiovascular surgery was performed in 22-27% of the BAV patients during follow-up.[3,4]

Familial BAV associated with dilatation of the aorta was first reported in literature over 40 years ago with the observation of a BAV in both a father and his son, who died in his sleep at the age of 19. No features of Marfan syndrome were observed. Autopsy confirmed an aortic dissection and a BAV.[39] The first systematic study on cardiological screening in asymptomatic relatives of BAV patients, to our knowledge, was published in 1978.[40] The authors screened 188 first-degree relatives of 41 BAV patients by auscultation and eccentricity index. BAV was diagnosed in 3.7-9.6% of the relatives and was familial in 14.6-31.7% of the 41 families (depending on the inclusion of doubtful cases). The familial clustering of BAV (+/-TAA) has since been confirmed by a number of studies, indicating a high heritability of BAV.[41] In addition, 20% of pediatric patients with a left ventricular outflow tract obstruction have (an) affected first-degree relative(s), frequently a previously undetected BAV further illustrating a strong genetic contribution to the origin of BAV.[42] However, to date, a genetic cause has been identified in only a minority of BAV families, mostly showing an autosomal dominant inheritance pattern with reduced penetrance. In 2005 *NOTCH1* mutations were reported to be involved in a spectrum of developmental aortic valve anomalies, including BAV and severe aortic valve calcification.[43] Sequence analysis of *NOTCH1* indicated a potential overrepresentation of non-synonymous missense variants among BAV (+/-TAA) patients, however *NOTCH1* mutations were found only in <5% of BAV cases in several subsequent studies.[43-49] Interestingly, *NOTCH1* mutations were recently shown to cause Adams Oliver syndrome. This is a rare developmental disorder with aplasia cutis of the scalp and transverse limb defects, frequently associated with cardiac defects, including BAV.[50] Based on studies in individual patients, linkage analysis in families and animal studies, several other genes and candidate loci have been implicated to be potentially involved in BAV.[51-58] These studies emphasize the genetic as well as phenotypic heterogeneity of BAV.

Whether dilatation of the proximal aorta in patients with BAV is a primary manifestation of an underlying genetic disorder, or secondary to hemodynamic effects related to the abnormal aortic valve remains controversial.[21,32,59,60] Martin *et al.* performed bivariate genetic analyses between aortic dimensions and BAV. Their results did not support a shared underlying genetic basis for BAV and aortic measures.[60] However, Loscalzo *et al*. conclude that BAV and TAA might be independent manifestations of a single gene defect with an autosomal dominant pattern of inheritance with incomplete penetrance. They studied segregation of BAV and TAA in 13 TAA families referred for analysis of known aneurysm, dissection or rupture. In total 110 first-degree relatives of index patients were included. In 15 (13.6%) BAV was seen, in 10 cases associated with TAA. Twenty four of the relatives (22%) were diagnosed with TAA in the presence of a normal tricuspid aortic valve.[32] In addition, Keane *et al.* reported that aortic size in BAV patients was larger than in control patients. They observed comparable degrees of aortic regurgitation, stenosis or mixed lesions, and concluded that intrinsic pathology appears to be responsible for aortic enlargement.[21] These observations support the hypothesis that BAV and TAA are independent manifestations of a single gene defect in a subset of BAV families.[21,32,61] The risk of dilatation of the thoracic aorta might therefore be increased in some first-degree relatives of BAV patients, even in the absence of a bicuspid aortic valve. Vice versa, a limited number of studies suggest that BAV might be more prevalent in familial thoracic aneurysms and dissections (FTAAD) and in some hereditary connective tissue disorders associated with an increased risk of aortic aneurysms (for example Marfan syndrome).[33,62-64] Several studies illustrate the value of DNA testing in families with BAV +/- TAA by the identification of pathogenic mutations in known TAAD genes in FTAAD families with affected individuals with BAV. This provides further evidence for the hypothesis that both TAA and BAV may be independent phenotypic manifestations of a single mutation.[31,33,62] BAV was identified in four FTAAD patients in three of 14 studied families with an autosomal dominant pattern of thoracic aortic dissections on the basis of a mutation in smooth muscle aortic actin (*ACTA2*) gene.[62] A mutation in transforming growth factor- receptor type II (*TGFBR2*) gene, associated with Loeys Dietz syndrome, was found in a 48-year-old woman with BAV and a proximal aortic aneurysm including the aortic root, measuring 56 mm. Her brother died suddenly at the age of 42 immediately after the onset of excruciating chest pain. Her father, who also carried the mutation, had a history of elective surgical replacement of the aortic valve and ascending aorta for BAV and ascending aortic aneurysm at the age of 72.[33] In several subsequent studies no *TGFBR1* or *TGFBR2* mutations were found in sporadic and familial BAV.[32,44,64] Recently, *FBN1*-mutations were linked to BAV as well by the identification of three *FBN1* mutations in two BAV patients with TAA (age at diagnosis 15 and 19 years) who did not fulfill the Marfan syndrome criteria according to the revised Ghent criteria.[64] In both patients the aortic diameter exceeded the threshold for surgery and the aortic size was largest at the level at the sinuses of Valsalva. Two of the three mutations (pArg529Gln and Arg2726Trp) were previously identified to be associated with variable/incomplete Marfan phenotype. In addition, the prevalence of BAV was reported to be increased in a cohort of 257 unrelated patients diagnosed with Marfan syndrome according to the updated Ghent criteria. Echocardiography showed BAV in 12 patients (4.7%).[63] In three of these 12 patients DNA testing was performed revealing a pathogenic *FBN1* mutation in two cases, supporting the hypothesis that *FBN1* mutations may not only be associated with an increased incidence TAA, but also with BAV.

