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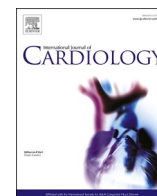
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Heritability of aortic valve stenosis and bicuspid enrichment in families with aortic valve stenosis

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ABSTRACT

Background: Although a familial component of calcific aortic valve stenosis (CAVS) has been described, its heritability remains unknown. Hence, we aim to assess the heritability of CAVS and the prevalence of bicuspid aortic valve among CAVS families.

Methods: Proband's were recruited following aortic valve replacement (AVR) for severe CAVS on either tricuspid (TAV) or bicuspid aortic valve (BAV). After screening, relatives underwent a Doppler-echocardiography to assess the aortic valve morphology as well as the presence and severity of CAVS. Families were classified in two types according to proband's aortic valve phenotype: TAV or BAV families. Control families were recruited and screened for the presence of BAV.

Results: Among the 2371 relatives from 138 CAVS families (pedigree cohort), heritability of CAVS was significant ($h^2 = 0.47$, $p < 0.0001$), in TAV ($h^2 = 0.49$, $p < 0.0001$) and BAV families ($h^2 = 0.50$, $p < 0.0001$). The prevalence of BAV in 790 relatives (phenotype cohort) was significantly increased in both TAV and BAV families compared to control families with a prevalence ratio of 2.6 ([95%CI:1.4–5.9]; $p = 0.005$) and 4.6 ([95% CI:2.4–13.4]; $p < 0.0001$), respectively. At least one relative had a BAV in 22.2% of tricuspid CAVS families.

Conclusions: Our study confirms the heritability of CAVS in both TAV and BAV families, suggesting a genetic background of this frequent valvular disease. In addition, BAV enrichment in TAV families suggests an interplay between tricuspid CAVS and BAV. Overall results support the need to improve phenotyping (i.e. BAV, TAV, risk factors) in CAVS families in order to enhance the identification of rare and causal genetic variants of CAVS.

Clinical Trials Identifier: NCT02890407.

1. Introduction

Calcific aortic valve stenosis (CAVS) is a progressive disease in which age is one of the most important risk factors [1]. Other factors associated

with the development and progression of CAVS are cardiovascular risk factors such as hypertension, dyslipidemia, diabetes, metabolic syndrome [2], as well as aortic valve anatomy and genetic markers [3]. Bicuspid aortic valve (BAV), the most common congenital abnormality

Abbreviations: AVR, Aortic valve replacement; BAV, Bicuspid aortic valve; CAVS, Calcific aortic valve stenosis; CI, Confidence Interval; EAE/ASE, European Association of Echocardiography and the American Society of Echocardiography guidelines; IQR, Inter Quartile Range; PR, Prevalence ratio; SOLAR, Sequential Oligogenic Linkage Analysis Routines; TAV, Tricuspid aortic valve; TTE, Trans-thoracic Doppler-echocardiography.

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with a prevalence estimated between 0.5 and 2% in the general population [4,5], accounts for 30 to 50% of patients referred for aortic valve replacement (AVR) for CAVS [6,7]. In addition, a genetic component was evoked as an important contributing factor involved in degenerative CAVS. Even though the heritability, i.e. the proportion of phenotypic variation explained by genetic variation, of CAVS remains unknown, few studies suggested the inheritance of CAVS [8,9]. Probst et al. identified five families with numerous relatives affected by tricuspid CAVS, demonstrating a familial aggregation of the disease [8]. In a large nationwide Swedish Register, a sibling history of CAVS was associated with increased risk of CAVS [9]. From a genome-wide scan of a 5-generation family, Garg et al. showed that *Notch1* might favor the development of CAVS in both tricuspid (TAV) and bicuspid aortic valve by impacting the development of the aortic cusps and the subsequent calcification process [10]. Indeed, *Notch1* encodes a single-pass transmembrane receptor and function, which plays critical roles in cell fate determination during organogenesis and normally represses the activity of RUNX2, a fundamental transcriptional regulator of osteoblast cell fate [10]. However, except in this family, identification of rare and causal genetic variants for this relatively frequent disease remains unsuccessful so far. By contrast, genome-wide association studies identified several common variants in patients with CAVS, the most powerful being the lipoprotein(a) [11–13].

The discrepancy between the high frequency of CAVS and the inability of identifying a strong genetic cause, the interplay with BAV abnormality and cardiovascular risk factors, are in agreement with the previously suggested complex pathophysiology [3,14]. Hence, in a large number of CAVS families, we sought to address the first estimate heritability of this frequent disease and the potential interplay between BAV and CAVS in TAV families. The aims of this multicenter study were i) to determine the heritability of CAVS in overall families and in TAV and BAV families, ii) to assess and compare the prevalence of BAV among relatives between TAV families, BAV families and controls.

