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CONTEMPORARY REVIEW

Relevance of Clonal Hematopoiesis in Immune Cells for Degenerative Aortic Valve Stenosis

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Abstract: Clonal hematopoiesis of indeterminate potential (CHIP), defined as the presence of somatic mutations in subjects without other hematologic abnormalities has been recently associated with several cardiovascular diseases. Degenerative aortic valve stenosis (AVS), a prototypical cardiovascular age-related disorder resulting from calcification and inflammation processes for which no medical treatment is currently available, has become the subject of scientific research in this field. In this review we describe the clinical relevance of CHIP in patients with AVS, as well as its effects on left ventricular dysfunction and heart failure, particularly about compensatory hypertrophy and diastolic dysfunction assessed by echocardiographic parameters. CHIP-driver mutations *DNMT3A* and *TET2* have been found in a third of all patients with severe AVS undergoing transcatheter aortic valve replacement in most studies; and several short-term and long-term studies have reported an increased mortality rate associated with the presence of CHIP in these patients. Additionally, we discuss potential mechanistic insights involving inflammatory pathways both from a clinical and from an experimental approach. These include primarily fluorescence-activated cell sorting (FACS) analyses or single-cell RNA sequencing (sc-RNA-seq) analyses from circulating leukocytes of patients with AVS, showing an increase in proinflammatory interleukines, and analyses on valve tissue exhibiting higher transcript levels of immunoglobulins, as well as murine models mimicking the consequences of AVS that could shed some light on potential causal link between CHIP and AVS. Lastly, we summarize prospects for novel therapeutic strategies, with efforts focusing on developing both general and more specific inflammatory therapies targeting inflammatory pathways. Future research will need to assess the incidence and progression of milder stages of aortic stenosis and evaluate potential interactions with different types of acquired mutations.

Key Words: aortic valve stenosis ■ clonal hematopoiesis ■ inflammation

In recent years, clonal hematopoiesis of indeterminate potential (CHIP), which was initially described in hematological diseases, gained increasing importance in cardiovascular disorders. It appears to be of particular relevance for degenerative aortic valve stenosis (AVS), a common disorder associated with increasing age, in which calcifying and immune-related processes are involved and for which no medical treatment is currently available. In the present review, the prevalence and impact of CHIP on clinical outcomes in patients with AVS (Figure 1),[1–6] as well as its potential mechanistic insights involving inflammatory pathways, and prospects for novel therapeutic strategies are summarized (Figure 2).[7–10]

THE CONCEPT: CLONAL HEMATOPOIESIS OF INDETERMINATE POTENTIAL

Clonal hematopoiesis of indeterminate potential (CHIP) is defined as the presence of somatic mutations in circulating hematopoietic cells in subjects without any evidence for other hematologic abnormalities.[11] These mutations

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Nonstandard Abbreviations and Acronyms

AVS	aortic valve stenosis
CHIP	clonal hematopoiesis of indeterminate potential
mLOY	mosaic loss of Y chromosome
NLRP3	NOD-, LRR- and pyrin domain-containing protein 3
SAVR	surgical aortic valve replacement
TAVR	transcatheter aortic valve replacement
VAF	variant allele frequency
VIC	valvular interstitial cells

accumulate throughout human lifespan and are highly prevalent in the elderly population, ie, greater than 10% over the age of 70 years.[12] Some of the mutated cells confer a survival advantage and cause a clonal expansion of the mutated leukocytes in peripheral blood.[11] They also trigger an abnormal immune response.[13]

Many CHIP-driver mutations have been described; they include mutations in epigenetic modulators (DNA methyltransferase 3 alpha [*DNMT3A*], tet methylcytosine dioxygenase 2 [*TET2*] or additional sex combs-like 1 [*ASXL1*]),[14] splicing factors (serine/arginine-rich splicing factor 2 [*SRSF2*] or splicing factor 3B subunit 1 [*SF3B1*]),[15] and genes involved in DNA damage response (tumor protein p53 [*TP53*], protein phosphatase Mg^{2+}/Mn^{2+} dependent 1D [*PPM1D*])[16] or in the immune signaling cascade (janus kinase 2 [*JAK2*], guanine nucleotide binding protein [*GNAS*], guanine nucleotide-binding protein G subunit beta 1 [*GNB1*], Casitas B-cell lymphoma [*CBL*] gene).[17,18] Some individuals can have more than 1 gene affected.

The classic definition of CHIP uses a variant allele frequency (VAF) cut-off value of ≥2% in peripheral blood cells.[11] When deeper sequencing is performed (ie, VAF as low as to 0.5%), a mutation can be detected in a higher number of subjects; however, it is still under investigation whether variants detected at this lower threshold have clinical relevance.[11] Some of the studies addressing the role of CHIP for a variety of diseases in the cohort of the UK biobank used a VAF threshold >10% because of the shallow sequencing method applied in the majority of subjects in the UK biobank.[19] Thus, when comparing different CHIP studies, it is mandatory to consider the method of sequencing, as it defines the detection threshold for CHIP and, thus, may significantly impact outcome.

