

Bicuspid Aortic Valve Is Heritable

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OBJECTIVES	Previous studies have established familial clustering of bicuspid aortic valve (BAV), presumably indicating genetic inheritance. Our objective was to statistically test whether the segregation pattern of BAV is consistent with genetic inheritance and to obtain an estimate of the size of the genetic effect (heritability).
BACKGROUND	Bicuspid aortic valve occurs in 1% of the population, making it the most common cardiovascular malformation (CVM). Bicuspid aortic valve is frequently an antecedent to aortic valve stenosis or insufficiency and is often associated with other CVMs, including aortic root dilation. The genetic and developmental significance of these findings remains obscure.
METHODS	In 50 probands with BAV, we obtained a three-generation family history and echocardiograms on first-degree relatives. Heritability (h^2) of BAV and BAV and/or other CVMs were estimated using maximum-likelihood-based variance decomposition extended to dichotomous traits implemented in the computer package Sequential Oligogenic Linkage Analysis Routines (SOLAR, San Antonio, Texas).
RESULTS	A total of 309 probands and relatives participated. Bicuspid aortic valve was identified in 74 individuals (prevalence = 24%). A total of 97 individuals had BAV and/or other CVM (prevalence = 31%), including aortic coarctation, ventricular or atrial septal defect, abnormal mitral valve, aortic root dilation, or hypoplastic left heart syndrome. The heritability (h^2) of BAV and BAV and/or other CVMs were 89% and 75%, respectively.
CONCLUSIONS	The high heritability of BAV suggests that in this study population BAV determination is almost entirely genetic. The heritability of BAV plus other cardiovascular anomalies suggests that valve malformation can be primary to defective valvulogenesis or secondary to other elements of cardiogenesis. (J Am Coll Cardiol 2004;44:138–43) © 2004 by the American College of Cardiology Foundation

Bicuspid (or bicommissural) aortic valve (BAV) describes an aortic valve with two rather than three leaflets. Based on autopsy and echocardiographic studies, a prevalence of 0.5% to 2.0% has been estimated, making BAV the most common cardiovascular malformation (CVM) in humans (1,2); based on these prevalence estimates, a figure of 1% is often cited (3–5). Bicuspid aortic valve is frequently found underlying aortic stenosis; in pediatric patients 70% to 85% of stenotic aortic valves are bicuspid (4), and at least 50% of adults with aortic stenosis have BAV (5). Bicuspid aortic valve is also recognized in association with other CVMs, including coarctation of the aorta (50% to 80%), interruption of the aorta (36%), and isolated ventricular septal defect (20%) (4,6), but BAV occurs infrequently with other forms of cardiovascular (CV) disease in the young. For example, BAV occurrence in individuals with D-transposition of the great vessels is no different from that of the general population (6). Aortic root dilation is common in BAV even when the valve is hemodynamically normal (7,8). Both the genetic significance and the developmental significance of the diverse phenotypes associated with BAV remain obscure.

Based on pedigree analysis of familial clustering, a genetic cause of BAV has been suspected. Most such reports describe findings in a single kindred (9–15), which is a limitation because it cannot be known to what extent clustering in a single family represents BAV inheritance in general. Two previous reports investigated a number of families and provided results on families with single as well as several affected individuals (11,14); in both studies elevated BAV prevalence in family members compared with the general population was interpreted as indicating genetic inheritance. Although these studies do not dismiss the possibility of genetic inheritance of BAV, neither study statistically tested for genetic inheritance, which would require demonstrating that the segregation of BAV is consistent with the segregation of genes.

To further clarify the genetic characteristics of the BAV phenotype, the present study employed echocardiography to evaluate 50 BAV probands and their relatives. Our objective was to statistically test whether the segregation pattern of BAV is consistent with genetic inheritance and to obtain an estimate of the size of the genetic effect (heritability). We estimated the heritability (h^2) of BAV using maximum-likelihood-based variance decomposition extended to dichotomous traits implemented in the computer package Sequential Oligogenic Linkage Analysis Routines (SOLAR, San Antonio, Texas). The advantage of this approach is that it does not rely on specification of an inheritance model.