2. Methods

2.1. Study population

Unrelated patients, without age limits, were recruited following AVR for severe and symptomatic (i.e. Class I indication) degenerative CAVS in 3 French university hospitals (Nantes, Angers, Rennes), from 2009 to 2017. After informed consent, these patients were considered as proband and a familial screening for CAVS was offered to all relatives. Those who agreed to participate were enrolled in the study and underwent clinical and echocardiographic assessment. Probands with history of rheumatic disease or chest radiotherapy or syndromic features were secondarily excluded, as well as those with undefined aortic valve phenotype (i.e. uncertain tricuspid or bicuspid aortic valve). A detailed familial pedigree was obtained for each proband (pedigree cohort) in order to estimate heritability.

A control group for the BAV prevalence study included already recruited probands diagnosed with non-syndromic isolated mitral valve prolapse, and their relatives. They were enrolled in the current study according to the same familial screening scheme than CAVS families [15].

This study was conformed to the ethical guidelines of the 1975 Declaration of Helsinki and conducted in accordance with French guidelines for clinical and genetic research. Local ethics committees, Comité de Protection des Personnes Ouest IV, approved the study and an informed written consent was obtained for each individual who agreed to participate.

2.2. Clinical data

Clinical data, recorded at the time of individuals' examination, included a medical history and physical examination as well as blood

sample analysis. Symptoms and cardiovascular risk factors such as obesity, hypertension, history of smoking, diabetes, dyslipidemia were recorded.

2.3. Doppler-echocardiographic data

All probands and relatives who agreed to participate to CAVS cohort and control cohort underwent a comprehensive trans-thoracic Doppler-echocardiography (TTE). All TTE acquisitions and interpretation in each lab were performed according to the European Association of Echocardiography and the American Society of Echocardiography guidelines (EAE/ASE) [16].

Tricuspid or bicuspid aortic valve morphology was determined in the parasternal short-axis view. The TAV was defined by the presence of three cusps without raphe. BAV was defined as the presence of two cusps and commissures with or without raphe. BAV were subdivided into 5 types: bicuspid without raphe, bicuspid with 1 raphe detailed as right and left (R-L) coronary cusps fusion, right coronary and non-coronary (R-N) cusps fusion, left coronary and non-coronary (L-N) cusps fusion and bicuspid with 2 raphe (Schematic diagram in Fig. 1S. in supplemental data) [17]. Aortic valve function included an evaluation of the presence and severity of CAVS, as well as aortic valve regurgitation. For the purpose of the study, aortic valves were stratified into 2 different types: normal aortic valve/sclerosis and moderate/severe stenosis defined by an aortic valve area $< 1.5 \text{ cm}^2$ and a mean aortic valve gradient $\geq 20 \text{ mmHg}$ with clear aortic cusps calcifications, thickening and reduced motion.

2.4. Definition of TAV or BAV families

Families were classified as TAV or BAV family according to the aortic valve phenotype of the proband. The morphology of the aortic valve of the proband, ascertained by different methods, allowed to classify the family as TAV or BAV family. For patients who underwent AVR, surgical and histopathological features were also used to confirm the aortic valve phenotype (i.e. TAV vs BAV).

2.5. Statistical analysis

Differences in descriptive characteristics (mean \pm SD for quantitative variables or n (%) for qualitative variables) between individuals from BAV families and TAV families were evaluated by univariable logistic and linear regression (for dichotomous and continuous variables). All regression analyses were performed using the generalized estimating equation method to account for family relations. The exchangeable correlation structure was used for these models. In order to confirm the accuracy of family classification as BAV or TAV, a random approach to class them as BAV or TAV families based on the choice of a random individual in each family was applied. The relative observed agreement between our family classification and the random approach was assessed by the accuracy correlation coefficient (the greater the absolute value of the accuracy correlation coefficient, the stronger is the relationship). The 95% CI of the accuracy correlation coefficient was determined in 1000 bootstraps sample.

Additive genetic heritability was estimated through variance decomposition using SOLAR (Sequential Oligogenic Linkage Analysis Routines) genetic statistics package. In this method, the distribution of the binary trait (affected/unaffected) was modelled as a latent continuous variable Y . The variance of the trait was estimated as a sum of environmental (σ_e^2) and genetic variance (σ_a^2), using twice the kinship coefficients matrix, inferred from familial links matrix (G). The latent phenotypic was the sum of additive genetic and environment component: $Y = A + E$ and its variance V is $[Gh^2 + I(1-h^2)]\sigma^2$, where A is the additive component, E the environment component, I is the identity matrix and the heritability $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$. The method compares the sporadic model ($h^2 = 0$) and the polygenic model ($h^2 = h_{\text{max}}^2$) and

compares log-likelihood under the two models [18,19]. The heritability estimate was done on the complete pedigree for each CAVS proband, including participants with missing data and phenotyped participants (based on clinical and echocardiographic data). For heritability of CAVS on TAV in TAV families, participants with BAV in TAV families were removed from the analysis.