The presence of CHIP increases with age, and in recent years it has been shown to be an important risk factor for cardiovascular diseases[20]. CHIP has been shown to play a major role in patients with atherosclerosis,[20] in heart failure, both with reduced[21,22] and with preserved ejection fraction,[23] and it has also been associated with increased risk of stroke,[24] and with established cardiovascular risk factors such as a higher incidence of type 2 diabetes[25] or chronic kidney disease (CKD) progression.[26]

CHIP IN DEGENERATIVE AORTIC VALVE STENOSIS

With aortic valve stenosis (AVS) being the prototypical cardiovascular age-related disease and CHIP being also linked to increasing age, AVS quickly became the subject of scientific research in this field.

Severe AVS is the most common acquired degenerative valve disease, with a prevalence of 3.4% in patients older than 75 years.[27] An active inflammatory process leads to valve calcification[28] causing narrowing of the valve with a left ventricular outflow tract obstruction, subsequent cardiac overload and compensatory left ventricular hypertrophy, resulting in heart failure[29]. Since no medical treatment for AVS is currently available, the only therapeutic option is transcatheter aortic valve replacement (TAVR) or surgical aortic valve replacement (SAVR) for patients in a serious clinical state. As a result, there is an unmet need for drug therapies that can stop or slow the progression of AVS at earlier stages.

The first study assessing CHIP prevalence and prognostic impact in AVS appeared in 2020 and analyzed the 2 most common CHIP-driver mutations and their clinical implications.[5] Subsequently, a long-term follow-up of the largest AVS population has been reported[6] as well as four studies[1–4] by other groups have been published. An overview of the current available literature in this field is shown in Table.[1–6]

Prevalence of CHIP-Driver Mutations in AVS

Prevalence of CHIP in severe AVS has been investigated in several studies using different next generation sequencing techniques. Although a high prevalence rate has been found in all of the studies, there is some variability across reported frequencies, ranging from 30.3% to 68.2%. The first study in this field assessing the most frequently occurring *DNMT3A* and *TET2* mutations in AVS patients undergoing TAVR reported a frequency of 33.3%.[5] Since subsequent studies in similar populations analyzed a larger spectrum of CHIP-driver mutations, the prevalence was higher in the majority of cases: 30.3% when testing for 9 genes,[1] 36.4% when testing for 16 genes,[3] 34% when testing for 48 genes,[4] and 68% when testing for 67 genes.[2] It is worth noting that different panels with different numbers of mutations have been used in all of these studies, and that standardization will be needed in the future to allow more accurate comparative studies.

With TAVR now being offered to younger and lower-risk patients,[30] comparison of CHIP prevalence in current