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Abbreviations and Acronyms

BAV	= bicuspid aortic valve
CoA	= coarctation of the aorta
CV	= cardiovascular
CVM	= cardiovascular malformation
h ²	= heritability
SOLAR	= Sequential Oligogenic Linkage Analysis Routines

METHODS

Proband recruitment and sequential sampling. To generate a cohort enriched for BAV and/or other CVMs, probands with BAV were recruited from the Division of Cardiology at Cincinnati Children’s Hospital. Families were informed of the study by their child’s cardiologist, and families expressing interest in the study were contacted by phone or during outpatient visits to request participation. A detailed family history (minimum of three generations) was obtained for each proband. Informed consent and a complete medical history were obtained from all participants. Each proband’s first-degree relatives willing to participate were enrolled into the study by the following method: for every new affected individual identified, all of that individual’s first-degree relatives were subsequently evaluated (i.e., sequential sampling). When family history identified affected second-degree relatives, sampling was extended to include their first-degree relatives. A single experienced echocardiographer (L.C.) interpreted all echocardiograms. The study was approved by the Institutional Review Board of Cincinnati Children’s Hospital.

Echocardiographic analysis. Standardized, complete two-dimensional and Doppler transthoracic echocardiograms were obtained on all participants through commercially available systems (Hewlett-Packard [Andover, Massachusetts] Sonos 5500, GE Vivid 7 or a GE [Waukesha, Wisconsin] Vivid 5) according to the protocol shown in Table 1. Aortic valve morphology was examined in both systole and diastole in the parasternal short-axis view. In all subjects, BAV was classified as either “right-left” or “anterior-posterior.” Valve leaflet fusion sites were assigned according to a standardized decision tree that identified two characteristic morphologies in patients with an anatomic or functionally bicuspid valve (Fig. 1). Supportive features of BAV included cusp redundancy, valve thickening, and eccentric valve leaflet closure. All additional anatomic or hemodynamic abnormalities were recorded in probands and relatives.

Proband inclusion and exclusion criteria. Probands were identified when the aortic valve appeared bicommissural in the parasternal short-axis view on echocardiogram. Potential probands with known cytogenetic abnormalities (e.g., Down syndrome), diagnosed Mendelian syndromes (e.g., Marfan syndrome), or “complex” cardiac anatomy (e.g., single ventricle) were excluded.

Table 1. Echocardiographic Protocol

Aortic valve and root evaluation
Two-dimensional measurements (end-diastole, parasternal long axis)
Aortic annulus
Sinuses of Valsalva
Supra-aortic ridge
Proximal ascending aorta
Morphology of the aortic valve
Valve thickened (yes, no)
Location of commissural fusion
Other details
Mitral and tricuspid valve morphology
Papillary muscle architecture
Prolapse
Myxomatous changes
Insufficiency
Stenosis
Pulmonary valve evaluation
Two-dimensional measurements (end-diastole, parasternal short axis)
Pulmonary annulus
Diameter of proximal pulmonary artery
Morphology of the pulmonary valve
Aortic arch evaluation
Two-dimensional measurements (suprasternal notch view)
Evaluation of the brachiocephalic vessels
Diameter of the transverse arch
Diameter of the descending aorta
General cardiac anatomy
Chamber dimensions (M-mode measurements)
Other malformations

Statistical analysis: heritability of BAV. Although previous research suggests that some families exhibit autosomal dominant inheritance of BAV, our focus was to determine the extent to which genes influence the development of BAV in all families, not just the multiplex. Therefore, we opted to utilize variance component methodology. This methodology was first applied to plant and animal studies of complex traits, but in the past 10 to 15 years it has become an effective way to estimate genetic effects in complex human disease. In short, the phenotypic variance (σ_p^2) is partitioned into components corresponding to the additive

