



# A new opening on aortic stenosis: predicting prognosis with clonal haematopoiesis

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**This editorial refers to 'Clonal haematopoiesis in patients with degenerative aortic valve stenosis undergoing transcatheter aortic valve implantation'<sup>†</sup>, by S. Mas-Peiro *et al.*, on page 933.**

Clonal haematopoiesis of indeterminant potential (CHIP) arises when haematopoietic stem cells (HSCs) of otherwise healthy individuals acquire somatic mutations in driver genes commonly mutated in haematological malignancies.<sup>1,2</sup> Cells that bear these mutations expand and can form clones detectable in peripheral blood. CHIP, defined as having >2% of mutated leucocytes in peripheral blood, increases with age, and affects >10% of unselected septuagenarians. Individuals with CHIP have increased all-cause mortality, surprisingly largely due to an increase in cardiovascular death rather than leukaemia or other myeloproliferative disorders.<sup>3</sup> People with CHIP have double the risk of cardiovascular disease (CVD), ischaemic stroke, and heart failure outcomes, independent of traditional risk factors.<sup>3,4</sup>

Valvular heart disease increases with age, in North America and Europe predominantly due to calcified aortic valve disease (CAVD).<sup>5</sup> CAVD describes a clinically vast spectrum of disease, ranging from early leaflet thickening and fibrosis to severe leaflet calcification and narrowing, haemodynamic compromise, and decline of ventricular function. Once attributed to age-related degeneration, we now understand that traditional cardiovascular risk factors are also associated with CAVD, such as atherosclerosis, elevated LDL, smoking, hypertension, and metabolic derangements including diabetes and chronic kidney disease<sup>5</sup> (*Take home figure*). Those with CAVD have a 50% increased risk of cardiovascular death, and an increased risk of atherosclerosis, myocardial infarction, and heart failure.

These observations suggest the operation of similar mechanisms in the pathogenesis of atherosclerosis and CAVD, including perturbation of inflammatory cells such as macrophages and T-cells that mediate leaflet thickening and calcification (*Take home figure*). Indeed, mice with mutations that increase susceptibility for atherosclerosis (Ldlr<sup>-/-</sup>) and engineered to have CHIP mutations in myeloid cells,

compared with mice without CHIP mutations, have increased atherosclerotic lesions when they consume an atherogenic diet.<sup>3,6</sup> These mice also have increased inflammatory cytokines, such as interleukin (IL)-1 $\beta$  and IL-6, but have similar haemograms. Could CHIP also contribute to CAVD?

In this issue of the *European Heart Journal*, Mas-Peiro *et al.* expand our understanding of the roles of CHIP and inflammation in heart disease, focusing on calcified valvular disease and severe aortic stenosis.<sup>7</sup> They show in a prospective study of 279 patients with aortic stenosis treated with transcatheter aortic valve implantation (TAVI) an enrichment of mutations in the two most common CHIP driver genes, *DNMT3a* and *TET2*, compared with age-matched populations without CHIP, and show that the presence of these mutations is associated with worse outcomes including increased mortality during a median 8-month follow-up. Of the 279 patients in the cohort, 93 (33%) had *DNMT3a* or *TET2* CHIP mutations. The patients with *DNMT3a* or *TET2* mutations had a three-fold increase in death compared with non-CHIP patients. There was a trend indicating that patients with a variant allele frequency (VAF) of  $\geq 0.1$  had reduced 8-month mortality when compared with CHIP patients with a VAF of  $\leq 0.1$ , suggesting a relationship between clone size and mortality, although this study was insufficiently powered to demonstrate statistical significance in this subgroup.