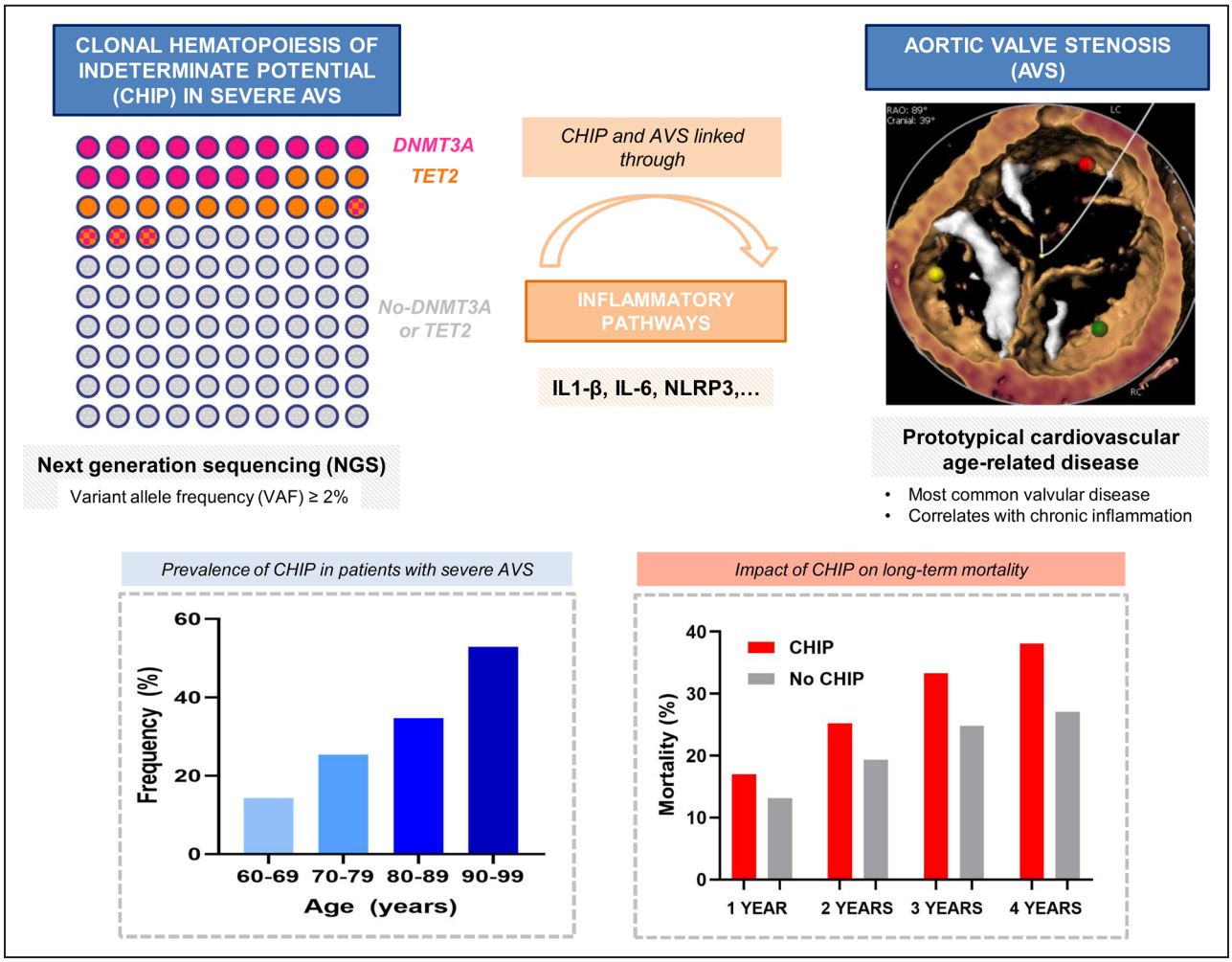

Figure 1. Prevalence of CHIP-driver mutations in severe aortic valve stenosis and impact on long-term mortality. *DNMT3A* and *TET2* are the genes most commonly involved in CHIP-driver mutations in patients with AVS,[1–4] the prototypical cardiovascular age-related disease and the most common heart valve disease. Inflammatory pathways appear to be the link between CHIP and AVS. Prevalence of CHIP-driver mutations shows a clear-cut increase with age (illustration developed by the authors based on data from ref. [5]), and main CHIP mutations are associated with a higher long-term mortality in patients with severe AVS (illustration developed by the authors based on data from ref. [6]).

AVS cohorts versus future cohorts will need to take into account differences in age and comorbidities. In one study, CHIP prevalence was explored in severe AVS in 2 different populations: (a) patients undergoing SAVR and (b) patients undergoing TAVR. The authors performed tests for just 9 CHIP-related genes but found 30.3% of patients with at least one mutation. Interestingly, patients who had undergone SAVR had a lower frequency of CHIP-driver mutations than patients receiving TAVR (25.9% vs 39.3%, respectively). This difference is to be expected, since the patients in SAVR cohorts are generally younger than in TAVR cohorts.[1]

The most common mutations found in all studies were in the following genes, in descending order of frequency, *DNMT3A*, *TET2*, and *ASXL1*. Other mutations involved *PPM1D*, *SF3B1*, *TP53*, *GNAS*, *CBL*, and *JAK2*, among other genes. Single nucleotide variants predominated, with deletions and insertions being observed less often.[4]

In studies assessing the whole spectrum of mutations, many individuals exhibited more than one CHIP-driver variant: 31.4% (16/51) in an Italian Cohort,[1] 40% (16/40) in an Asian cohort,[3] and 59.6% (105/176) in a French cohort.[2] Again, the variability in the percentage of concomitant mutations across studies can be explained by different sequencing methods and by the number of genes tested in each panel.

Differences have also been found in median VAF values in these studies. With VAF ranging usually up to a maximum of about 40%, median VAF of the CHIP variants was 3.49% in the study from Taiwan,[31] and it was similar in the study by Lassalle et al (3%),[2] although interestingly, cases of VAF ≥10% were excluded from the analysis in this latter study. Other investigations

have found somewhat higher median VAF values such as 7.33%[4] and 8.9%.[1] In general, though, most CHIP carriers had relatively small clones. Notably, the currently used VAF threshold of 2% is arbitrary, and a more precise definition will be required in the future.

Clinical and Laboratory Parameters in Patients With CHIP and AVS

As expected, median age in CHIP-carriers was higher than in non-carriers, and prevalence of CHIP-driver mutations was shown to increase with age.[1,2,5] Although only comparable to a certain extent because of the use of whole-exome sequencing of prior landmark studies, the frequency of CHIP-driver mutations in severe AVS patients using targeted sequencing was higher than in a normal population[32] or in patients with coronary artery disease.[20] As for other demographic and baseline clinical characteristics, no significant differences were detected between CHIP-carriers and non-CHIP-carriers in any reported AVS cohort. Strikingly, no significant differences in circulating inflammatory markers were identified either. However, some studies found specific differences in laboratory parameters: slightly worse baseline renal function in CHIP-carriers,[4] slightly lower hemoglobin levels in CHIP-carriers[1] or higher serum ferritin levels in CHIP-carriers,[3] this latter observation possibly indicating an hyperinflammation state.

Potential Impact of CHIP-Induced CKD on Development of AVS

CHIP has also been shown to be a key contributor to diverse kidney-related conditions. Vlasschaert et al.