Prevalence of relatives with BAV phenotype was calculated for each group: controls, TAV families and BAV families. The difference in BAV prevalence between relatives of TAV or BAV families versus controls was explored using univariable logistic regression analysis, adjusted with weight of family members. Prevalence ratio (PR) was calculated using the method described by Santos [20]. Further analyses were performed stratifying prevalence of relatives with BAV by proband’s age-class (<75 and ≥ 75 years). The 95% CI of the prevalence was determined in 1000 bootstraps sample.

All tests were 2-sided, and a $p \leq 0.05$ was considered as statistically significant. Statistical analyses were performed using the R statistical package, version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Patient’s characteristics

A flow diagram with details of the pedigree cohort used for the heritability analysis and the phenotype cohort used for the prevalence analysis is shown in Fig. 1.

3.1.1. Calcified aortic valve stenosis population

A familial screening with clinical and echocardiographic assessment was done for relatives of 187 probands. Among them 38 families were

excluded for a history of rheumatic fever or chest radiotherapy for the proband and 11 for uncertain proband aortic valve morphology (Fig. 1). The mean age of the 138 included probands was 70 years old (ranged from 48 to 87) and 89 (64.5%) were men.

For the heritability study, the 138 probands and all identified relatives (2371) were used to construct pedigrees (pedigree cohort) and estimate Kinship matrix (G/2). Among relatives, 917 (38.6%) individuals died before familial screening initiation and 662 (27.9%) were not available (refused to participate, unable to reach or did not attend appointments). Finally, 790 relatives of the 138 probands (phenotype cohort) were phenotyped and included in the prevalence study (Fig. 1). Of the phenotyped relatives, participants’ age ranged from 3 to 95 years (IQR: 53–73) and 49.9% were men. CAVS was identified in 165 relatives (20.8%), and moderate or severe aortic insufficiency in 5 (0.6%).

3.1.2. Control population

The control population for BAV prevalence included 67 probands and 361 relatives (n = 428). Mean age was 46 ± 19 years old (ranged from 3 to 90, IQR: 30–60), and 197 (46%) were men. All controls probands had normal TAV.

3.1.3. CAVS families ‘characteristics

Among the 138 probands, 90 probands had CAVS on tricuspid aortic valve thus defining 90 TAV families, with 5.9 (IQR: 2–6) relatives per families. In the same way, 48 had CAVS on bicuspid aortic valve thus defining 48 BAV families, with 5.3 (IQR: 2–6) relatives per family (Table 1). Pedigrees depicting BAV and TAV families out of the 138 families are shown in Fig. 2S in supplemental data. TAV families were older and presented higher prevalence of dyslipidaemia than BAV families, all other clinical factors or echocardiographic parameters were similar in both groups (Table 1).