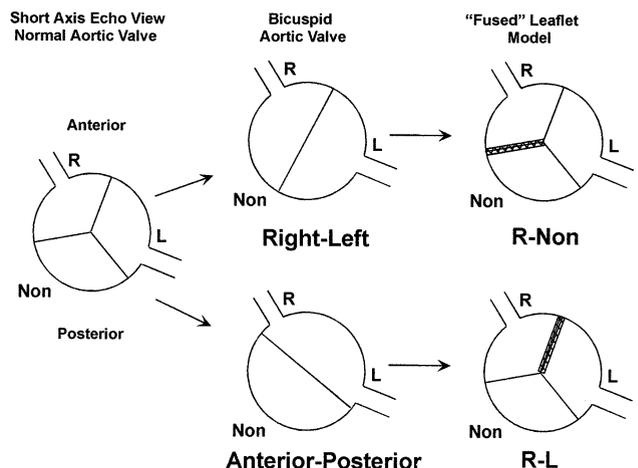


Figure 1. Schematic depiction of echocardiographic two-dimensional cross-sections of the aortic valve in the parasternal short axis used for morphologic assessment and nomenclature. L = left; R = right.

genetic (σ_G^2) and residual (i.e., environmental) (σ_E^2) effects. Because these components are additive, such that

$$\sigma_P^2 = \sigma_G^2 + \sigma_E^2$$

h^2 is estimated as $h^2 = \sigma_G^2/\sigma_P^2$. The proportion of the phenotypic variance attributable to nongenetic factors (e) is estimated as $e^2 = 1 - h^2$. To estimate the genetic and environmental variances, we used maximum-likelihood-based variance decomposition implemented in the computer package SOLAR, which allows us to simultaneously consider all possible relative pairs (16).

The ascertainment strategy of this project created a sample that, when compared with the general population, was enriched for BAV. Although for relatively rare conditions such as BAV, ascertainment is necessary to identify sufficient numbers of affected individuals, the increased prevalence can impair the ability to detect genes. Therefore, in all models analyzing BAV or BAV/other CVMs, the appropriate population prevalence is constrained in the model. We used a population prevalence of 1% for BAV and 2% for BAV/other; the latter choice is additive for BAV prevalence plus an estimate of 1% for CVMs exclusive of BAV (17). Although it is recognized that males have a higher prevalence of BAV than females, good estimates of gender-specific prevalence are not available; therefore, we constrained the prevalence of the sample so that it was equal to the prevalence in the general population to correct for ascertainment bias. Simulation analyses have demonstrated the effectiveness of this approach (J. Blangero, unpublished data, 2003).

Significance of the heritability estimates was assessed by likelihood ratio tests (18). The maximum likelihood for the general model in which all parameters were estimated are compared to those for restricted models in which the value of the parameter to be tested will be held constant at some value (usually zero). Twice the difference in the \ln likelihoods of the two models to be compared is distributed asymptotically, approximately as a 1/2:1/2 mixture of chi-square and a point mass at zero (19). Degrees of freedom can be obtained as the difference in the number of estimated parameters in the two models (19). Values of $p \leq 0.05$ were considered to be significant evidence for additive genetic effects.

RESULTS

Proband recruitment and sequential sampling. Among 64 potential recruits, 50 probands and their families were recruited (79%) in a 13-month period from August 2001 to September 2002. Two potential probands were excluded for medical reasons, eight families self-excluded, and four other families repeatedly missed appointments for the evaluation of family members. Among the 50 probands, 49 were white and 1 was Asian; there were 33 males and 17 females. Sequential sampling of first-degree relatives led to enrollment of 259 subjects; 14 first-degree relatives elected not to

participate. Sampling was extended to second-degree relatives when medical history or study echocardiography identified additional affected individuals. A total of 309 family members participated. Participants ranged in age from 1 year to 78 years (150 males, 159 females, with a gender ratio male:female of 0.94:1) (Fig. 2).

Phenotype characterization. Exemplary echocardiographic findings of aortic valve morphology in the short parasternal axis are shown in Figure 3. Among the 50 probands, BAV was an isolated finding (38 probands) or associated with coarctation of the aorta (7 probands), atrial septal defect (2 probands), ventricular septal defect (2 probands), or mild subaortic stenosis (1 proband). Among 309 participants (including the 50 probands), BAV was identified in 74 individuals (44 males, 30 females) (prevalence = 24%, 29% for males and 19% for females). Among 259 participants (excluding probands), 24 (9.3%) had BAV, a finding similar to that reported by Huntington et al. (14). In 16 of 50 kindreds (32%), two or more individuals were affected with BAV, a result nearly identical to that previously reported by Huntington et al. (14). Bicuspid aortic valve was an isolated finding in 52 of 74 individuals. Among 74 individuals with BAV the leaflet pattern was anterior-posterior (44 cases, 59%), right-left (21 cases, 28%) and indeterminate (9 cases, 12%). The designation “indeterminate” was utilized when a BAV diagnosis was determined from echocardiographic, angiographic, or autopsy reports with insufficient information to classify as anterior-posterior or right-left pattern.