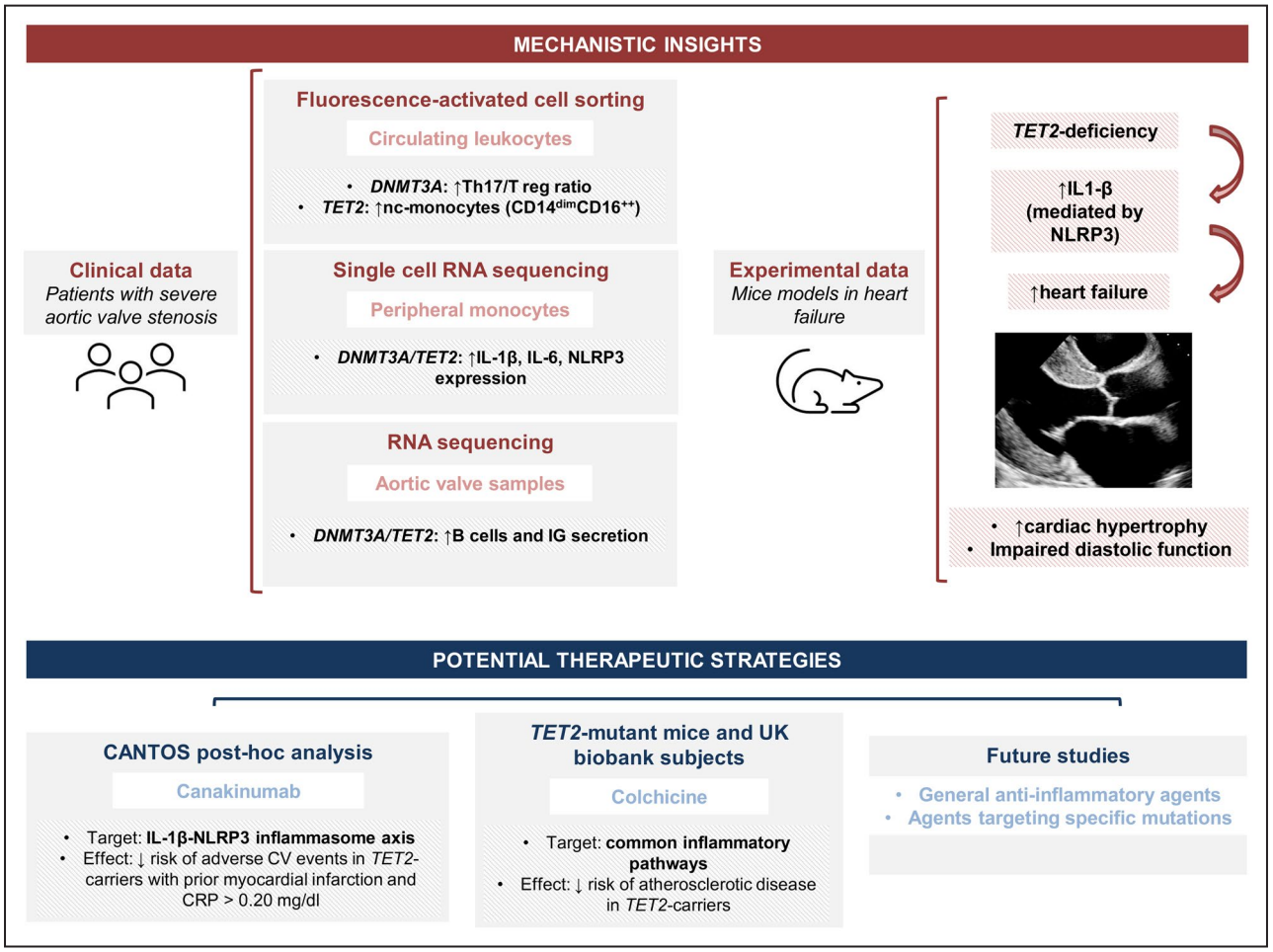

Figure 2. Inflammatory pathways linking CHIP to aortic valve stenosis.
Clinical data suggesting a link between CHIP and AVS are based on RNA sequencing in aortic valve samples,[1] single cell RNA sequencing of peripheral monocytes[7] and fluorescence-activated cell sorting of circulating leukocytes.[5] Experimental data have involved mice models in heart failure relating *TET2* deficiency with inflammatory mediators and heart failure.[8] Potential therapeutic strategies include the use of canakinumab[9] and colchicine.[10] Further studies are warranted with anti-inflammatory agents and potential CHIP-driver mutations tailored agents. CHIP indicates clonal hematopoiesis of indeterminate potential; CRP, C-reactive protein; CV, cardiovascular; DNMT3A, DNA methyltransferase 3 alpha; IG: immunoglobulins; IL-6, interleukin-6; IL-1β (interleukin-1 beta); nc-monocytes, non-classical monocytes; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; TET2, tet methylcytosine dioxygenase 2.