Overview of the literature on family screening in first-degree relatives of bicuspid aortic valve patients

We performed a PubMed search using the term *bicuspid aortic valve* in combination with at least one of the following terms in title and/or abstract: *gene(s), genetic(s), syndrome(s), family, relatives, family screening, pedigree analysis, inherited, aortic aneurysm, aortic dilatation or aortic dissection*. Inclusion

criteria were: studies providing data on systematic cardiological screening using echocardiography in first degree relatives of at least 20 BAV index patients, English language, published before November 2014. The number of at least 20 probands was arbitrarily chosen as we estimated that smaller cohorts or case series might introduce uncontrollable bias.

Using the abovementioned search strategy, we identified 683 articles. These articles were assessed for eligibility by reading title, abstract and/or full text. Of these, 27 contained data on family screening. Nine studies met our inclusion criteria. The results of these studies are summarized in Table 1.[26,28,41,42,54,61,65-69] In 5.8-47.7% of the families BAV was shown to be familial (defined as BAV diagnosed in at least one first degree relative of the index patient). Of the screened first-degree relatives of BAV patients 1.8-11% was found to be affected with BAV. The results regarding the risk of TAA with TAV in first degree relatives are conflicting. One study reported the presence of TAA in 32% of first degree relatives with TAV.[61] In this study, 53% of the BAV index patients had a dilated aortic root. Dilatation of the aortic root is described to be relatively rare in BAV and is proposed to be the form of bicuspid aortopathy most likely to be associated with a genetic cause.[3,13,20,24,31-33] The other studies reported percentages of TAA in the first-degree TAV relatives of 3-4%.[54,65-68] This is around population risk since 2.3% of the general population, by definition, is expected to have z scores >2.[28,70,71] In the large study by Robledo-Carmona *et al.* only mild aortic dilatation (<4 cm) at older age (>50 years), was observed among nine out of 270 first degree relatives with a TAV, comparable to the observations in the control cohort.[68] They concluded that if their findings are confirmed by other studies, echocardiographic follow up of the aortic dimensions of TAV first-degree relatives might not be necessary.[68] In addition, in a recent study by Dayan *et al* among first-degree relatives of BAV patients without TAA, normal aortic dimensions were seen in all relatives with a tricuspid valve.[72]

A proposed model for clinical and genetic work-up in bicuspid aortic valve (+/-TAA) families based on literature