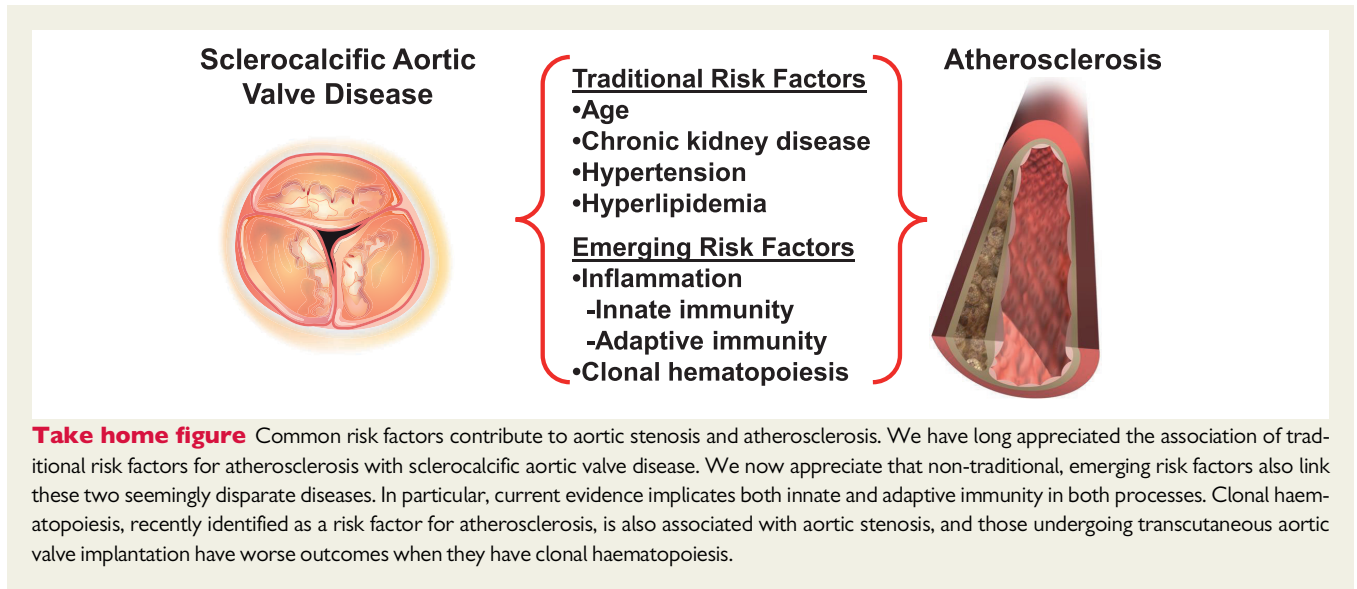
This study was limited to sequencing mutations in the two most common CHIP genes, *DNMT3a* and *TET2*, so the full profile of CHIP driver genes and their clinical association with CAVD are not known. While CHIP mutations are associated with increased cardiovascular death, and in the Mas-Peiro study with CAVD, these mutations act through divergent mechanisms that converge on augmenting various aspects of inflammation. For instance, the *JAK2 V617F* mutation is associated with increased thrombosis in CAD via enhanced formation of neutrophil extracellular traps.<sup>8</sup> Larger and more comprehensive cohort studies would provide additional mechanistic insights and may also reveal subtle and distinct clinical subgroups.

The opinions expressed in this article are not necessarily those of the Editors of the *European Heart Journal* or of the European Society of Cardiology.

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Mas-Peiro *et al.* analysed leucocyte subsets, showing that patients with CHIP mutations had increases in proinflammatory cell populations. Patients with *DNMT3a* mutations had an increase in the Th17 to regulatory T-cell ratio, while those with *TET2* mutations had an increase in non-classical monocytes (CD14<sup>dim</sup>CD16<sup>+</sup>). This difference of affected inflammatory cell populations is an intriguing finding. There is growing literature showing that the innate and adaptive immune cells that reside in the normal aortic valve can drive inflammatory responses preceding and probably propagating active valvular calcification.<sup>9,10</sup> Recent work in mice showed that Th17 T-cells and the overproduction of IL-17 can lead to chronic inflammation, specifically increased collagen production and atherosclerotic plaque stabilization.<sup>11,12</sup> Fundamentally, *DNMT3a* and *TET2* encode proteins with distinct effects on DNA methylation, and their distinct effects on inflammatory cell populations indicate that the mutations may cause different clinical and biological consequences. While significant focus remains on the role of CHIP in myeloid lineages and distinct roles of macrophages in proinflammatory mechanisms of atherosclerosis, a full spectrum of T-cells from effector to regulatory T-cells may contribute to the inflammatory response, valve fibrosis, and accelerated calcification.<sup>5,10,13</sup> Adding to this, recent work by Yue *et al.* also implicates a role for *TET2* in regulatory T-cell function,<sup>14</sup> again emphasizing that there is still much work to be done in this field.

This study affords striking insight and major contributions into the mechanisms both of how somatic mutations in HSCs that drive CHIP affect cardiovascular outcomes and of inflammation in atherosclerosis. It also suggests novel predictive biomarkers to aid decision-making in the management of the growing population of candidates for TAVR. A recent clinical trial has shown improved patient outcomes with known CAD and increased inflammatory markers treated with targeted IL-1β antibody inhibition, findings concordant with mechanistic studies in mice with experimental atherosclerosis or heart failure.<sup>15,16</sup> These findings suggest that targeted inhibition of proinflammatory pathways such as IL-1β or its activator, the inflammasome, could improve outcomes in TAVR patients with CHIP, a conjecture that merits further investigation.

The intimate relationship between inflammation, immune regulation, and CVD represents the crux of our current understanding of the pathogenesis of atherosclerotic disease. With advances in our understanding of the role of CHIP and coordinated patient clinical trials and experimental studies, we have the potential to better understand this mechanistic link and inform advances in clinical care.

