Discordant Aortic Valve Morphology in Monozygotic Twins
A Clinical Case Series

Dawn S. Hui, MD; Robert O. Bonow, MD, MS; Joshua M. Stolker, MD; Stephen R. Braddock, MD; Richard Lee, MD, MBA

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.
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 (5.0-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).

Figure 1. Echocardiographic Short-Axis View of the Aortic Valves in Systole From 2 Sets of Monozygotic Twins


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 1C). 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 1D). 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, SLC2A10, SMAD3, SMAD4, SMAD6, TGFBR2, TGFB1, 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. 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. Recently, nonsynonymous variation within GATA5 transcriptional activation domains were reported in 4 sporadic cases of patients with a BAV but not confirmed in another study of familial BAV. 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. 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. 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 al prospectively evaluated family members of 13 individuals with both BAVs and TAA, 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 BAV as well as in patients with a BAV with concomitant aortic root aneurysms without other phenotypic features of Marfan syndrome. However, in a large cohort of patients with FBN1 mutations, all patients with a BAV and TAA met international criteria for Marfan syndrome.20

**Figure 2. Computed Tomography of the Chest, Coronal Views**

A, Aneurysm of the ascending aorta (5-cm diameter; arrows) and aortic root (3.5-cm diameter). B, Noncontrasted scan showing postoperatively replaced aorta (3-cm diameter, arrows) and aortic valve (arrowhead).
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>
</tr>
</thead>
<tbody>
<tr>
<td>NOSCH1</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^2^</td>
</tr>
<tr>
<td>Hoxa1</td>
<td>Bosley-Salih-Alorainy syndrome; athabascan brainstem dysgenesis syndrome; associations with interrupted aortic arch, aberrant subclavian artery, ventricular septal defect, and tetralogy of Fallot^2^</td>
</tr>
<tr>
<td>Kcnj2</td>
<td>Andersen syndrome; associated with a BAV, BAV with coarctation, and pulmonary stenosis^3^</td>
</tr>
<tr>
<td>ACTA2</td>
<td>10% to 15% of familial nonsyndromic TAAD^6^</td>
</tr>
<tr>
<td>FBN1</td>
<td>Single-nucleotide polymorphisms associated with sporadic TAAD^13,14^</td>
</tr>
<tr>
<td>SMAD3</td>
<td>Familial TAAD^15^</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>Familial TAAD^15^</td>
</tr>
<tr>
<td>TGFBR1</td>
<td>Approximately 2% of TAAD^6^</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>Approximately 2% of TAAD^6^</td>
</tr>
<tr>
<td>Notch1</td>
<td>Familial TAAD^15^</td>
</tr>
<tr>
<td>Myh11</td>
<td>Approximately 1% to 2% of familial TAAD^6^</td>
</tr>
<tr>
<td>Mylk</td>
<td>Approximately 1% of familial TAAD^6^</td>
</tr>
<tr>
<td>COL3A1, COL5A1, and COL5A2</td>
<td>Ehlers-Danlos syndrome</td>
</tr>
<tr>
<td>FBN1</td>
<td>Marfan syndrome</td>
</tr>
<tr>
<td>TGFBR1</td>
<td>Loeys-Dietz syndrome</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>Loeys-Dietz syndrome</td>
</tr>
<tr>
<td>Cg5</td>
<td>Homocystinuria</td>
</tr>
<tr>
<td>Med12</td>
<td>Lujan-Fyrs syndrome</td>
</tr>
<tr>
<td>Slc2A10</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^15^</td>
</tr>
<tr>
<td>NOSCH1</td>
<td>Nonsynonymous variations in 4% to 10% of coexisting BAV and TAA^12,18^</td>
</tr>
<tr>
<td>GATA5</td>
<td>Transcriptional activation domains of 4% of sporadic cases of BAV and TAA^11^</td>
</tr>
<tr>
<td>Fbn1</td>
<td>FBN1 mutations in 2 patients with a BAV and aortic aneurysm without MFS^15^</td>
</tr>
<tr>
<td>Tgfbr2</td>
<td>Single-nucleotide substitution in nonsyndromic BAV and proximal TAA^11,15^; in a group with TGFBR2 mutations, all patients with a BAV also had TAA and moderate skeletal features^2^</td>
</tr>
<tr>
<td>Mat2a</td>
<td>18 Individuals with MAT2A rare variant; 8 diagnosed with TAA and 4 diagnosed with a BAV^22</td>
</tr>
</tbody>
</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 monozygotic 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.
Discordant Aortic Valve Morphology in Monozygotic Twins

ARTICLE INFORMATION
Accepted for Publication: June 2, 2016.
Published Online: August 31, 2016.

Author Contributions: Dr Hui had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Hui, Stolker, Braddock, Lee. Acquisition, analysis, or interpretation of data: Hui, Bonov, Braddock, Lee. Drafting of the manuscript: Hui, Stolker. Critical revision of the manuscript for important intellectual content: All Authors. Obtaining funding: Stolker. Administrative, technical, or material support: Braddock. Study supervision: Hui, Stolker, Braddock, Lee.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Disclaimer: Dr Bonow is the Editor of JAMA Cardiology but was not involved in the editorial review or decision to accept the manuscript for publication.

Additional Contributions: We thank Abhay Ladda, MD (Center for Comprehensive Cardiovascular Care, Saint Louis University School of Medicine, St Louis, MO), for preparation of the echocardiogram image and Katherine M. Christensen, MS (Division of Medical Genetics, Saint Louis University; St Louis, MO), for genetic counseling. Neither contributor was compensated for their work.

REFERENCES