Table. Studies of CHIP-Driver Mutations in Patients With Severe Aortic Valve Stenosis

	No. of patients	Studied mutations in CHIP-driver genes	Threshold for variant allele frequency (VAF)	CHIP prevalence	Follow-up	Patients excluded for survival analysis	Treatment	Geographic area	Main outcomes
Mas-Peiro et al. 2020[5]	279	DNMT3A, TET2	VAF ≥2%	33.3%	8 months	Death <30 days post-procedure	TAVR	Germany	• CHIP-driver mutations occur frequently • Increased proinflammatory leucocyte subsets • Increased mid-term mortality
Mas-Peiro et al. 2023[6]	453	DNMT3A, TET2	VAF ≥2%	32.4%	48 months	Death <30 days post-procedure	TAVR	Germany	• Higher long-term mortality, also in never smokers
Vieceli Dalla Sega et al. 2022[1]	168	9 genes, including DNMT3A, TET2 as most common mutated genes	VAF >2%	30.3%	12 months	Death ≤30 days post-procedure	TAVR and SAVR	Italy	• CHIP-driver mutations are common • Increased mortality at 12 months • B-cells are potential effectors of CHIP-induced inflammation
Lassalle et al. 2023[2]	258	67 genes, including DNMT3A, TET2 as most common mutated genes	VAF (2%–10%)	68%	60 months	Death ≤30 days post-procedure	TAVR	France	• Decrease in overall 5-year mortality in TET2-carriers with VAF between 2% and 10%
Yao et al. 2025[3]	110	16 genes, including DNMT3A, TET2 as most common mutated genes	VAF >2%	36.4%	55.2 months	none	TAVR	Taiwan	• Long-term clinical impact of CHIP in AVS possibly attributed to maladaptive remodeling
Jamin et al. 2025[4]	194	48 genes, including DNMT3A, TET2 as most common mutated genes	VAF ≥2%	34%	36 months	Death ≤90 days post-procedure	TAVR	Germany	• Increased mortality in CHIP-carriers other than DNMT3A • No association with mortality of CHIP-driver mutations with low VAF

CHIP indicates clonal hematopoiesis of indeterminate potential; DNMT3A, DNA methyltransferase 3 alpha; SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement; TET2, tet methylcytosine dioxygenase 2; and VAF, variant allele frequency.

showed that CHIP is associated with an increased risk of acute kidney injury and impaired recovery after renal injury, especially in subjects with CHIP-driver mutations other than *DNMT3A*. Mechanistically, in murine models of acute kidney injury, *TET2*-CHIP and *JAK2*^{V617F}-CHIP carriers showed an aberrant inflammatory response via an increased proinflammatory macrophage infiltration, as well as higher levels of fibrosis after acute kidney injury.[33]

A further study in individuals with preexisting CKD, ie, adults with an estimated glomerular filtration rate <60 mL/min per 1.73, revealed that patients with a CHIP-driver mutation showed a worse baseline renal function as reflected by the Kidney Failure Risk Equation score, and were more prone to progress to a worse CKD stage.[34]

With CKD being a well-known risk factor for AVS,[35,36] CHIP-induced CKD could be a contributor to the progression of AVS, potentially mediated by an aberrant macrophage inflammatory response. In summary, future longitudinal studies are needed to understand the dynamic effects of CHIP on both AVS and CKD and its potential role in mediating or modifying such effects.

CHIP in Incident Aortic Valve Stenosis and in Aortic Valve Sclerosis

No studies have yet investigated the association of CHIP with incident AVS, except for some preliminary research that has only been presented at the American Heart Association Congress in 2021. In a cohort of 6866 individuals above the age of 40 years, the authors found that CHIP mutations were identified in 3.5% of the population and that those harboring a CHIP-driver mutation showed a higher frequency of incident AVS, both assessed by maximal transvalvular velocity (V max) (hazard ratio [HR] = 1.82 [1.03–3.20]; $P = 0.039$) and by aortic valve area (HR = 1.65 [1.07–2.54]; $P = 0.023$).[37] These results could pave the way for exploring the impact of CHIP-driver mutations' inflammatory effects on the pathophysiology of AVS.

Research on the effects of CHIP on mild or moderate AVS is still lacking. However, a recent prospective case–control study has assessed the impact of CHIP on aortic valve sclerosis, which is defined as an aortic valve leaflet thickening or calcification not yet causing hemodynamically relevant stenosis (Vmax <2.5 m/s).[38] Prevalence of aortic valve sclerosis ranges from 9% in patients with a mean age of 54 years to 42% in patients with a mean age of 81 years, with a 1.8% to 1.9% progression to aortic stenosis per year.[39] In this single-center study comprising 187 subjects, it was observed that individuals with aortic valve sclerosis had larger CHIP clones than age- and sex-matched controls. Additionally, aortic valve sclerosis patients also had more CHIP variants. Distributions were as follows: 52.8% versus 48.4% when using a VAF cut-off value ≥0.5%, 39.2% versus 21.0% with VAF ≥1% and 20.0% versus 6.5% with VAF ≥2%, after testing for a total of 24 genes.[40] It is important to highlight that median age of this population was 72.6 ± 8.5 years, which is lower than the age of a typical AVS cohort. This could explain, at least in part, the lower frequency of CHIP-driver mutations found in this entity. Future studies will need to evaluate whether a causal relationship exists between aortic valve sclerosis and CHIP, and whether lower cut-off values should be used for this often considered "pre-stage" of aortic stenosis.