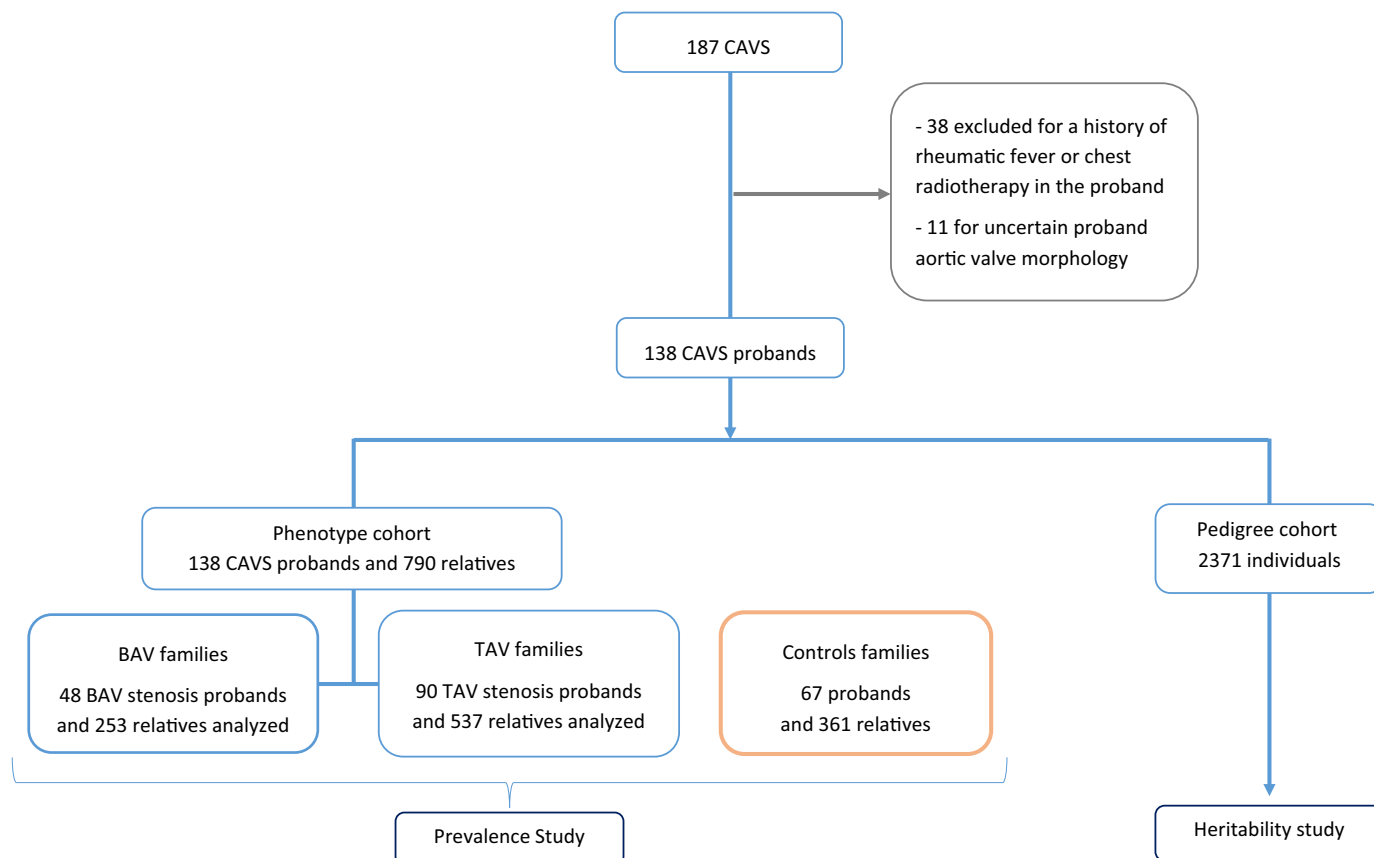


Fig. 1. Flow diagram.

Caption: Selection process of the studied families. BAV: Bicuspid aortic valve, CAVS: Calcific aortic valve stenosis, TAV: Tricuspid aortic valve.

Table 1
Baseline characteristics of the phenotyped population study according to family sub-groups.

	Overall	BAV families	TAV families	p-value
Families, n	138	48	90	
Individuals, n	928	301	627	
Relatives / family, median [IQR]	5.7 (2–6)	5.3 (2–6)	5.9 (2–6)	0.7
Age, years, mean [SD], years	64 ± 15	63 ± 13	64 ± 16	0.002
Male, n (%)	464 (50.0)	149 (49.2)	315 (50.2)	0.94
BSA, mean [SD], m ²	1.8 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	0.39
Hypertension, n (%)	360 (38.8)	113 (37.3)	247 (39.4)	0.08
Diabetes, n (%)	68 (7.3)	22 (7.3)	46 (7.3)	0.98
Dyslipidemia, n (%)	358 (38.6)	99 (32.7)	259 (41.3)	0.009
Obesity, n (%)	139 (14.9)	55 (18.1)	84 (13.4)	0.43
Smoker, n (%)	193 (20.8)	47 (15.5)	146 (23.2)	0.46
Number of CV risk factors, median [IQR]	1.3 (0–2)	1.2 (0–2)	1.3 (0–2)	0.10
Mean gradient, mean [SD], mmHg	6.4 (3.9–43.8)	5.8 (3.9–47.7)	6.6 (3.9–43.0)	0.71
Aortic valve area, mean [SD], cm ²	1.9 (0.8–2.5)	1.9 (0.7–2.5)	1.8 (0.8–2.5)	0.84
Peak aortic jet velocity, mean [SD], m/s	1.6 (1.3–3.8)	1.5 (1.2–4)	1.7 (1.3–3.6)	0.86
LVEF, mean [SD], %	62 ± 7	62 ± 7	62 ± 7	0.25
Valve function				
Non-severe dysfunction, n (%)	627 (67.6)	192 (63.8)	429 (68.4)	0.44
CAVS, n (%)	303 (32.6)	103 (34.2)	200 (31.9)	0.45
Tricuspid CAVS	222 (73.3)	41 (39.8)	181 (90.5)	
Bicuspid CAVS	81 (26.7)	62 (60.2)	19 (9.5)	
Aortic regurgitation ≥3, n (%)	8 (0.8)	6 (2.0)	2 (0.3)	0.94
Aortic valve surgery, n (%)	281 (30.2)	95 (31.6)	186 (29.6)	0.37

BAV: Bicuspid Aortic Valve, BSA: Body Surface Area, CAVS: moderate/severe Calcific Aortic Valve stenosis, LVEF: Left Ventricular Ejection Fraction, TAV: Tricuspid Aortic Valve.