We classified affected individuals as having isolated BAV, having BAV with other CVMs, or having other CVMs; 97 affected individuals (59 males and 38 females) were identified (prevalence = 31%, 39% for males, 24% for females) (Fig. 4). Affected status was determined from echocardiographic studies performed at our institution (85 subjects) or review of archived echocardiographic, operative, angiographic, or autopsy reports (12 subjects). In 23 of 50 kindreds (46%), two or more individuals were affected with a CVM (with or without BAV) including mitral valve abnormality (10 individuals), coarctation of the aorta (11 individuals), ventricular septal defect (perimembranous or muscular, 9 individuals), subaortic stenosis (4 individuals), and ventricular septal defect with mitral valve abnormality (perimembranous, 2 individuals). One individual each had hypoplastic left heart syndrome, secundum atrial septal defect, pulmonary valve stenosis, patent ductus arteriosus, D-transposition of the great arteries, or isolated persistent left superior vena cava. Persistent left superior vena cava has been identified in 0.3% to 0.5% of the population (20). Relatively few individuals had aortic root dilation (8 individuals); a single individual had undergone aortic reconstructive surgery for aortic root dilation. Valve surgery had been performed in 14 individuals (age range 12 to 74 years) including aortic valve replacement (9 individuals), aortic valvulotomy (2 individuals), mitral valve replacement (2 individuals), and mitral valve reconstruction (1 individual).