Impact of CHIP on Systolic and Diastolic Dysfunction and Heart Failure in AVS

Little is known about the effects of CHIP on cardiac function in patients with AVS. Only 1 study has found that patients harboring a CHIP-driver mutation have slightly worse baseline left ventricular ejection fraction than those without such mutations (51.6% versus 55.6%, respectively; $P = 0.019$).[4]

Diastolic dysfunction in patients with severe AVS is the result of left ventricular (LV) hypertrophy caused by a constant pressure overload as a compensatory mechanism for the obstruction. Since baseline diastolic dysfunction has been associated with worse clinical outcomes in patients with AVS even after undergoing TAVR,[41] a recent study has focused on analyzing specific diastolic echocardiographic parameters in these high-risk patients. In an Asian population with a total of 110 patients, it was observed that individuals harboring a CHIP variant had smaller left ventricular end-diastolic diameter and left ventricular end-diastolic volume, as well as thicker interventricular septum and thicker posterior wall, indicating an increased LV hypertrophy. Additionally, there was a trend toward an increased E/e' ratio [ratio between the value of early diastolic flow velocity (E velocity) and mitral annulus early diastolic velocity (e')] and there were more cases of severe diastolic dysfunction in CHIP-carriers, suggesting that some of these patients respond to the stenosis with stronger compensatory mechanisms: more hypertrophy, worse LV remodeling and, consequently, worse diastolic function. This could explain the higher heart failure hospitalization rates in these patients, and possibly justify a closer post-TAVR monitoring.[3] Prior investigations assessing diastolic dysfunction in heart failure with preserved ejection fraction from both a clinical and experimental perspective have found similar findings, with CHIP-carriers showing worse parameters of diastolic dysfunction compared with non-CHIP carriers, as assessed by E/e' (14.9 versus 11.7, respectively; $P = 0.0096$) and E/A [ratio between E velocity and late diastolic transmitral flow velocity (A velocity)] (1.69 versus 0.89, respectively; $P = 0.0206$).[42]

Some laboratory parameters could further support the hypothesis of heart failure being one of the causes of worse prognosis in CHIP-carriers, since prior studies found higher NT-proBNP (N-terminal pro-B-type natriuretic peptide)[5] and higher troponin levels,[4] 2 well-established markers of myocardial damage, in these patients.

Although the potential effect of CHIP in AVS has been specifically associated with diastolic dysfunction, additional studies will also be needed to gain further insights on the potential role of systolic dysfunction. Whether patients harboring a CHIP-driver mutation have worse recovery of their cardiac function following LV remodeling remains also to be established; such potential association could translate into worse clinical outcomes and survival.

Association of CHIP With Clinical Outcomes in Patients With Severe AVS

Eugene Braunwald described in 1968 the typical unfavorable prognosis of patients with severe AVS after a long latent period followed by the onset of severe symptoms such as angina, syncope or heart failure.[43] A large study performed in 2018 analyzing echocardiographic

reports of a total of 1 085 850 patients from 24 U.S. hospitals found that among 595 120 patients with available AVS severity assessment, patients with severe AVS had a 4-year all-cause mortality of 44.9% if left untreated.[44] The same authors observed that rates of aortic valve replacement are still low and suggest that further studies are warranted to assess the timing and approach for these therapies.

With high mortality rates being found in patients with aortic valve stenosis and CHIP-driver mutations having been shown to have an impact on mortality in several cardiovascular diseases, extensive research has tried to shed some light into a possible association of CHIP with clinical outcomes in patients with AVS.

The first study that assessed the impact of CHIP variants in 279 patients with severe AVS undergoing TAVR showed that patients with a *DNMT3A* or a *TET2*-CHIP-driver mutation had a worse mid-term survival (8 months after TAVR) than those without a CHIP-driver mutation, even after adjusting for other potentially relevant factors.[5] In a follow-up study with the largest reported cohort of 453 patients, it became apparent that in never smokers, only *TET2*-carriers showed an impaired survival at 4 years.[6] Since these seminal studies, other groups have confirmed similar results in the long-term. Using a panel of 9 genes, a study in a total of 168 patients showed a worse clinical outcome at 12 months after TAVR in patients carrying a CHIP-driver mutation, even after adjusting for potential confounding factors. In fact, this association was also significant when only assessing death attributable to cardiovascular events.[1] Other groups have analyzed CHIP-driver mutations separately and have shown that not all variants have the same impact on long-term prognosis. A study including 258 patients found a significant reduction in mortality 5 years after TAVR in patients with a *TET2*-CHIP driver mutation, alone or associated with other CHIP-driver mutations, with a VAF between 2% and 10%. The authors further observed that patients with large VAF (>10%–40%) did not show this difference in survival,[2] which differs from previous findings in patients with coronary artery disease in which larger clones (VAF > 10%) were associated with an increased risk of cardiovascular events[45]. Interestingly, the same group observed that patients harboring both *DNMT3A* and *TET2* CHIP-driver variants exhibited better survival post-TAVR compared with those with only the *TET2* variant. However, it should be noted that the number of patients in this subgroup is rather small, suggesting a play-by-chance result rather than suggesting a potential protective effect of *DNMT3A* in this subgroup of patients. A more recent study assessed a cohort of 194 patients with a follow-up of up to 4 years and found that patients with a CHIP-driver mutation other than *DNMT3A* had an impact on prognosis. The authors suggest that a possible explanation for this, is that clinical studies rarely differentiate between R882H and non-R882H mutations, whereas experimental studies showing cardiovascular consequences in murine models have primarily focused on the R882H variant.[4] It is important to highlight this difference since mechanistic studies in *DNMT3A*^{R882H} mice have been shown to disrupt epigenetic regulation leading to increased inflammatory and fibrotic responses, an effect that has not yet been observed in other variants.[46] Another potential explanation is that impact on mortality may be primarily driven by *TET2* in most studies. Contrary to the results found in other disorders such as heart failure,[47] low VAF values (0.5%–2.0%) have not shown any clinical significance or impact on mortality in patients with severe AVS so far.[4] Lastly, there is 1 study that has not shown an association of CHIP with mortality but rather demonstrated significantly higher levels of heart failure hospitalization rates in a follow-up of up to 55.2 months in patients carrying a CHIP-driver mutation.[3]