Based on our review of the literature and our clinical practice, we propose the model shown in Figure 1 as a tool for the clinical and genetic screening of BAV patients and their relatives. The main purpose of this model is identifying at risk relatives for BAV and TAA. Following the ACC/AHA guidelines and the recommendations of the authors of the nine studies on family screening, we recommend cardiological screening including echocardiography in all first degree relatives (>18 years).[7-9] We do not perform screening in asymptomatic children given the low chance of significant abnormalities not detected during the routinely performed prenatal ultrasound at 20 weeks gestation and cardiac auscultation after birth. After reviewing the available family history, patient characteristics and the results of cardiological screening in relatives, follow-up in relatives with a TAV and/or DNA testing may be advised (Figure 1). In case of sporadic or familial cases of BAV without TAA or aortic dissection in relatives, we do not recommend follow up in first-degree relatives with a tricuspid valve and normal aortic diameters. In these families, we only recommend DNA diagnostics in the index patient and echocardiographic follow up in the first-degree relatives (*e.g.* every five years, depending on the age and echocardiographic findings) when dilatation of the aortic root in the BAV index patient develops before the age of 60 years. Based on our review of literature, with most studies showing only a slightly increased risk of TAA in relatives with TAV, we advise clinical follow up of first-degree relatives with TAV only in the following situations; sporadic BAV with aortic root involvement before the age of 60 years, TAA in a relative with TAV before the age of 60, and in families with two or more persons with (suspected) TAA and a TAV irrespective of age. The cut off at the age of 60 years is more or less arbitrary and is chosen since increased aortic diameters are more likely to be associated with older age and hypertension then with genetic factors when observed later in life.[73] In contrast, aortic root involvement at young age is relatively rare in BAV, but relatively frequent in FTAAD and syndrome associated TAAD, and might be associated with mutations in TAAD genes.[33,62,64,74,75] First-degree relatives of young BAV patients with dilatation of the aortic root may therefore have an increased risk of TAA in absence of BAV.[22,32,61,62] In our clinical genetics department DNA diagnostics of 13 TAAD genes (*ACTA2, COL3A1, EFEMP2, ELN, FBN1, FBN2, MYH11, MYLK, PLOD1, SLC2A10, SMAD3, TGFBR1, TGFBR2*) and *NOTCH1* is offered in the following three situations: BAV patients with aortic root dilatation (z>2 or an aortic diameter >4.0 cm) before the age of 60, in case of TAA in a relative with TAV before the age of 60 years, and in familial TAA. Furthermore, in confirmed familial BAV cases not fulfilling the aforementioned criteria, sequencing of the *NOTCH1* gene may be considered. In sporadic cases of BAV, or sporadic BAV patients with TAA <60 years not involving the aortic root, the chance of identifying a pathogenic mutation in *NOTCH1* is likely to be low. *NOTCH1* mutation screening in these patients may be considered, depending on the social context of the patient, but is not routinely recommended by us (Figure 1). In familial BAV without TAA, *NOTCH1* mutation screening can be helpful to identify at risk relatives, especially in patients with calcified bicuspid valves.[43] We currently advise follow-up of all relatives carrying a pathogenic *NOTCH1* mutation irrespective of the presence or absence of BAV given a potentially increased risk of TAA.[48] Future phenotype-genotype studies may potentially enable the identification of specific *NOTCH1* mutations associated with an increased risk for TAA. When using our proposed model it is important to consider all available clinical data of a family in its entirety. In case of new information during follow-up one should reconsider the situation using the model and adjust the clinical and genetic work-up accordingly (for example in case of the development of an aortic root aneurysm in a BAV patient or a newly diagnosed family member with BAV). This model is meant as a tool for non-syndromic BAV (+/-TAA) patients and their families. In case of evidence for a hereditary connective tissue disorder we recommend a custom multidisciplinary work-up and targeted analysis of candidate genes for the suspected underlying syndrome instead of using the presented model. For example in cases of extreme degrees of aortic dilatation, and/or the presence of other symptoms or features. The individual work-up in patients with (suspected) genetic syndromes associated with an increased occurrence of BAV, such as Turner syndrome, is outside the scope of our study.

