Discordant Aortic Valve Morphology in Monozygotic Twins
A Clinical Case Series

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**IMPORTANCE** Bicuspid aortic valve (BAV) is considered an autosomal dominant condition, which is commonly associated with thoracic aortic aneurysm. Both conditions pose the risk of valvular and aortic complications not only for affected patients but also for genetically related persons as well. The genetic underpinnings of these disease processes, which are in various stages of elucidation, have implications for screening and risk prognostication.

**OBJECTIVE** To analyze genetic differences between 2 pairs of monozygotic twins that had discordant aortic valve morphology, with 1 twin in each pair having a BAV and the other having a trileaflet aortic valve.

**DESIGN, SETTING, AND PARTICIPANTS** Two pairs of twins that were objectively determined to be monozygotic were examined at a tertiary care medical center associated with an academic medical center. Aortic valves that were surgically excised for clinical indications were examined for morphology. Whole-exome sequencing was performed for the twin pair that had discordance of aortic valve and aortic aneurysm. In the second pair, targeted gene sequencing of 25 genes known to be associated with BAV and/or thoracic aortic aneurysm was performed. In each pair, the twin with a BAV underwent surgical aortic valve replacement for clinical indications.

**MAIN OUTCOMES AND MEASURES** Genetic coding variations between monozygotic twins using whole-exome sequencing and targeted gene sequencing.

**RESULTS** This case series included 2 pairs of male monozygotic twins; one pair was aged 51 years and the other aged 59 years. Genetic sequencing methods identified no pathogenic sequence changes between the twins in each pair.

**CONCLUSIONS AND RELEVANCE** Our findings challenge the traditional view of BAV as a condition with an entirely autosomal dominant inheritance pattern and emphasize the variability of penetrance of both BAV and thoracic aortic aneurysm as well as the variability of the association of the 2 conditions. Continued work to elucidate the genetic basis may lead to the refinement of risk stratification for affected patients and relatives.

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The most common congenital cardiac valve abnormality, bicuspid aortic valve (BAV), is associated with valvular and aortic complications requiring intervention at a younger age than the general population. There appears to be a heritable pattern, and guidelines recommend screening first-degree relatives for a BAV and thoracic aortic aneurysm (TAA). However, the genetics of BAV and TAA are incompletely defined. We report 2 pairs of monozygotic twins with discordant aortic valve morphology, with one pair also having discordant aortic pathology. A waiver of review was granted by the Saint Louis University Institutional Review Board. Written consent was granted by the individuals presented in this case series.

Report of Cases

Case 1
A man in his 50s presented with hemianopsia of the left eye. Brain imaging demonstrated multiple small bilateral lesions consistent with embolic infarcts. Further workup included blood cultures, which grew *Streptococcus sanguinis*, and transesophageal echocardiogram demonstrating a calcified BAV with a leaflet perforation, small perivalvular abscess, and a fistula extending from the right sinus of Valsalva to the right atrium (Figure 1A). Computed tomography of the chest demonstrated aneurysms of the aortic root (5.5-cm diameter) and ascending aorta (5-cm diameter) (Figure 2). At operation, the valve was confirmed to be bicuspid, with fusion of the left and right cusps. The fistula arose from the left ventricular outflow tract rather than the right atrium, as suggested on the preoperative echocardiogram. The patient underwent autologous pericardial patch repair of the fistula and replacement of the aortic valve, aortic root, and ascending aorta (Figure 2).

Key Points

**Question** Can genetic testing in monozygotic twins with discordant aortic valve morphology (ie, bicuspid and tricuspid aortic valves) contribute to our knowledge of the genetic mechanism of bicuspid aortic valve?

**Findings** Whole-exome sequencing from one pair of monozygotic twins and targeted gene testing of a second pair of monozygotic twins identified no genetic alterations.