The differences in the impact on mortality can be explained by the heterogeneity within the cohorts; follow-up is different in all studies, and more importantly, the exclusion of patients differs across studies (either no exclusion period, 30 or 90 days exclusion period after the procedure to remove mortality because of peri-procedural complications). The number of CHIP-driver mutations assessed in each study is also variable, with some research groups focusing on the 2 most common ones and others assessing a combination of them. Similarly, for VAF, different thresholds have been used. Ultimately, there is a definite need for standardization to enable comparison between studies.

Currently available results seem to suggest that CHIP-driver mutations with a VAF ≥2% play an important role in clinical outcomes in AVS and that this effect might be driven by *TET2* CHIP-driver mutations. The mechanisms by which CHIP contributes to adverse outcomes in AVS remain to be fully elucidated.

MECHANISTIC INSIGHTS

Mechanisms Underlying Aortic Valve Stenosis

The development of calcified AVS seems to initially derive from changes in mechanical forces and shear stress on the leaflets because of oscillatory movement, that causes an impaired function of valvular endothelial cells (VECs). This is the basis for the endothelial dysfunction that results firstly in infiltration and deposition of lipoproteins (including low-density lipoprotein and Lp(a)) and, secondly, in immune cells reaching the interstitial space in valve tissue.[48] Then, the oxidation of such lipoproteins induced by an endothelial nitric oxide synthase alteration seems to promote apoptosis of

valvular interstitial cells (VICs), resulting in calcification. Cell differentiation into osteogenic phenotypes and the interaction between VICs and VECs in this process appear to be crucial in valve calcification.[49]

Oxidized lipids also result in valve tissue inflammation through the activation of local immune cells infiltrating the tissue.[48] Multiple studies led to the concept that osteoblastic VICs phenotype promoted by cytokines secreted by immune cells is a key factor to initiate calcification.[50] The calcific effect is partly attributable to the activation of toll-like receptors, with inflammatory stimulation inducing the differentiation of aortic valve VICs into an osteogenic phenotype.[51] Such differentiation is also stimulated by immune cells through a process involving cytokines such as TNF (tumor necrosis factor) and IL-1β (interleukin-1 beta) and resulting in calcification.[52,53] The presence of CHIP in circulating cells is known to induce an inflammatory state and this suggests that the potential acceleration of the calcification process in AVS may be attributable to an effect of CHIP on the inflammatory phase of its pathobiology. However, the precise role of CHIP in the homeostasis of VICs and valvular endothelial cell phenotypes is still unclear.

The pathological processes linking CHIP to adverse outcomes in AVS patients probably differ from the putative causal mechanisms through which CHIP contributes to the onset or progression of AVS. While worsening of the systolic or diastolic LVEF and its detrimental consequences such as decompensated heart failure seem to account for a worse prognosis in patients with severe AVS, endothelial dysfunction, matrix remodeling, lipid accumulation or calcification are different physiopathological mechanisms that are involved in the development of AVS. To date, current literature suggests that CHIP seems to be linked to AVS through inflammatory pathways (Figure 2).

Divergent Lineage of the 2 Most Common Mutations: DNMT3A and TET2

The 2 most common genes, *DNMT3A* and *TET2*, are both epigenetic regulators but have distinctive effects on hematopoietic stem cells.[54] Although they have comparable disease phenotypes with loss-of-function effects, they exhibit antagonistic biochemical activities and promote inflammatory states through divergent mechanisms. Both mutations are involved in distinct patterns of lineage restriction. While *DNMT3A* mutations impact multiple hematopoietic lineages, including T cells, *TET2* mutations are restricted to myeloid lineages, primarily affecting monocytes but not T cells.[55,56] Thus, different consequences of the mutations on innate versus adaptive immune mechanisms need to be considered separately. Additionally, the impact of the mutation may be different in each patient depending on the hematopoietic differentiation state at which the mutation originated.