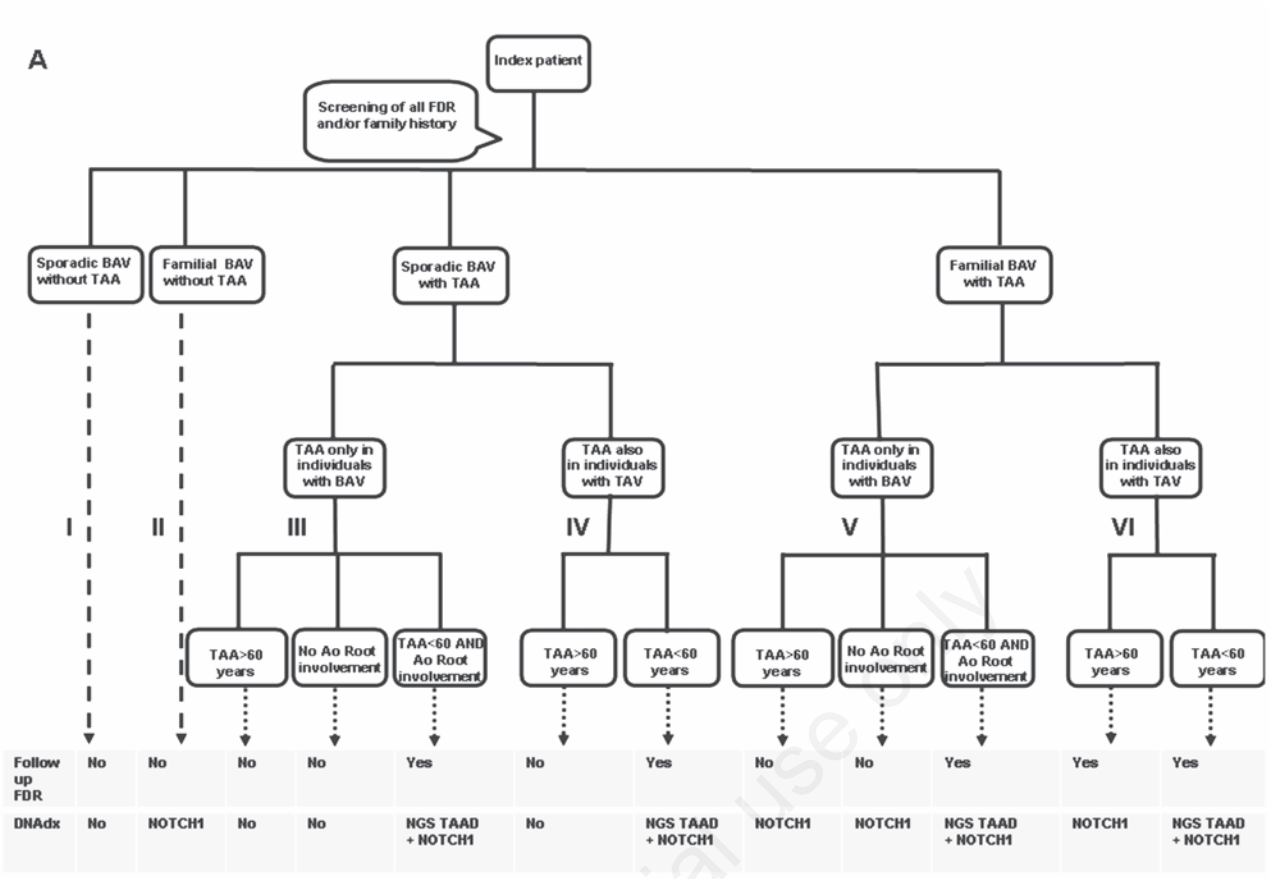
Discussion and Conclusions

We propose a model for the clinical and genetic screening in non-syndromic BAV (+/-TAA) families based on a review of the literature. The percentage of BAV in first-degree relatives of BAV index patients was 1.8-11% and BAV was found to be familial in 5.8-47.4% of the families in the nine included studies. The different percentages reported in literature might be explained by the small number of index patients and screened first-degree relatives in some studies. Furthermore, the participation rates of the first-degree relatives in cardiological screening varied between studies and were not mentioned in all papers. This could have resulted in a selection bias. In addition, different patient groups with potentially different prevalences of BAV were included (*e.g.* pediatric patients with/without additional congenital cardiac defects *versus* adult index patients). Furthermore, the prevalence of (familial) BAV might differ between geographical regions as suggested by Robledo-Carmona *et al.*[68] In all studies, the importance of screen-

Table 1. Overview of literature on family screening.