**Meaning** In addition to gene inheritance, epigenetic effects, somatic mutations, and environmental factors may play a more important role than previously suspected, which may limit the usefulness of genetic screening in risk stratification.
Family history included 3 relatives who had undergone aortic aneurysm repair and with no known history of BAVs. Trans-thoracic echocardiogram of his asymptomatic twin brother (Figure IB) demonstrated a noncalcified trileaflet aortic valve and mild dilation of the aortic root (4.3-cm diameter) and ascending aorta (4.2-cm diameter). Because the twins were thought to be identical but their aortic valves clearly had different structures, zygosity was questioned. Genetic testing with polymerase chain reaction followed by capillary electrophoresis of 15 autosomal short tandem repeat markers and 1 sex marker showed greater than 99% chance of monozygosity (ARUP Laboratories). Whole-exome sequencing was performed. No pathogenic sequence changes were identified in either twin.

Case 2
A man in his 50s underwent bioprosthetic aortic valve replacement for symptomatic severe aortic stenosis with normal ascending aortic dimension (2.5 cm) (Figure IC). At operation, he was found to have a BAV with heavily calcified, dystrophic leaflets and fusion of the left and right cusps. Family history was unremarkable for BAV or aortopathy. Four months previously, his twin brother had undergone trans-thoracic echocardiography for a syncopal episode. This demonstrated a trileaflet aortic valve without calcification or stenosis (Figure ID). Again, zygosity was queried with genetic testing, which showed greater than 99% chance of monozygosity (ARUP Laboratories). Targeted exon regions from 25 genes that have been implicated in BAV and/or thoracic aortopathy (ACTA2, CBS, COL3A1, COL5A1, COL5A2, FBN1, FBN2, GATA5, GATA6, HOXA1, KCNJ2, MAT2A, MEDI2, MYH11, MYLK, NOTCH1, PRKGI, SKI, SLC2A10, SMAD3, SMAD4, SMAD6, TGFBR2, TGFBR1, and TGFBR2) were sequenced, mapped, and analyzed. The DNA sample from the twin with the bicuspid valve was compared with the hg 19 reference sequence (GeneDx), a human genome build published by the University of California, Santa Cruz. No pathogenic sequence alterations were found.

Discussion
Bicuspid aortic valve is the most common congenital heart anomaly, with a prevalence of 0.5% to 2% in the population, based on autopsy and echocardiographic studies. Prospectively studied first-degree relatives showed an incidence of 9.1%, and authors have suggested an autosomal dominant pattern with reduced penetrance.2 Other studies using statistical modeling techniques have shown a high heritability, also consistent with an almost entirely genetic determination of BAV. Bicuspid aortic valve is also a well-known finding in females with the chromosomal condition Turner syndrome. In a small number of nonsyndromic, autosomal dominant human pedigrees, NOTCH1 mutations have been implicated. In mouse models, targeted deletion of Gata5 leads to partially penetrant BAV with fusion of the right and non-coronary leaflets.3 Recently, nonsynonymous variation within GATA5 transcriptional activation domains were reported in 4 sporadic cases of patients with a BAV4 but not confirmed in another study of familial BAV.5 Animal models have identified other potential gene candidates yet to be confirmed in human genetic studies, including eNOS (nos3), Nkx2-5, and Hoxa1.3 The association between BAV and TAA continues to be debated. Eleven of 12 genes identified in syndromic and nonsyndromic thoracic aortic disease are transmitted in an autosomal dominant pattern.6 Plasma and aortic tissue specimens of patients undergoing aortic aneurysm resection have shown differential profiles of matrix metalloproteinases, tissue inhibitors, and microRNA expression levels in patients with a BAV vs trileaflet aortic valves.7,8 Although these studies downplay, if not outright negate, the contribution of hemodynamic factors, recent cardiovascular magnetic resonance studies have identified abnormal flow patterns in the proximal aorta in patients with a BAV that are associated with increases in aortic wall shear stress and aortic dilation that differ according to the pattern of cusp fusion.9 It is noteworthy that the twin with the trileaflet aortic valve in case 1 had aortic ectasia (4.3 cm). Loscalzo et al10 prospectively evaluated family members of 13 individuals with both BAVs and TAs, finding that all families had at least 1 member with TAA alone, 35% had a BAV and TAA or TAA, and 85% had some form of aortic dilation above the sinotubular junction. The authors concluded that BAV and TAA were independent manifestations of a single gene defect.10 Others suggest distinct genetic etiologies for BAV and TAA.11 There is growing evidence that genes typically associated with either BAV, TAA, or syndromic conditions may be implicated in nonsyndromic and/or a concomitant BAV and TAA phenotype (Table). For example, variants in FBN1, which is associated with Marfan syndrome, have been reported in patients with sporadic TAA and thoracic aortic dissection without a BAV13,14 as well as in patients with both a BAV and TAA15.16 Other studies investigated TGFBR2, another gene associated with syndromic (Loeys-Dietz syndrome) and nonsyndromic (familial) TAA and dissection, was described in 2 first-degree relatives without features of Loeys-Dietz syndrome who underwent surgery for BAV.
and TAA.20 In a large cohort of patients with TGFB2 mutations, of which only 10% had Marfan syndrome, all 7% of those with a BAV also had TAA and moderate skeletal features. Mutations in the 2 genes with known associations with BAV in humans have been described in concomitant BAV and TAA.21 Four NOTCH1 variants were identified in 10.4% of patients with a BAV and TAA compared with 2.1% of control patients, and 3 of 4 individuals with GATA5 mutations with BAV had some degree of aortic dilation, with 2 presenting concurrently and 1 undergoing surgery 30 years after aortic valve replacement.22