The relative observed agreement between our family classification and the random approach was fair (74.6% [95%CI, 74.5%–74.7%]).

3.2. Calcified aortic valve stenosis heritability

A high heritability was found for CAVS in overall families regardless of the proband aortic valve morphology ($h^2 = 0.47$, $p < 0.001$). CAVS heritability was also significant in the 90 TAV families ($h^2 = 0.49$, $p < 0.001$) and in the 48 BAV families ($h^2 = 0.50$, $p < 0.001$). The heritability of CAVS on tricuspid aortic valve was also significant in TAV families ($h^2 = 0.21$, $p = 0.013$).

3.3. Bicuspid aortic valve individuals' phenotypes in each family subgroup

At the time of evaluation, in the BAV families, 26 relatives had BAV of whom 21 had CAVS (80.8%) and surgical aortic dilatation in 5 individuals (19.2%). BAV heritability was marginally significant in the 48 BAV families ($h^2 = 0.32$, $p = 0.05$). Complete BAV phenotypes are described in Table 2.

In the TAV families, 32 individuals had BAV (6.0% of 537 relatives) of whom 19 had CAVS (59.3%). Only one (3.1%) had surgical aortic dilatation.

In the control relatives, 9 (2.5% of 361 relatives) were identified as having a BAV, distributed in 7 families. The mean age of these 9 individuals was 45.4 ± 17.4 years old and none had CAVS or was referred to aortic valve surgery at the time of assessment.

3.4. Bicuspid aortic valve enrichment in families

Among the 48 BAV families, the 26 relatives with BAV (10.2%) were

distributed as follows: 29 probands had no relatives with BAV, 13 had one relative with BAV, 5 had two relatives with BAV and 1 had 3 relatives with BAV. Overall, 19 out of the 48 BAV probands (39.6%) had at least one relative with BAV. In 90 TAV families, 32 relatives with BAV (6.0%) were identified, 70 probands had no relatives with BAV, 15 had one relative with BAV, 3 had two relatives with BAV and 2 had 3 relatives with BAV. Overall, 20 out of 90 TAV families (22.2%) had at least one relative with a BAV compared to 7 out of the 67 control families (10.4%). In TAV families, the mean age of probands with relatives with BAV was 70 years-old compared to 74 years-old in probands without BAV relatives (p -value = 0.02).

As expected, the prevalence of BAV was significantly higher in BAV families vs. control families (10.2 [95%CI, 7.0%–14.5%], vs. 2.5 [95%CI, 1.3%–4.7%]; PR = 4.6 [95%CI, 2.4–13.4]; $p < 0.001$). The prevalence of BAV was also significantly higher in TAV families vs. control families (6.0 [95%CI, 4.2%–8.3%], vs. 2.5 [95%CI, 1.3%–4.7%]; Prevalence Ratio = 2.6 [95%CI, 1.4–5.9]; $p = 0.005$) (Fig. 2).

In addition, we performed the subgroup analyses stratified by age of the proband in TAV families. The prevalence of BAV was significantly higher in relatives of younger TAV probands (<75 years old) than in relatives of older TAV probands (≥ 75 y.o.), 6.82 [95%CI, 4.6%–10.0%], vs. 4.5 [95%CI, 2.3%–8.37%]) (Fig. 3.S in supplemental data).

4. Discussion

This large multicenter study confirmed the familial clustering of CAVS especially in patients with tricuspid CAVS and provided the first significant estimate of disease heritability reinforcing the current acceptance of a genetic background in this so-called degenerative disease. The accuracy of family classification (i.e. TAV or BAV) according to proband phenotype was statistically confirmed by a bootstrap method. Thanks to this cautious familial approach, the 2.6-fold higher prevalence of BAV in TAV families (6.0%) compared to control families (2.5%) suggests a complex and frequent interplay between BAV and CAVS.