Studies	BAV index patients (n)	Phenotype	Familial BAV/ families screened (%)	First-degree relatives with BAV/ first-degree relatives screened (%)	First-degree relatives with BAV+TAA/first degree relatives with BAV (%)	TAA definition	First-degree relatives with TAV+TAA/first degree relatives with TAV (%)
Huntington[66]	30	Adults, isolated BAV (n=18), BAV + TAA (n=7), BAV + CVM (n=5)	11/30 (36.7%)	17/186 (9.1%)	N/A	N/A	5/169 (3.0%)
Cripe[41]	50	Pediatric patients, BAV (n=38), BAV + CVM (n=12)	16/50 (32%)	24/259 (9.3%)	N/A	-	N/A
Martin[54]	38	Pediatric patients, BAV (n=24), BAV + CMV (n=14)*	18/38 (47.4%)	36/315 (11%)	3/36 (8%)	N/A	7/279 (2.5%)
Biner[61]	49	Adults°	N/A	5/53 (9.4%)	N/A	Maximal dimension at any level of the root 95% CI of the diameter at the sinuses of Valsalva of a normal reference population[26]	14/44 (32%)
Kerstjens-Frederikse[42]	50	Pediatric patients, BAV/ aortic valve stenosis	14/50 (28%)	N/A	N/A	-	N/A
Panayotova[67]	24	Adults, undergoing surgery for BAV or BAV + TAA	4/24 (16.7%)	4/52 (8%)#	2/4 (50%)	Definition TAA: increase in aortic size >50% of the upper limit of normal as per standard nomograms taking into account BSA, age and gender	2/48 (4.2%)
Demir[65]	66	Pediatric patients, isolated BAV (n=52)	3/52 (5.8%)	3/168 (1.8%)	N/A	z score >2	5/163 (3.1%)
		Pediatric patients, BAV + CoA (n=14)	1/14 (7.1%)	1/38 (2.6%)	N/A	z score >2	4/37 (11%)
Robledo-Carmona[68]	100	Adults, BAV + CMV (n=13), BAV + TAA (n=42)	15/100 (15%)	16/348 (4.6%)	2/13 (15.4%)	Indexed sinus diameter >2.1 cm/m^2 and tubular diameter >2 cm/m^2[28]	9/270 (3.3%)^
Hales[69]	181	Pediatric patients	N/A	21/207 (10.1%)	N/A		N/A

BAV, bicuspid aortic valve; TAA, thoracic aortic aneurysm; TAV, tricuspid aortic valve; CVM, cardiovascular malformation; N/A, not available; CI, confidence interval; BSA, body surface area; CoA, coartaction of the aorta. *Second degree relatives were included in some families, we only included families with 2 affected first degree relatives to calculate the % familial BAV. Several families were reported previously in Cripe *et al.*[41]; °5 first-degree relatives diagnosed with BAV were added to the BAV index group; #on the basis of family history aortic valve disease was present in a further four relatives (8%); ^only mild dilatation (<4 cm) not significantly different to control group.