Despite advances in our contemporary understanding of BAV and its heterogeneous presentations, major gaps in both basic and clinical research persist, and the prospect of registry data holds the greatest promise as called for by the International Bicuspid Aortic Valve Consortium.23 A link between congenital heart defects and twin gestation has been postulated. Among 41525 twins in the Danish Registry of Twins, the incidence of congenital heart disease was 63% higher compared with singletons.24 The first reported case of BAV in twins in 198725 followed an earlier report of 6 patients with congenital aortic stenosis whose twin sibling had normal aortic valves,26 but none of these twin pairs were monzygotic (ie, identical twins). Zafar and Roberts27 reported brothers with BAVs, one with coarctation and a normal aortic valve and the other with no coarctation but severe aortic stenosis.

While reports of monzygotic twins with the same BAV phenotype have been published,28,29,30 we are aware of only 1 report of monzygotic twins with discordant valve morphology,30 in which identical (and presumed monzygotic) twin brothers each presented at age 62 years with symptomatic aortic stenosis. At operation, one had a calcified BAV and the other a calcified trileaflet valve. However, valve morphology was based on retrospective review of the surgeons' operative reports, with no objective proof of monzygosity.

Our report objectively documents discordant valve morphology in monzygotic twins in whom neither calcification nor infective endocarditis had progressed to the point of obscuring the native valve morphology. Further, the trileaflet valves had not calcified to the point that congenital commissural fusion could not be differentiated from advanced calcification of a commissure on echocardiogram. In the first case, mild aortic dilation may be a phenotypic variation of the BAV and TAA syndrome. Phenotypic differences in monzygotic twins may be due to epigenetic effects, somatic mutations, and environmental factors.31 Whether similar effects, including in utero events, could explain the phenotypic difference in valve morphology of these 4 patients remains unclear.