4.1. CAVS heritability

Previous studies reported the familial clustering of CAVS. In 2006, a highly heterogeneous geographic distribution of patients referred for CAVS surgery was showed in the western part of France [8]. In addition, 5 families with many affected members were identified, demonstrating a clear familial aggregation of the disease. In 2017, Martinsson et al. showed that a CAVS history in sibling was associated with a familial increased risk of CAVS [9]. Spouses of patients with CAVS had a modest risk increase, suggesting that shared adult environmental factors contribute less to the development of CAVS than genetic factors [9]. Heritability describes how much of the variation in a phenotype can be attributed to genetic variation. Our results confirm the current acceptance of an inherited genetic background of CAVS, including for patients with TAV. Nevertheless, the moderate heritability in our population is concordant with the idea that CAVS on TAV is due to combination of genetic and potentially uncaptured environmental or diet factors [3]. Pathophysiological mechanisms explaining CAVS heritability are complex. Heritable components of aging as reduced telomere length have been associated with an increased incidence of CAVS with age [21]. In the same way, low-density lipoprotein, triglycerides and lipoprotein (a) [22] have a high heritability contributing in a direct or indirect mechanism to the observed heritability for CAVS. In addition to the well-known heritability of BAV, our study demonstrates the heritability of CAVS in both tricuspid and bicuspid aortic valve, and suggests that BAV could contribute to the heritability of CAVS in TAV families [23–26]. Finally, numerous genes, at least common variants, may contribute to heritability of CAVS. Indeed, genome-wide association studies identified multiple common variants associated with CAVS, the most powerful being the lipoprotein(a) [11–13]. By contrast, causal mutations have been rarely identified in CAVS. Using a positional cloning approach,

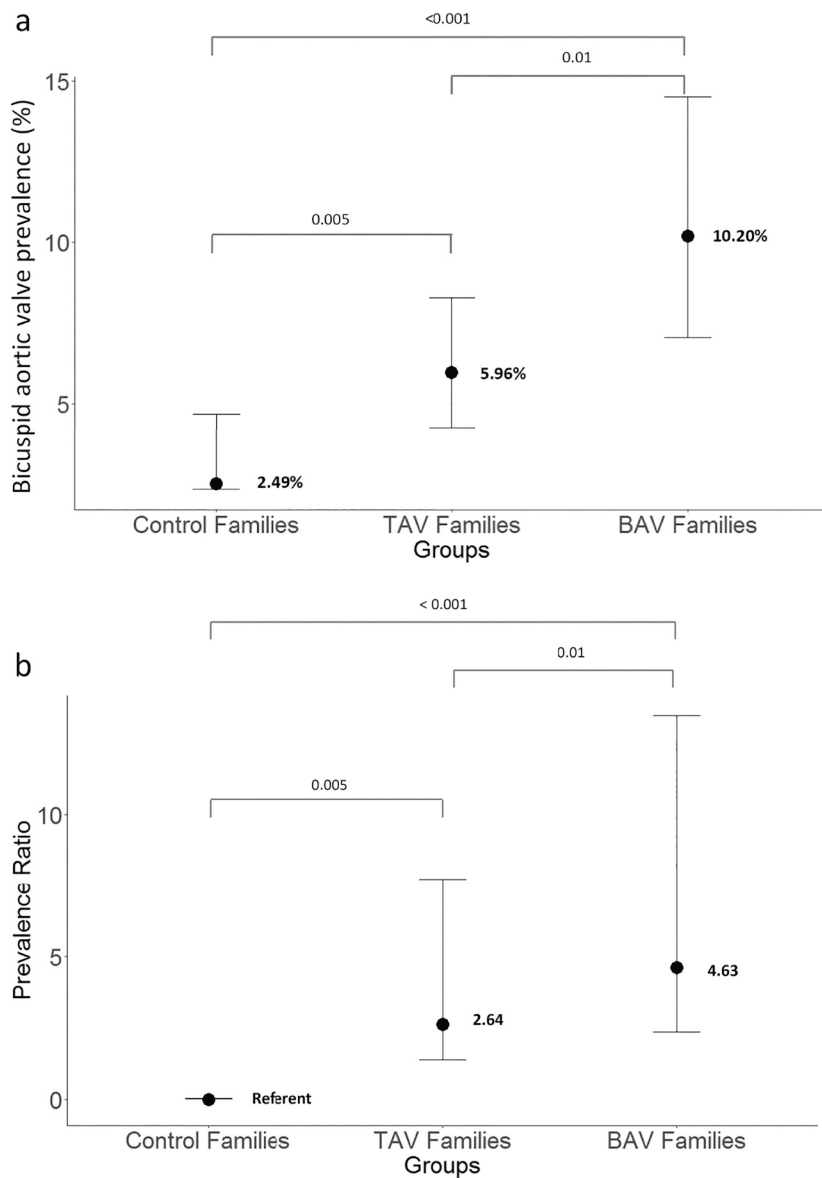


Fig. 2. Prevalence rates and ratio of bicuspid aortic valve in each family subgroups.
Caption: (A) Prevalence rates of BAV and 95% confidence intervals (CIs) and (B) prevalence ratio of BAV and 95% confidence intervals (CIs) in subgroups (i.e. controls, TAV families and BAV families). The prevalence of BAV was significantly higher in BAV vs. control families and the prevalence of BAV was also significantly higher in TAV vs. control families. BAV: bicuspid aortic valve, TAV: tricuspid aortic valve.

mutations in the signaling and transcriptional regulator NOTCH-1 found to be involved in the development of CAVS [10].