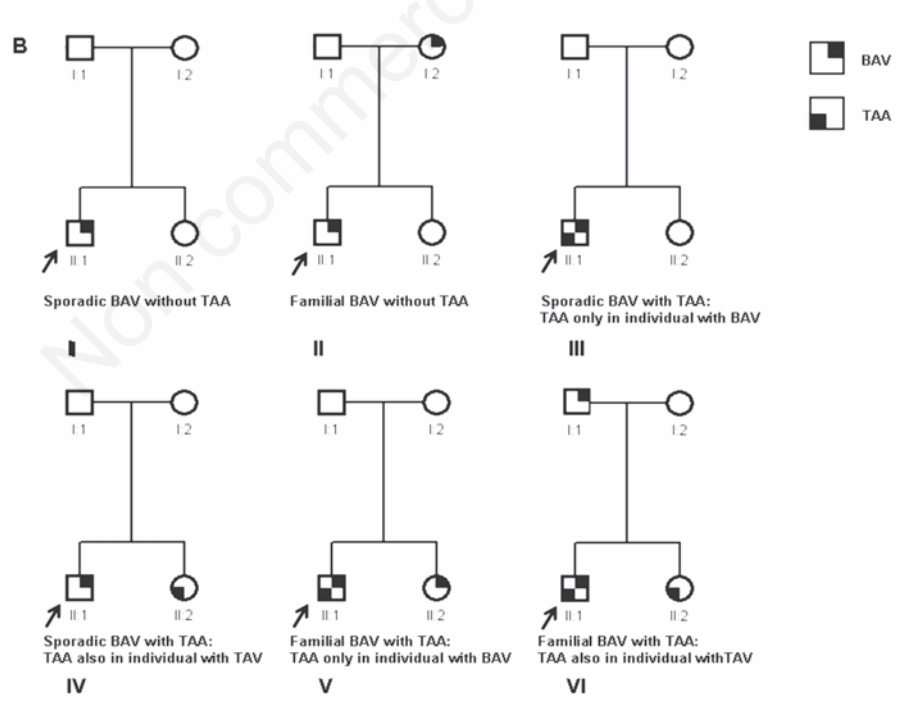


Figure 1. A) Flow chart illustrating a proposed model for genetic testing, and clinical work-up of first-degree relatives (FDR) of patients with bicuspid aortic valve (BAV). Clinical follow up of first-degree relatives comprises echocardiographic screening at a low frequency (*e.g.*, every five years) starting at the age of 18 years. In our center NGS TAAD analysis includes: *ACTA2, COL3A1, EFEMP2, ELN, FBN1, FBN2, MYH11, MYLK, PLOD1, SLC2A10, SMAD3, TGFBR1* and *TGFBR2*. B) Simplified pedigrees illustrating examples of the different familial situations in the flow chart. Men are encoded with squares, women with circles. Index patients are indicated with an arrow. Ao root involvement, aortic root involvement; NGS, next generation sequencing; TAA, thoracic aortic aneurysm; TAV, tricuspid aortic valve.

ing in first-degree relatives of BAV patients was underlined by the authors. One study addressed the cost-effectiveness of cardiological screening in siblings of children with BAV. It was concluded that screening is effective and inexpensive and should be incorporated into clinical care.[69] We feel confident that, using our model, the majority of high-risk families can be identified. In our opinion, repeated follow up in all first-degree relatives of all BAV patients poses an unjustifiable burden on health resources. Although based on a few studies only, DNA sequencing appears to be a promising tool in the identification of a subset of high-risk families. Aortic dissections at a young age are suggestive for a connective tissue disorder or FTAAD, whereas dissections in BAV patients usually occur later in life. The mean age at presentation (either detection of an aneurysm or after presentation with a complication) was lowest in syndrome associated and monogenic aneurysm patients at around 25-27 years *versus* 55-57 years in familial aneurysms and 64-66 years in the sporadic aneurysm group, and 55 years in the BAV group.[76] However, the ages at presentation within these groups were highly variable and largely overlapping, further complicating differentiating between sporadic BAV, familial BAV (+/-TAA), FTAAD and syndromal TAAD when faced with an individual patient without screening of relatives.[75-78] The reported location of aortic dilatation in the families with an identifiable pathogenic mutation in *FBN1*, *ACTA2* or *TGFBR2* included the aortic root and dissections were observed at young age. Mutations in these genes are associated with a high risk of aortic dilatation and dissection at young age, in most cases in patients with tricuspid aortic valves. Although evaluation in larger cohorts is required, DNA diagnostics appears to be a promising and valuable tool in identifying a minority of high-risk families presenting with an index patient with BAV (+/-TAA). The identification of a pathogenic mutation enables genetic testing of family members and selective clinical follow up of at risk relatives. Furthermore, DNA testing is becoming widely available at rapidly declining costs and is increasingly incorporated into standard clinical practice.[74,75] Novel techniques such as whole exome- and whole genome sequencing are likely to be valuable in the identification of novel genes in BAV and TAA. Therefore, it is likely that DNA testing will take a more prominent part in risk stratification in BAV (+/-TAA) patients and their families in the near future. In the Netherlands, genetic counseling and DNA testing in patients with suspected inherited cardiovascular disease is performed mainly by clinical geneticists and genetic counselors working in multidisciplinary teams also including pediatric cardiologists, cardiologists and social workers specialized in cardiogenetic disorders. These outpatient clinics provide well-equipped setting for the coordination of family screening, collecting of clinical data and, when indicated, performing dysmorphologic examination and DNA testing. BAV patients can be referred to these outpatient clinics when there is an indication for DNA testing and/or clinical follow-up of first-degree relatives on the basis of our flowchart or when a genetic syndrome is suspected. Other reasons for referral can for example be questions about the inheritance of BAV, including potential implications for family members and/or (future) offspring. Further insight into the genetic and pathophysiological mechanisms leading to BAV and/or TAA is required to enable the identification of novel factors causing health risks in BAV patients and their relatives. These include aortic medial degeneration, vascular smooth muscle cell apoptosis and the interplay between BAV morphology, sheer stress and valvular dysfunction and TAA in BAV patients,

Limitations of our model include the limited number of studies, different patient groups and the absence of DNA testing in the majority of publications. When using our proposed model it is important to consider all available clinical data of a family in its entirety. This may be difficult due to loss of follow up of the index patient (*e.g.* after moving or due to non compliance) and because of difficulties to obtain all relevant medical records of relatives (*e.g.* because not all first degree relatives are informed by the index patient and/or not all relatives are participating in cardiological screening). Long term follow up in well-characterized BAV cohorts is required to test the feasibility, sensitivity and specificity of our model which should be adjusted accordingly when necessary.

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