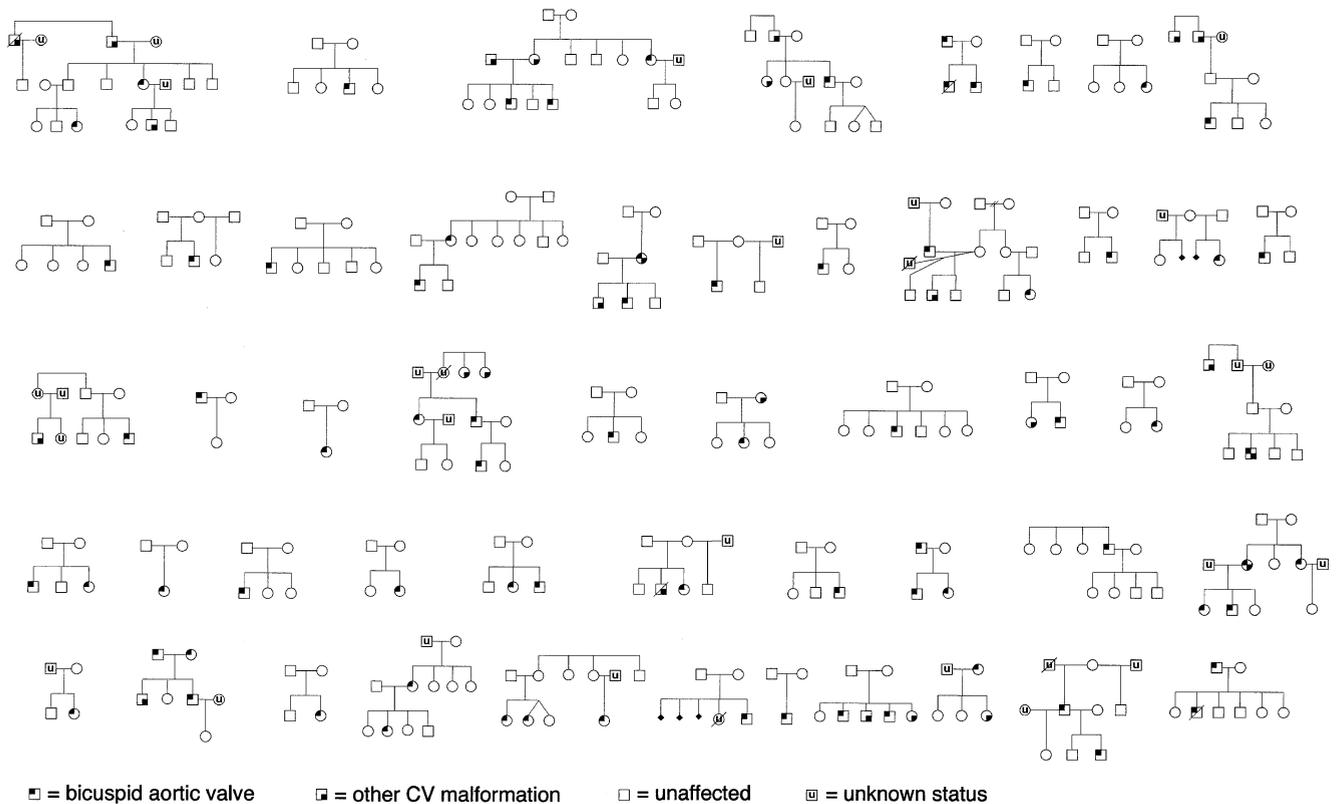


Figure 2. Pedigrees depicting 50 kindreds used in analysis. **Squares** = males; **circles** = females. **Darkened left upper quadrant** = bicuspid aortic valve; **darkened right lower quadrant** = other cardiovascular (CV) malformation.

Heritability. The sequential sampling ascertainment permitted collection of information about extended families which included information on more than 800 relative pairs distributed nearly equally between first-degree relatives and

higher order relatives (e.g., grandparents-grandchild, avuncular, and so forth) (Table 2).

We used maximum likelihood based variance decomposition extended to dichotomous traits implemented in the computer package SOLAR to estimate heritability. Given that this was a highly ascertained sample enriched for BAV, we

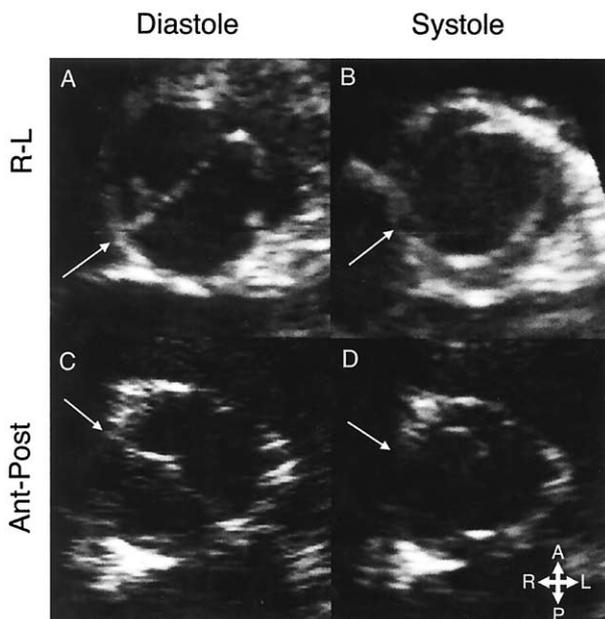


Figure 3. Echocardiographic images of bicuspid aortic valve. (A) R-Non, diastole; (B) R-Non, systole; (C) R-L, diastole; (D) R-L, systole. **Arrows** = commissure location and orientation. **Orientation arrows in D** correspond to echocardiographic cross-sections for all panels. L = left; R = right.

