The genetics of bicuspid aortic valve disease: Shall we take it down a *NOTCH*?

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Central Message

NOTCH1 mutations are found in some patients with bicuspid aortic valves. Investigators explore the mechanistic basis of bicuspid aortic valve aortopathy with a novel in vitro model.

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Bicuspid aortic valve (BAV) disease is a common disorder, affecting 1% to 2% of the population. It can occur in conjunction with other congenital heart defects or as an isolated lesion in nonsyndromic cases. Surgeons are familiar with the heterogeneity of BAV disease, which includes several valve morphologies, different propensities and modes of valvular degeneration, and a predisposition toward proximal thoracic aortic aneurysms.

The genetic basis of nonsyndromic BAV disease has been supported by genetic linkage studies, in which BAV disease segregates in an autosomal dominant pattern with incomplete penetrance. Garg and coworkers¹ reported the association between loss of function mutations in the *NOTCH1* gene in 2 different families with nonsyndromic BAV disease, and a follow-up study by Mohamed and associates² implicated *NOTCH1* gene mutations in 4% of sporadic BAV cases as well. McKellar and coworkers³ also found *NOTCH1* gene variants in 10% of patients with concomitant BAV and thoracic aortic aneurysms, compared with 2.1% in control subjects. The *NOTCH1* gene encodes a transmembrane receptor that is involved in cell fate determination and morphogenesis of the cardiac chambers and valves.⁴ It promotes epithelial to mesenchymal transition during valvulogenesis, and in the adult it is involved in valvular and vascular homeostasis. On ligand binding, cleavage of the NOTCH1 receptor releases the protein's intracellular domain, which then translocates to the nucleus and triggers gene transcription. The low frequency of *NOTCH1* mutations in the population with BAV disease suggests that *NOTCH1* is part, but not all, of the mechanistic story behind BAV development.

In this issue of the *Journal*, Jiao and colleagues⁵ reevaluate the contribution of *NOTCH1* to the pathogenesis of BAV disease, albeit from a different angle. They are specifically interested in the aortopathy associated with BAV disease, which preferentially involves the proximal thoracic aorta and leads to aneurysm and increased risk of dissection. They focus on the differentiation potential of precursor cells that populate the proximal thoracic aorta and the effect of

NOTCH1 loss of function on this process. Their hypothesis is that loss of *NOTCH1* function will impair cellular differentiation, and in vivo, this could result in an aortopathy. Rather than focus on the organism as a whole, however, they take a microscopic view and use advanced techniques in stem cell biology and gene editing to answer their questions. Their model consists of in vitro induced pluripotent stem cells derived from peripheral blood mononuclear cells of a single donor with a normal trileaflet aortic valve. Jiao and colleagues⁵ use CRISPR/Cas9 gene editing to generate iPSCs with *NOTCH1* loss of function. With this model, Jiao and colleagues⁵ find impaired differentiation of *NOTCH1* loss of function iPSCs along 2 pathways: (1) iPSC to neural crest stem cell to smooth muscle cell and (2) iPSC to cardiovascular progenitor cell to endothelial cell. They conclude that *NOTCH1* is likely a key factor in the differentiation of proximal aortic smooth muscle cells and endothelial cells from their stem cell precursors.

This study is an interesting extension of the previous work of Jiao and colleagues,⁶ which also used an iPSC model to identify defects in differentiation and function of smooth muscle cells from patients with BAVs and thoracic aortic aneurysms.⁶ They identified abnormalities in the differentiation pathway from iPSC to neural crest stem cell to smooth muscle cell, which models characteristics in the proximal aorta. In contrast, the differentiation pathway from iPSC to paraxial mesoderm cell to smooth muscle cell (which models characteristics in the descending aorta)

was not affected. These in vitro studies support the hypothesis that smooth muscle cell phenotypes of the proximal (as opposed to descending) aorta behave abnormally in patients with BAVs and thoracic aortic aneurysms versus those in control patients with tricuspid aortic valves.

The study of Jiao and colleagues⁵ has refocused attention on *NOTCH1* as a genetic regulator of BAV, providing mechanistic insights into how defective *NOTCH1* signaling may lead to aortopathy. But is this where we should be looking? In trying to support this direction of research, Jiao and colleagues⁵ examined a panel of 108 patients with BAVs, and they found no missense or nonsense mutations in *NOTCH1*. This finding points to the reality that *NOTCH1* is at best a supporting actor in the aortopathy of BAV disease, and the leading actors remain unknown.

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