Bicuspid aortic valve (BAV) is the most common congenital heart defect, occurring in 1% to 2% of the general population. Given the high incidence of sequelae, including aortic valve calcification and dysfunction, congenital aortic malformations, and aortic dilatation and dissection, it may account for considerable morbidity and mortality compared with other congenital cardiac abnormalities. The aortic dilatation that occurs with BAV occurs more frequently and at a younger age than it does in patients with trileaflet aortic valves, and the clinical significance of the correlation between BAV and ascending aortic dilatation is based on the potential for aortic dissection and rupture. Defining a potential molecular biological basis for aneurysm formation in BAV is critical for understanding disease progression and designing strategies for intervention. Medical treatments that might mitigate or halt the progression of aneurysmal expansion may significantly decrease the incidence of rupture and death and reduce the number of patients requiring high-risk surgical interventions. Potential targets for medical therapy have only recently begun to be elucidated, and much remains unclear about the mechanism of aneurysm formation and expansion.

Unfortunately, the precise mechanisms underlying the development and progression of thoracic aortic disease in BAV patients have not been clearly delineated. Two logical considerations are most frequently believed to play a major role in this context: that the increased hemodynamic load placed on the proximal aorta may result in progressive aortic dilatation and that an as-yet unidentified genetic or developmental abnormality in the proximal aortic tissue clinically results in altered integrity of the extracellular matrix with consequent weakness of the aortic wall.

Although the first hypothesis has the advantage of relative simplicity, several studies suggest that hemodynamic alterations alone cannot be responsible for aortic dilatation in these patients. In fact, aortic dilatation occurs in the presence of normally functioning BAV. Furthermore, Yasuda and coworkers have demonstrated that aortic valve replacement alone does not prevent aortic dilatation in patients with BAV.

However, the presence of aortic valve dysfunction is not without consequence to the proximal aorta. In patients with BAV, concomitant aortic stenosis increases the growth rate of the proximal ascending aorta and significantly increases the risk of rupture or dissection. Although the mechanism for this effect is unknown, one might postulate that the altered hemodynamics resulting from a high-pressure aortic ejection jet may place a greater burden on the proximal aorta.

The other hypothesis for the high incidence of aortic disease in BAV patients—an inborn congenital defect in aortic structure—appears more compelling. The association may involve a broader spectrum of developmental anomalies involving the great vessels. The aortic valve and ascending aorta share a common embryological origin in that both develop from neural crest cells. The pulmonary trunk also has been shown to undergo histopathological changes similar to those of the ascending aorta in BAV patients. The association between BAV and coarctation of the aorta with and without Turner syndrome may point toward BAV disease involving the ascending aorta and aortic arch.

Like isolated thoracic aortic aneurysms, aortic dilatation in BAV disease has confirmed familial clustering, with the primary mode of inheritance being autosomal dominant. Genetic heterogeneity is apparent, however, in that X-linked dominant and recessive modes also are evident. Mutations in the NOTCH1 gene have recently been identified that lead to signaling abnormalities that may be related to the development of a bileaflet aortic valve and possibly accelerated calcium deposition. In addition, missense mutations in the ACTA2 gene, which encodes vascular smooth muscle α-actin, has been identified as being associated with familial thoracic aortic aneurysms and dissections in BAV.

From a histopathological perspective, BAV aortic disease shares similarities with other collagen vascular disorders like Marfan syndrome, with cystic medial degeneration, an increase in matrix metalloproteinase (MMP) expression, and decreased fibrillin-1 content in the aortic wall. Although the fibrillin-1 content has been shown to be significantly decreased in BAV compared with tricuspid valves aortas, mutations in the FBN1 gene encoding this gene have not been found to be associated with BAV disease.

Whatever the underlying cause, MMP expression in ascending aortic aneurysms associated with BAV has increased expression of different and distinct MMPs and tissue inhibitors of MMPs (TIMPs) compared with patients with tricuspid aortic valves. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are commonly implicated as being elevated in aneurysms associated with BAV. This has not uniformly been the
case, however, with some researchers demonstrating MMP-2 expression in BAV and MMP-9 in trileaflet aortic valves.\textsuperscript{14} Marfan syndrome, on the other hand, demonstrates increased expression of MMP-12 (elastase) and TIMP-2, with decreased MMP-2 and TIMP-3.\textsuperscript{15} These different patterns of MMP and TIMP expression may reflect varying mechanisms of pathophysiology, depending on the origin of the aortic disease.