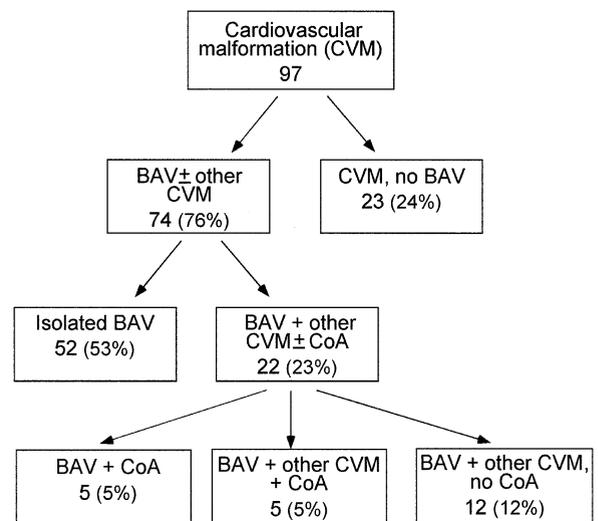


Figure 4. Flow chart illustrating phenotype classification numbers of 97 subjects with bicuspid aortic valve and/or cardiovascular malformation in study sample. Percents are relative to the total of 97 individuals with cardiovascular malformation. BAV = bicuspid aortic valve; CoA = coarctation of the aorta, CVM = cardiovascular malformation.

Table 2. Distribution of Relative Pairs Entering the Analysis

Relationship	Number
Parent-offspring	305
Siblings	177
Grandparent-grandchild	84
Avuncular	134
Half-siblings	24
Grand-avuncular	19
Half-avuncular	12
1st cousins	48
1st cousins, once removed	6
Total	809

constrained the prevalence of the sample to be equal to the prevalence in the general population to correct for ascertainment bias. Using this approach, we found that both BAV and BAV and/or other CVMs were strongly determined by additive genetic effects with heritability estimates of 0.89 ± 0.06 ($p < 0.000001$) and 0.75 ± 0.0002 ($p < 0.000001$) for BAV and BAV and/or other CVMs respectively. To confirm the robustness of this estimate, we also estimated the heritability when the population prevalence was constrained to 0.5 and 2 ($h^2 = 1.0 \pm 0.06$ and 0.40 ± 0.23 , respectively). These estimates of heritability are highly significant ($p < 0.0005$).

DISCUSSION

Results of the present study confirm previous reports of familial clustering of BAV (11,14) but also provide the first estimate of the genetic effect size (heritability). Using a maximum-likelihood-based variance decomposition method, and after correcting for ascertainment bias, we estimated the heritability of BAV to be 0.89, suggesting that in this population, determination of BAV is almost entirely genetic. In addition to familial cases of BAV, we also identified family members with other CVMs, and after correcting for ascertainment bias, we estimated the heritability of BAV and/or other CVMs to be 0.75. Although on the basis of previous reports we expected to find hypoplastic left heart syndrome more frequently (21), the finding of BAV in association with other CVMs is not unexpected and suggests that valve malformation can be primary to defective valvulogenesis or secondary to other elements of CV system development. Previous studies have estimated strong heritabilities for various CVMs, including aortic coarctation (0.58) (22) and congenital heart disease (0.50 to 0.95, mean 0.65) (23), although no study has estimated the heritability of the phenotype of BAV and/or other CVMs.

Although previous studies have suggested an autosomal dominant inheritance pattern of BAV (10,15,24), genetic heterogeneity has been a common finding in genetic studies of CV disease in the young (25). Therefore, it is likely that mutations in diverse genes with dissimilar inheritance patterns are responsible for the development of BAV in different families. Given this complexity, we chose to analyze the data using variance components analysis, which

does not incorporate a specified model of inheritance. Furthermore, the variance components package we utilized permitted us to analyze extended family data; this is extremely important because studies limited to sibling analyses are confounded by common family environment. Although cardiogenesis commences early in fetal development, common family environment still can affect similarity between siblings, as full siblings develop in a common uterus, albeit at different times. The inclusion of second-degree relatives permits us to tease apart the genetic variance from the common family environment, thus obtaining a better estimate of heritability.

Bicuspid aortic valve is often considered a benign lesion early in life, but complications of BAV, including aortic stenosis, aortic regurgitation, infective endocarditis, and aortic dilation and dissection, result in considerable morbidity and mortality later in life (5,26–29). Because many of these BAV-related complications can be predicted or prevented, the identification of BAV heritability supports the previous recommendation (14) that echocardiographic screening of first-degree relatives of patients with BAV is warranted in order to identify persons with structural cardiac abnormalities.

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