**Conflict of interest:** B.L.E. reports grants from Celgene and Deerfield, and personal fees from Grail. In addition, he has a patent, PCT/US2015/062787, pending. P.L. has received laboratory support from Novartis. A.E.L. reports other support from the John S. LaDue Memorial Fellowship during the conduct of the study.

## References

1. Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, Chambert K, Mick E, Neale BM, Fromer M, Purcell SM, Svantesson O, Landén M, Höglund M, Lehmann S, Gabriel SB, Moran JL, Lander ES, Sullivan PF, Sklar P, Grönberg H, Hultman CM, McCarrroll SA. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 2014;**371**:2477–2487.
2. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, Lindsley RC, Mermel CH, Burt N, Chavez A, Higgins JM, Moltchanov V, Kuo FC, Kluk MJ, Henderson B, Kinnunen L, Koistinen HA, Ladenvall C, Getz G, Correa A, Banahan BF, Gabriel S, Kathiresan S, Stringham HM, McCarthy MI, Boehnke M, Tuomilehto J, Haiman C, Groop L, Atzmon G, Wilson JG, Neuberg D, Altshuler D, Ebert BL. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 2014;**371**:2488–2498.
3. Jaiswal S, Natarajan P, Silver AJ, Gibson CJ, Bick AG, Shvartz E, McConkey M, Gupta N, Gabriel S, Ardissino D, Baber U, Mehran R, Fuster V, Danesh J, Frossard P, Saleheen D, Melander O, Sukhova GK, Neuberg D, Libby P, Kathiresan S, Ebert BL. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med* 2017;**377**:111–121.
4. Dorsheimer L, Assmus B, Rasper T, Ortmann CA, Ecker A, Abou-El-Ardat K, Schmid T, Brüne B, Wagner S, Serve H, Hoffmann J, Seeger F, Dimmeler S, Zeiher AM, Rieger MA. Association of mutations contributing to clonal hematopoiesis with prognosis in chronic ischemic heart failure. *JAMA Cardiol* 2019;**4**:25–33.
5. Otto CM, Prendergast B. Aortic-valve stenosis—from patients at risk to severe valve obstruction. *N Engl J Med* 2014;**371**:744–756.
6. Fuster JJ, MacLauchlan S, Zuriaga MA, Polackal MN, Ostriker AC, Chakraborty R, Wu CL, Sano S, Muralidharan S, Rius C, Vuong J, Jacob S, Muralidhar V, Robertson AA, Cooper MA, Andrés V, Hirschi KK, Martin KA, Walsh K. Clonal hematopoiesis associated with *TET2* deficiency accelerates atherosclerosis development in mice. *Science* 2017;**355**:842–847.
7. Mas-Peiro S, Hoffmann J, Fichtlscherer S, Dorsheimer L, Rieger MA, Dimmeler S, Vasa-Nicotera M, Zeiher AM. Clonal haematopoiesis in patients with degenerative aortic valve stenosis undergoing transcatheter aortic valve implantation. *Eur Heart J* 2020;**41**:933–939.

8. Wolach O, Sellar RS, Martinod K, Cherpokova D, McConkey M, Chappell RJ, Silver AJ, Adams D, Castellano CA, Schneider RK, Padera RF, DeAngelo DJ, Wadleigh M, Steensma DP, Galinsky I, Stone RM, Genovese G, McCarroll SA, Iliadou B, Hultman C, Neuberger D, Mullally A, Wagner DD, Ebert BL. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. *Sci Transl Med* 2018;**10**:ean8292.
9. Dweck MR, Jones C, Joshi NV, Fletcher AM, Richardson H, White A, Marsden M, Pessotto R, Clark JC, Wallace WA, Salter DM, McKillop G, van Beek EJ, Boon NA, Rudd JH, Newby DE. Assessment of valvular calcification and inflammation by positron emission tomography in patients with aortic stenosis. *Circulation* 2012;**125**:76–86.
10. New SE, Aikawa E. Cardiovascular calcification: an inflammatory disease. *Circ J* 2011;**75**:1305–1313.
11. Brauner S, Jiang X, Thorlacius GE, Lundberg AM, Östberg T, Yan ZQ, Kuchroo VK, Hansson GK, Wahren-Herlenius M. Augmented Th17 differentiation in Trim21 deficiency promotes a stable phenotype of atherosclerotic plaques with high collagen content. *Cardiovasc Res* 2018;**114**:158–167.
12. Gistera A, Robertson AK, Andersson J, Ketelhuth DF, Ovchinnikova O, Nilsson SK, Lundberg AM, Li MO, Flavell RA, Hansson GK. Transforming growth factor- $\beta$  signaling in T cells promotes stabilization of atherosclerotic plaques through an interleukin-17-dependent pathway. *Sci Transl Med* 2013;**5**:196ra100.
13. Nagy E, Lei Y, Martínez-Martínez E, Body SC, Schlotter F, Creager M, Assmann A, Khabbaz K, Libby P, Hansson GK, Aikawa E. Interferon-gamma released by activated CD8<sup>+</sup> T lymphocytes impairs the calcium resorption potential of osteoclasts in calcified human aortic valves. *Am J Pathol* 2017;**187**:1413–1425.
14. Yue X, Lio CJ, Samaniego-Castruita D, Li X, Rao A. Loss of TET2 and TET3 in regulatory T cells unleashes effector function. *Nat Commun* 2019;**10**:2011.
15. Sano S, Oshima K, Wang Y, MacLauchlan S, Katanasaka Y, Sano M, Zuriaga MA, Yoshiyama M, Goukassian D, Cooper MA, Fuster JJ, Walsh K. Tet2-mediated clonal hematopoiesis accelerates heart failure through a mechanism involving the IL-1 $\beta$ /NLRP3 inflammasome. *J Am Coll Cardiol* 2018;**71**:875–886.
16. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P, Glynn RJ; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;**377**:1119–1131.