**Table. Associations of Genetic Mutations With Isolated BAV, Isolated TAAD, and Coexistent BAV and TAAD**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genetic Associations</th>
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<tbody>
<tr>
<td><strong>With a BAV</strong></td>
<td></td>
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<tr>
<td>NOSTCH1</td>
<td>Limited number of familial cases and 4% of sporadic cases</td>
</tr>
<tr>
<td>GATA5</td>
<td>Rare nonsynonymous variations in BAV disease</td>
</tr>
<tr>
<td>SMAD6</td>
<td>Variants found in 1 patient with a BAV, AS, and coarctation and in 1 infant with a BAV and moderate AS</td>
</tr>
<tr>
<td><strong>Syndromic</strong></td>
<td></td>
</tr>
<tr>
<td>HOX1</td>
<td>Bosley-Salih-Alarainy syndrome; athabascan brainstem dysgenesis syndrome; associations with interrupted aortic arch, aberrant subclavian artery, ventricular septal defect, and tetralogy of Fallot</td>
</tr>
<tr>
<td>KCN1J2</td>
<td>Andersen syndrome; associated with a BAV, AVW with coarctation, and pulmonary stenosis</td>
</tr>
<tr>
<td><strong>With TAAD</strong></td>
<td></td>
</tr>
<tr>
<td>ACTA2</td>
<td>10% to 15% of familial nonsyndromic TAAD</td>
</tr>
<tr>
<td>FBN1</td>
<td>Single-nucleotide polymorphisms associated with sporadic TAAD</td>
</tr>
<tr>
<td>SMAD3</td>
<td>Familial TAAD</td>
</tr>
<tr>
<td>TGFB2</td>
<td>Familial TAAD</td>
</tr>
<tr>
<td>TGFB2</td>
<td>Approximately 2% of TAAD</td>
</tr>
<tr>
<td>PRKG1</td>
<td>Familial TAAD</td>
</tr>
<tr>
<td>MVH11</td>
<td>Approximately 1% to 2% of familial TAAD</td>
</tr>
<tr>
<td>MYLK</td>
<td>Approximately 1% of familial TAAD</td>
</tr>
<tr>
<td><strong>Syndromic</strong></td>
<td></td>
</tr>
<tr>
<td>COL3A1, COL5A1, COL5A2</td>
<td>Ehlers-Danlos syndrome</td>
</tr>
<tr>
<td>FBN1</td>
<td>Marfan syndrome</td>
</tr>
<tr>
<td>TGFB1</td>
<td>Loeys-Dietz syndrome</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>Loeys-Dietz syndrome</td>
</tr>
<tr>
<td>CBS</td>
<td>Homocystinuria</td>
</tr>
<tr>
<td>MED12</td>
<td>Lujan-Fryns syndrome</td>
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<tr>
<td>SLCA10</td>
<td>Arterial tortuosity syndrome with recessive inheritance</td>
</tr>
<tr>
<td>SKI</td>
<td>Shprintzen-Goldberg syndrome</td>
</tr>
<tr>
<td>SMAD4</td>
<td>Nonsense mutation segregated in a family with juvenile polyposis, aortopathy, and mitral valve dysfunction</td>
</tr>
<tr>
<td><strong>With Coexistent BAV and TAAD</strong></td>
<td></td>
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<tr>
<td>NOSTCH1</td>
<td>Nonsynonymous variations in 4% to 10% of coexisting BAV and TAA</td>
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<tr>
<td>GATA5</td>
<td>Transcriptional activation domains of 4% of sporadic cases of BAV and TAAD</td>
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<tr>
<td>FBN1</td>
<td>FBN1 mutations in 2 patients with a BAV and aortic root aneurysm without MFS</td>
</tr>
<tr>
<td>TGFB2</td>
<td>Single-nucleotide substitution in nonsyndromic BAV and proximal TAAD</td>
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<tr>
<td>MAT2A</td>
<td>18 Individuals with MAT2A rare variant; 8 diagnosed with TAA and 4 diagnosed with a BAV</td>
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</table>

Abbreviations: AS, aortic stenosis; BAV, bicuspid aortic valve; MFS, Marfan syndrome; TAA, thoracic aortic aneurysm; TAAD, thoracic aortic aneurysm and dissection.

**Conclusions**

These 2 cases highlight the gap in knowledge of the genetic underpinnings of BAV. Genetic testing in 2 pairs of monzygotic twins with discordant phenotypes suggests that in addition to gene inheritance, epigenetic effects, somatic mutations, and environmental factors may play a more important role than previously suspected. This may limit the usefulness of genetic screening in risk stratification. Because understanding of the molecular mechanisms of thoracic aortic disease has outpaced that of BAV, these cases highlight the need for ongoing investigation of the pathogenesis of BAV and its associated syndromes.
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Brief Report Research

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Data analysis.
the integrity of the data and the accuracy of the data in the study and takes responsibility for the publication.

Author Contributions:


