VALVULAR DISEASE

Calcific aortic valve disease: turning therapeutic discovery up a notch

Suya Wang and William T. Pu

Valve replacement is currently the only treatment for calcific aortic valve disease. Studies of an uncommon, genetic form of aortic valve disease have yielded in vitro and mouse models of the disease and a transcriptomic disease signature. Machine learning-driven screens for compounds that normalize this signature promise to enable medical management of aortic valve disease.

Refers to Theodoris, C. V. et al. Network-based screen in iPSC-derived cells reveals therapeutic candidate for heart valve disease. *Science* https://doi.org/10.1126/science.abd0724 (2020).

Calcific aortic valve disease (CAVD) results in progressive dysfunction of the aortic valve (AV) (FIG. 1a), which can cause cardiac hypertrophy, failure and arrhythmia[1]. CAVD is the most prevalent form of heart valve disease in developed countries[2], with a prevalence of ~12% among the elderly population. AV replacement is currently the only available therapy, despite progress in understanding the underlying disease mechanisms[3]. In addition to procedural morbidity and cost, AV prostheses have a limited lifespan and a risk of complications, which include thrombosis and endocarditis. CAVD progresses over many years, which suggests a window for effective medical intervention. In a study published in *Science*, Theodoris and colleagues make important progress towards this goal, by use of a human disease-in-a-dish model and an innovative gene network-based screen to identify a small molecule that ameliorates disease in both in vitro and mouse models[4].

The study from Theodoris and co-workers[4] is the latest in a series of studies from the Srivastava laboratory and other groups that together beautifully illustrate how the investigation of uncommon genetic conditions and cutting-edge biomedical advances can drive progress in the understanding and treatment of more prevalent diseases. A crucial risk factor for CAVD is a developmental AV abnormality that results in two (bicuspid) rather than three (tricuspid) valve leaflets (FIG. 1a). About 33% of patients with this common cardiac malformation (prevalence ~1%) develop AV stenosis, and the majority of patients with early-onset CAVD have bicuspid AVs[1]. Conversely, relatives of patients with severe forms of left-sided obstructive congenital heart disease, including hypoplastic left heart syndrome, have an increased prevalence of bicuspid AV. In 2005, Garg and colleagues reported two multigenerational families affected by congenital heart disease, including bicuspid AV and CAVD congenital heart disease and AV disease, which were caused by heterozygous variants in *NOTCH1* (REF.[5]). Further work showed that NOTCH1 signalling in valve endothelial cells (ECs) was important in disease pathogenesis and confirmed the presence of rare *NOTCH1* variants in ~4% of individuals with sporadic bicuspid AV[6]. In 2015, Theodoris and co-workers generated human induced pluripotent stem cell (iPSC)-derived ECs with an engineered *NOTCH1* mutation to establish an in vitro model of CAVD, which they used to identify a gene network in which NOTCH1 suppresses an osteogenic and inflammatory sub-circuit controlled by transcription factor SOX7 and transcription factor 4 (TCF4)[7] (FIG. 1b). A further crucial building block was the establishment of a mouse model of CAVD caused by *Notch1* haploinsufficiency, in which shortening of murine telomeres to a length similar to that of human telomeres was necessary for the development of AV disease[8]. This model showed that telomere length is related to AV disease severity, which suggests that telomere length is a factor in human age-dependent CAVD progression.

> “a machine learning classifier was developed that accurately labelled wild-type or *NOTCH1* heterozygous iPSC-ECs”

This robust foundation set the stage for the current study. Using the human iPSC-EC disease-in-a-dish model, the researchers performed an innovative, moderate-throughput screen of 1,595 small molecules, including a library of pharmacologically active compounds. Rather than target a small number of biomarkers or a physiological phenotype, the investigators assayed the expression of 119 highly connected members of their previously identified NOTCH1-regulated gene network as the screen readout (FIG. 1c). On the basis of the expression of these signature genes, a machine learning classifier was developed that accurately labelled wild-type or *NOTCH1* heterozygous iPSC-ECs. The *NOTCH1*$^{+/-}$ iPSC-ECs were then treated with the compound library, and their gene-expression signatures were classified as wild-type or diseased. Eight compounds caused *NOTCH1*$^{+/-}$ cells to be classified as wild-type and passed subsequent validation assays. Follow-up whole-transcriptome analyses focused attention on one compound, XCT790, which normalized the expression of most genes, including network drivers *SOX7* and *TCF4*. To extend their findings beyond CAVD caused by *NOTCH1* variants, the investigators next studied primary ECs from normal tricuspid AVs, calcified bicuspid AVs and calcified tricuspid AVs. Transcriptome analysis identified a shared set of dysregulated genes in calcific ECs, which overlapped with many misexpressed genes in *NOTCH1*$^{+/-}$ cells, including *SOX7* and *TCF4*. XCT790 effectively normalized the expression of the dysregulated gene network, demonstrating that sporadic and *NOTCH1*$^{+/-}$ CAVD have shared features that are effectively targeted by XCT790. Finally, Theodoris and colleagues found that XCT790 reduced AV stenosis and

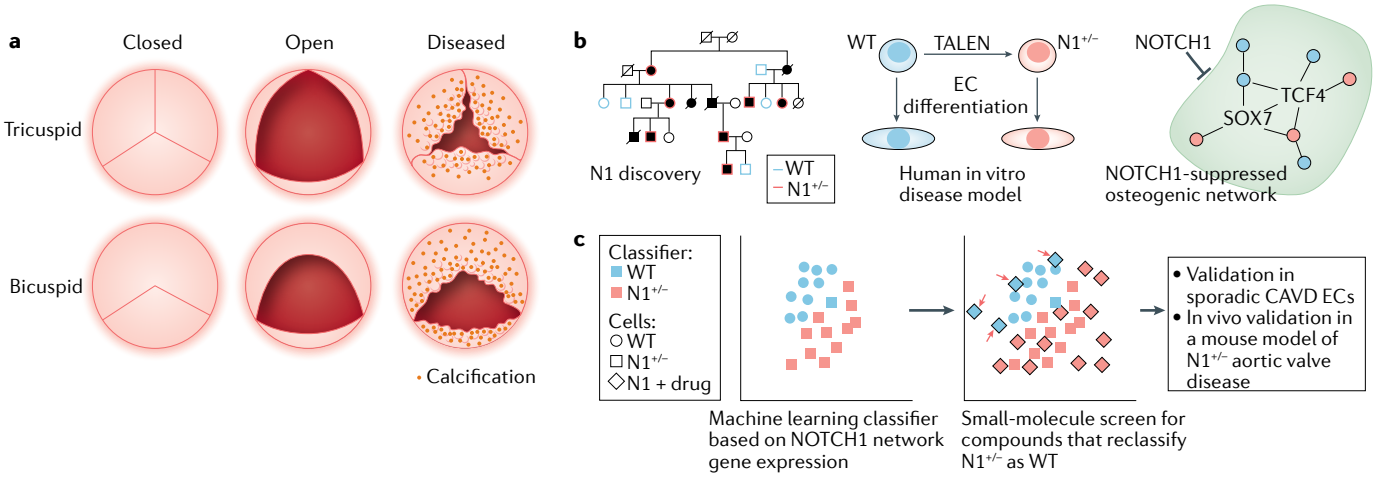

Fig. 1 | **Small-molecule screen targeting a NOTCH1-suppressed osteogenic gene network. a** | Illustrations of tricuspid and bicuspid aortic valves in closed, open and diseased states. **b** | Establishment of the foundations for the small-molecule screen. Genetic mapping in multigenerational human pedigrees with aortic valve disease linked to *NOTCH1* mutation (left). Patient-derived and genome-edited induced pluripotent stem cell (iPSC)–endothelial cell (EC) disease models were established (middle) and used to discover a NOTCH1-suppressed osteogenic gene network (right). **c** | On the basis of targeted gene-expression measurements, a machine learning classifier was trained that accurately distinguished between wild-type (WT) and *NOTCH1* heterozygous ($N1^{+/-}$) iPSC-ECs (left). The classifier was used to identify small molecules that caused some $N1^{+/-}$ iPSC-ECs to be labelled as WT (middle, red arrows). Screen hits were validated in primary ECs from patients with calcific aortic valve disease (CAVD) and in a mouse model of $N1^{+/-}$ aortic valve disease. Square, male; circle, female; filled, heart disease. SOX7, transcription factor SOX7; TALEN, transcription activator-like effector nucleases; TCF4, transcription factor 4.

CAVD in the telomere-shortened *Notch1*$^{+/-}$ mouse model.

XCT790 is a putative inverse agonist of oestrogen-related receptor-α (ERRα), an orphan nuclear receptor that promotes WNT signalling and osteogenesis[9]. Depletion of ERRα by siRNA in wild-type iPSC-ECs reduced the expression of *TCF4* but did not restore the expression of *TCF4* or *SOX7* in *NOTCH1*$^{+/-}$ cells[4], suggesting that the activity of XCT790 is at least in part independent of ERRα. ERRα also promotes mitochondrial oxidative metabolism in cardiac and skeletal muscle and is a potent mitochondrial electron transport chain uncoupler[10]. Although treatment of mice with XCT790 did not cause overt adverse effects[4], both of these on-target and off-target activities of XCT790 will complicate its direct development as a therapeutic agent. Further exploitation of the advances made by Theodoris and colleagues will require understanding of the mechanism of action of XCT790 to permit the development of more clinically applicable compounds.

The AV is a complex structure populated by several cell types in addition to ECs, including several different subtypes of valve interstitial cells (VICs) and immune cells. Although Theodoris and colleagues focused on ECs, the non-EC subsets and the interactions between these cells undoubtedly contribute to the pathogenesis of CAVD. Indeed, *NOTCH1* has been implicated in suppressing VIC osteogenic activity[3]. It will be interesting to explore the activity of XCT790 in VICs and, more broadly, to apply the transcriptomic-signature-based profiling strategy to disease models containing VICs or even a full range of cell types relevant to CAVD.

> “XCT790 effectively normalized the expression of the dysregulated gene network”

The work of Theodoris and co-workers[4] exemplifies how the study of an uncommon genetic disease using disease-in-a-dish technologies and an innovative screening strategy based on the transcriptional signature of a disease-driving genetic network can advance mechanistic understanding and therapeutic development for common, multifactorial diseases. This same strategy of screening by targeting a transcriptional signature could be readily deployed for a wide variety of other diseases. Within AV disease, the researchers identified a lead compound and established a screening platform for the discovery of others drugs. These efforts might yield compounds that will permit medical therapy to prevent or slow CAVD. Of note, bicuspid AV and *NOTCH1* variants are also associated with severe forms of congenital heart disease, including critical neonatal aortic stenosis and hypoplastic left heart syndrome, raising the possibility that these same approaches could also be used to discover new treatments for these devastating congenital malformations.

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https://doi.org/10.1038/s41569-021-00528-1

1. Rajamannan, N. M. et al. Calcific aortic valve disease: not simply a degenerative process. *Circulation* **124**, 1783–1791 (2011).
2. Osnabrugge, R. L. J. et al. Aortic stenosis in the elderly: disease prevalence and number of candidates for transcatheter aortic valve replacement: a meta-analysis and modeling study. *J. Am. Coll. Cardiol.* **62**, 1002–1012 (2013).
3. Goody, P. R. et al. Aortic valve stenosis: from basic mechanisms to novel therapeutic targets. *Arterioscler. Thromb. Vasc. Biol.* **40**, 885–900 (2020).
4. Theodoris, C. V. et al. Network-based screen in iPSC-derived cells reveals therapeutic candidate for heart valve disease. *Science* https://doi.org/10.1126/science.abd0724 (2020).
5. Garg, V. et al. Mutations in *NOTCH1* cause aortic valve disease. *Nature* **437**, 270–274 (2005).
6. McKellar, S. H. et al. Novel *NOTCH1* mutations in patients with bicuspid aortic valve disease and thoracic aortic aneurysms. *J. Thorac. Cardiovasc. Surg.* **134**, 290–296 (2007).
7. Theodoris, C. V. et al. Human disease modeling reveals integrated transcriptional and epigenetic mechanisms of NOTCH1 haploinsufficiency. *Cell* **160**, 1072–1086 (2015).
8. Theodoris, C. V. et al. Long telomeres protect against age-dependent cardiac disease caused by NOTCH1 haploinsufficiency. *J. Clin. Invest.* **127**, 1683–1688 (2017).
9. Auld, K. L. et al. Estrogen-related receptor a regulates osteoblast differentiation via Wnt/b-catenin signaling. *J. Mol. Endocrinol.* **48**, 177–191 (2012).
10. Eskiocak, B., Ali, A. & White, M. A. The estrogen-related receptor α inverse agonist XCT 790 is a nanomolar mitochondrial uncoupler. *Biochemistry* **53**, 4839–4846 (2014).

Acknowledgements
W.T.P. is supported by grants R01 HL138571 and UM1 HL098166.

Competing interests
The authors declare no competing interests.