4.2. Bicuspid aortic valve familial clustering and BAV heritability

As in our study, previously published data reported a prevalence of BAV in first-degree relatives nearly 10-fold higher (4.6 to 11%) than in the general population (1–2%) [23,25,27–29]. BAV morphological characteristics has similar prevalence to those previously described, with the most frequent form consisting of one raphe positioned between the left and right coronary sinuses [17]. In our study, there is only a trend for BAV heritability, which can be explained by the overall small number of individuals with BAV in BAV families. A larger previous study estimated the BAV heritability, with or without other cardiovascular malformations, at 89% [23]. By contrast, Galian-Gay et al. reported a moderate heritability of BAV at 47%, and like in our study, only sporadic BAV without other congenital defects were included [25].

4.3. Bicuspid aortic valve enrichment in families: interplay between BAV and Tricuspid CAVS

In order to further explore the interplay between BAV, bicuspid and tricuspid CAVS, our results provide the evidence of BAV enrichment in CAVS families of TAV phenotype. Indeed, we can hypothesize that some common or rare genetic variants could favor the development of BAV and CAVS on aortic valve and therefore induce a possible CAVS onset at a younger age. Previous studies found common genetic variants for BAV, bicuspid CAVS and tricuspid CAVS. First, Garg et al. showed from a 5-generation family, that Notch1 mutation might favor the development of CAVS on both BAV and TAV phenotype [10]. Notch1 mutation acts during foetal development leading to BAV phenotype, but also after birth by impairing RUNX2 factor eliciting an abnormal degenerative calcification process of the aortic valve [30]. Second, Helgadottir et al. identified two CAVS loci in a large genome-wide association study (rs7543130 near PALMD, rs1830321 in TEX41) which were also associated with BAV [31]. The genetic basis of BAV has been extensively studied in recent years with mutations in the ACTA2 gene, SMAD6 variants, ROBO4, and other candidate genes [10,26,32–34]. Future studies analyzing the pathways’ deregulation induced by these gene

Table 2
Aortic valve phenotypes.

	Controls	BAV families	TAV families
Families	67	48	90
Individuals*	428	301	627
Total tricuspid aortic valve, n (%)	419 (97.9)	227 (75.4)	595 (94.8)
Total bicuspid aortic valve, n (%)	9 (2.1)	74 (24.4)	32 (5.1)
Relatives	361	253	537
BAV relative, n (%)	9 (2.5)	26 (10.2)	32 (6.0)
Bicuspid valve characteristics in Relatives			
BAV right and left cusps fusion, n (%)	6 (66.7)	18 (69.2)	25 (78.2)
BAV right and non-coronary cusps fusion, n (%)	0	3 (11.5)	2 (6.2)
BAV left and non-coronary cusps fusion, n (%)	3 (33.3)	1 (3.9)	5 (15.6)
BAV without raphe, n (%)	0	4 (15.4)	0
Aortic valve function in Relatives			
CAVS, n (%)		79 (31.2)	190 (35.4)
Tricuspid CAVS		58 (22.9)	167 (31.1)
Bicuspid CAVS		21 (8.3)	23 (4.3)
Aortic regurgitation ≥ 3 , n (%)		3 (1.2)	2 (0.4)
Aortic valve surgery, n (%)		51 (20.1)	101 (18.8)

BAV: Bicuspid Aortic Valve, TAV: Tricuspid Aortic Valve.

* Individuals = Proband and relatives.

variations may include analyzes on both BAV and CAVS individuals.

It is noteworthy that the familial recurrence of BAV is relatively low, around 10% in previous studies [23,27], in agreement with our findings. With respect to a mendelian or more complex genetic model, and as suggested by our study, we can hypothesize that some common or rare genetic variants could favor the development of CAVS and BAV phenotype within a same family.

4.4. Clinical and research implications

CAVS is not only a familial but also an inheritable condition. At least one relative had a BAV in around one fourth of tricuspid CAVS probands. These findings have research implications.

First, genetic research could focus in sub-groups of families with both TAV and BAV individuals in order to understand the reasons behind the interplay between BAV and CAVS with a focus on genes involved in the pathogenesis of BAV or in the pathogenesis of CAVS. CAVS is the result of an active inflammatory cellular process characterized by lipoprotein deposition and molecular mediators of inflammation, calcification and oxidative stress including its effects on mitochondrial dysfunction [14,35,36].