The article by Phillippi et al\textsuperscript{16} in the current issue of \textit{Circulation} confirms the findings of others that MMP-9 expression is increased in BAV patients with concomitant ascending aneurysmal aortic disease but presents interesting data that shed new light on the complex disease process in BAV-associated ascending aortic aneurysms. The initial goal of their project was to study dysregulated genes in BAV-associated ascending aortic aneurysms compared with aneurysms in patients with trileaflet aortic valves and others with nondiseased aortas (controls) by microarray analysis. Interestingly, the group found that a high percentage of genes in the metallothionein family (stress response proteins) are dysregulated in patients with BAV-associated ascending aortic aneurysms, demonstrating markedly lower expression compared with controls. This was confirmed at the protein level by Western blot analysis. Cultures of smooth muscle cells isolated from BAV aortas demonstrated reduced induction of metallothionein and the poorest resistance to oxidative stress in vitro.

This finding of dysregulated metallothionein genes in BAV-associated tissue and its inferior response to oxidative stress is provocative and noteworthy because it may provide a missing piece of the complex puzzle of aneurysm progression in this disease process. Oxidative stress is invariably increased and contributes to the pathophysiology of inflammation. It can represent tissue damage occurring secondary to increased production and/or decreased destruction of reactive oxygen species. Reactive oxygen species and reactive nitrogen species have been implicated as the enzymatic source in a variety of disease processes from diabetes, atherosclerosis, and aging to the pathogenesis abdominal aortic aneurysms. Their metabolism involves many enzymes, including catalase, superoxide dismutase, and reductase enzymes. A proteomic study of the aortic media in thoracic aortic dissections demonstrated increased lipid peroxidation and decreased superoxide dismutase activity, indicating the importance of oxidative stress in this disease process.\textsuperscript{17}

The importance of the discovery of dysregulated metallothionein in BAV-associated aortic disease may explain the progression of aortic dilatation because metallothionein scavenges a wide range of reactive oxygen species (ie, superoxide, nitric oxide, hydrogen peroxide, hydroxy radicals, and peroxynitrite), which is followed by the release of zinc.\textsuperscript{18} Metallothionein, discovered in 1957, is a cysteine-rich protein that binds zinc and an array of other metal ions and is more effective in quenching radicals than GSH (glutathione), a major antioxidant, enabling cells to deal with pro-oxidant conditions (oxidative stress). Reactive oxygen species and oxidative stress also increase the expression of metallothionein-1 and metallothionein-2 by way of the metal response element-binding transcription factor-1 (a zinc binding protein), which is the predominant regulatory protein mediating metallothionein induction.\textsuperscript{19}

In summary, the work of Phillippi and colleagues is the first report of the dysregulation of metallothionein expression in ascending aneurysmal formation in the setting of BAV. In analyzing the Phillippi et al study, however, one must understand that microarray technology for gene expression profiling is still in its infancy and that the limitations of this study, like so many others, include a limited number of samples and the ability to minimize variance.

Most microarray data generated and reported in the literature (particularly gene expression data) have not been reproducible.\textsuperscript{20} Variance arises from technical features, ie, RNA isolation and handling, chip-to-chip, and hybridization conditions; scanner characteristics; and biological features, ie, specimen-to-specimen and experimental conditions. Aortic tissue consists of a mixture of different cell types. Therefore, changes in gene expression patterns in comparisons of 2 different tissue biopsy samples are a manifestation of all the cell types present in that sample. This issue can render the analysis inaccurate.

In the Phillippi study, due to limited tissue availability, there exists insufficient numbers to completely match for age and comorbid factors. Within the group’s entire tissue bank, there remains a difference in age between groups (see Table 1 in the article). In addition, principal-components analysis revealed that 20% of the samples were significantly different from the other 40 samples and were excluded from further analysis. Phillippi et al., however, made considerable efforts to corroborate their microarray findings of altered Metallothionein expression with PCR and western blotting assays of aortic samples in an effort to validate their original discovery. Despite these potential limitations, however, the article adds significantly to the growing evidence that there may be an altered response to oxidative stress in aneurysmal disease that affects the integrity of the extracellular matrix of the aortic wall. In the ascending aorta, metallothionein may well play an important role in the normal maintenance of the extracellular matrix in response to oxidative stress, including regulation of MMP expression. The diminished expression of metallothionein in BAV-associated ascending aneurysmal disease may indeed be a critical discovery in our understanding of the regulatory response of the aorta to oxidative stress.

Although the direct cause for ascending aortic aneurysm formation in the setting of BAV has not been elucidated, Phillippi et al propose that this defect in the cellular micro-environment, perhaps as a result of genetically predetermined abnormalities in the aortic wall, may well be an important link in the development of ascending aneurysm formation associated with BAV. Additional studies are necessary, however, to validate these novel findings.

\textbf{Disclosures}

None.

\textbf{References}


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Metallothionein Link to Bicuspid Aortic Valve–Associated Ascending Aortic Dilatation
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