## CARDIOVASCULAR FLASHLIGHT

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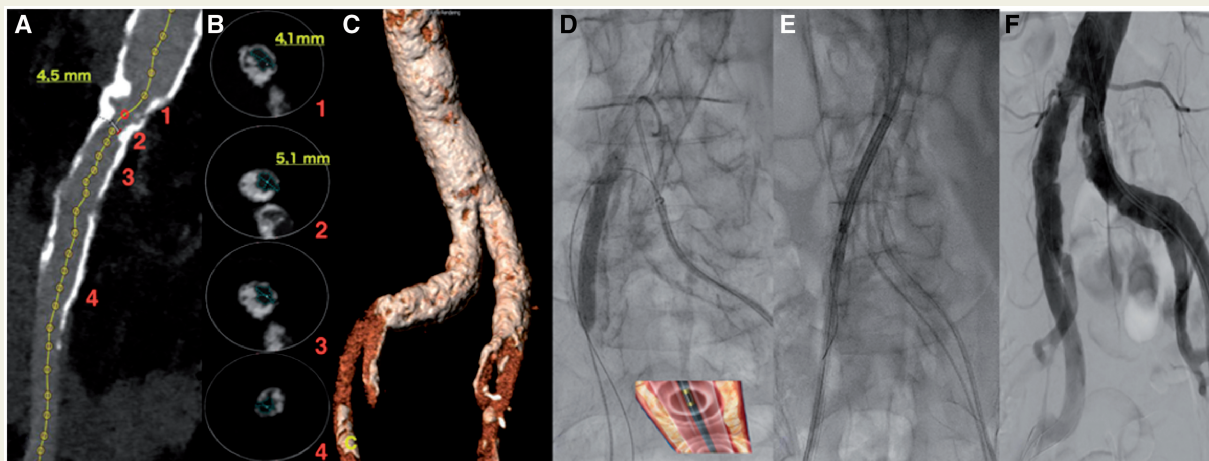
### Lithoplasty-assisted transfemoral aortic valve implantation

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An 85-year-old man with previous left anterior descending (LAD) and left circumflex (LCX) stenting and low left ventricular ejection fraction (35%) developed acute pulmonary oedema because of severe aortic valve stenosis (0.7 cm<sup>2</sup>). Computed tomography showed bilateral calcific atheromas of both external iliac arteries with circumferential thick calcium plates restricting the lumen diameter to 4.1 mm. The case was refused for surgery and initially discarded for transcatheter aortic valve implantation (TAVI) because of poor vascular access. After a new episode of pulmonary oedema refractory to medical treatment and requiring ultrafiltration, a transfemoral TAVI was attempted. The calcified stenosis of the right external iliac artery was dilated with a 7.0 mm lithoplasty balloon (Shockwave Medical, Fremont, CA, USA). After six lithotripsy erogations, there was good balloon expansion at low pressure (4–6 atm). Gentle twist and push over a Confida wire drove the 18-Fr delivery system of a 29 mm Evolut-R Medtronic valve through the narrowest segment of the iliac artery. After valve



deployment half a diamond below the aortic annulus, there was trivial aortic regurgitation with no ruptures or dissections at the access site. (Panel A) Computed tomography longitudinal image of the severely calcified right external iliac. (Panel B) Multiple cross-sections with near circumferential calcification and thick protruding nodules. (Panel C) 3D image of the iliac bifurcation showing severe tortuosity and calcification of both iliac arteries. (Panel D) Shockwaves delivered via a 7 mm lithoplasty balloon inflated at 4 atm. (Panel E) 18-Fr delivery system of the Evolut-R Corevalve across the calcified segment. (Panel F) Final aortogram with no dissection or extravasation.