Second, our results support the need to improve phenotyping (i.e. BAV, TAV, risk factors) in CAVS families in order to enhance the identification of rare and causal genetic variants of CAVS. These results have also clinical implication, they support the need of familial screening for BAV and CAVS in relatives of CAVS patients, at least by questioning patients about familial history of CAVS, to allow identification of relatives at risk of developing aortic valve disease or BAV-related complications [6]. In the large nationwide Swedish Register, even though the family history of aortic stenosis was a risk factor for CAVS, the increase in risk was modest [9]. However, the approach between our study and the Swedish register is very different, as we performed a systematic echocardiographic screening of relatives and were therefore able to detect early, still uncomplicated, BAV or CAVS. Considering the larger number of patients with CAVS, further studies are needed to specify the target population for echocardiography screening based on age or number of siblings affected.

4.5. Strengths and study limitations

Our study has a several limitations. First, patients and relatives attended tertiary centers. Only 33% of possible candidates have undergone echocardiographic screening. This rate is similar to previous studies [25] and is explained by numerous deaths among relatives due to the old age of disease onset or geographic reasons. Second, even though the mean number of participants in each family was similar in BAV and TAV families, a detection bias cannot be excluded in the participants screening after BAV diagnosis in relatives. BAV vs. TAV phenotype definition may be difficult with the superimposed calcification occurring in CAVS. Proband was excluded if the aortic valve phenotype was uncertain. For relatives, surgical and histopathological features were used in addition to echocardiography to determine the morphology of the aortic valve. Recent data suggested the potential interest of CT-scan in exploring the aortic valve phenotype, but were published long after the start of this study, and after enrollment of all probands [24]. Although an underestimation of BAV is possible, this underestimation should be the same in each group, or more pronounced in CAVS, which should accentuate the difference with the control group. Further, this limitation does not affect probands characterization as aortic valve morphology was examined during surgery and by histology.

Third, family-based heritability analyses can overestimate heritability compared to SNP-based heritability due to shared environment effects. The amount of overestimation seems to be less substantial if extended families are utilized [37]. This was one of the explanation for choosing a fourth degree pedigree.

At last, the control group for BAV prevalence was constituted by already recruited probands diagnosed with mitral valve prolapse and their relatives. This control group was chosen because individuals were enrolled according to the same familial screening scheme than CAVS families and there is currently no known increase association between non-syndromic mitral valve prolapse and BAV or CAVS, this association being regarded as sporadic [38]. Indeed, in our study, the prevalence of BAV in relatives was in the expected range of a general population (95% CI, 1.3%–4.7%) [4,5]. However, no analysis on CAVS in this control group could be done due to the younger age of the included individuals.

5. Conclusion

Beyond a simple familial clustering of CAVS, the heritability of CAVS in this large family study confirms the genetic background of this disease, both in TAV and BAV families. The bicuspid enrichment in TAV families, identified in more than 20% of CAVS families, suggests a complex interplay and a common genetic predisposition between BAV and CAVS in TAV individuals. This emphasize the needs to improve phenotyping in CAVS families, adding age of CAVS onset and cardiovascular risk factor in addition to aortic valve morphology in order to enhance the identification of rare and causal genetic variants of CAVS in different sub-groups. Furthermore, genetic research could focus in family's sub-groups with both TAV and BAV individuals in order to understand the reasons behind the interplay between BAV and CAVS on TAV.

Taking all together, these findings would encourage echocardiography screening of relatives of CAVS patients for identifying bicuspid aortic valve disease or asymptomatic CAVS in old patients. Considering the larger number of patients with CAVS, further studies are needed to specify the target population for screening.

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Declarations

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by local Ethics Committees of Nantes University, Comité de Protection des Personnes (CPP) Ouest IV. (Date 2016, Num: 33/02).

Contributorship statement

Anne Sophie Boureau: wrote the paper.
 Matilde Karakatchoff: analysis tool.
 Solena Le Scouarnec: analysis tool.
 Romain Capoulade: analysis tool.
 Caroline Cuffe: collected the data.
 Laure de Decker: analysis tool.
 Thomas Senage: collected the data.
 Jean-Philippe Verhoye: collected the data.
 Christophe Baufreton: collected the data.
 Jean-Christian Roussel: collected the data.
 Christian Dina: analysis tool.
 Vincent Probst: collected the data.
 JJ Schott: conceived and designed the analysis, wrote the paper.
 Thierry Le Tourneau: conceived and designed the analysis, wrote the paper.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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All author take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijcard.2022.04.022>.

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