Clinical Data: Inflammatory Signatures

Fluorescence-activated cell sorting analyses on inflammatory phenotypes of specific subsets of circulating leucocytes have shown that in patients with AVS, *DNMT3A*-carriers exhibited a significantly elevated Th17/Treg ratio, indicative of a proinflammatory T-cell polarization. Conversely, patients with a *TET2* CHIP-driver mutation showed elevated levels of circulating non-classical monocytes ($CD14^{dim}CD16^{++}$), which are known to produce high amounts of proinflammatory cytokines such as TNF-α (tumor necrosis factor alpha), IL-1β, and interleukin-8 (IL-8).[5]

In single-cell RNA sequencing (sc-RNA-seq) analyses of circulating peripheral monocytes in individuals with AVS and carriers of a *DNMT3A* or *TET2* CHIP-driver mutation, an increased expression of IL-1β, IL-6 (interleukin-6) receptor, and the NOD-, LRR- and NLRP3 (pyrin domain-containing protein 3) inflammasome complex was found. Additionally, they exhibited an upregulated expression of CD163, which is a cellular receptor involved in the macrophage activation syndrome, as well as other genes that have been associated with cytokine release syndrome.[7]

A further sc-RNA-seq study from the same group assessing the cell intrinsic effects of patients with heart failure harboring *DNMT3A* mutations demonstrated that while mutant monocytes exhibited upregulation of genes linked to inflammation and phagocytosis, T cells and natural killer cells displayed enhanced activation signatures and effector functions.[57]

In line with human data demonstrating an increased IL-6/IL-1β expression in CHIP-carriers, an analysis of the UK Biobank assessing exome sequencing data from 35 416 participants showed that in carriers of large *DNMT3A*- and *TET2* clones (VAF >10%), genetically reduced IL-6 signaling attenuated the risk of cardiovascular disease.[45]

More recent data in CHIP-mutated monocytes from heart failure patients showed, using transcriptomic data from peripheral blood mononuclear cells, that inactivation of *DNMT3A* in macrophages activates cardiac fibroblasts resulting in cardiac fibrosis.[46]

A further study has proposed humoral immunity as a potential contributor of CHIP-driven inflammation. In RNA-Seq analyses of AVS patients harboring *TET2* or *DNMT3A*-CHIP-driver mutations, an increase of B cells and immunoglobulin secretion was observed in comparison with samples with no CHIP or no AVS. This finding was further supported by analysis of a larger cohort of aortic valves, where CHIP carriers exhibited higher transcript levels of immunoglobulins IGKC and IGHG1 compared with non-CHIP samples.[1] These

results are consistent with previous studies suggesting a detrimental effect of the presence of B cells in valve tissue on the progression of the valve disease,[58] with a higher mortality in AVS patients. To the best of our knowledge, the aforementioned study by Sega et al. is the only one, to date, assessing the phenotypic impact of CHIP-driver mutations on the valve tissue itself.

Although *DNMT3A* and *TET2* have opposing enzymatic activities and thus different effects on DNA methylation, several studies have shown similar inflammatory signatures. It is yet to be confirmed whether the harmful effects of CHIP-driver mutations are predominantly caused by a common proinflammatory pathway or functional changes are specific to each mutation. Further mutation-tailored studies are needed to further characterize the specific cellular pathways of each CHIP-driver mutation and enable evaluation of the clinical consequences. This will allow for more general or mutation-specific treatments in the cardiovascular field.

Experimental Data: Causality in Murine Models

To our knowledge, no specific animal models have been yet developed to investigate a possible causal link between CHIP and AVS development or progression. However, murine models of heart failure mimicking the consequences of AVS can point us toward potential common mechanistic pathways at this time.

Experimentally, murine models with transaortic constriction and subsequent heart failure showed that *TET2* deficiency in hematopoietic cells is associated with an impaired cardiac remodeling and worse cardiac function because of increased secretion of IL-1β mediated by NLRP3, suggesting that individuals harboring *TET2*-CHIP-driver mutations can have a higher risk of developing heart failure. Additionally, it was shown that treatment with an IL-1β –NLRP3 inflammasome inhibitor was a protector of development of heart failure in these models.[8] In a murine model with calcified AVS, inhibition of the NLRP3 inflammasome complex was shown to reduce M1 macrophage polarization, downregulate proinflammatory factor levels of IL-6 and TNF-α as well as to mitigate osteogenic calcification.[59] Given the observed diverse effects of the inflammasome complex on AVS, it would be interesting to unravel, in future studies, whether CHIP-induced mouse models would reveal more significant effects along these pathways.

More recently, in a *TET2*-mediated CHIP murine model, several features of heart failure with preserved ejection fraction were exacerbated including cardiac hypertrophy by heart weight/tibia length and cardiomyocyte size, diastolic dysfunction by E/e' and left ventricular end-diastolic pressure, as well as cardiac fibrosis.[42]

Experimental models using clustered regularly interspaced short palindromic repeats (CRISPR)-mediated gene editing have also suggested that both *DNMT3A*- and *TET2*-CHIP-driver mutations contribute to cardiac dysfunction. Even though their pathways to produce such effects may be different since they have divergent effects on leukocytes, both mutations seem to lead to similar increases in cytokine levels. Sano *et al*. demonstrated that *TET2* inactivation produces higher levels of IL-1β, IL-6, and CC motif chemokine ligand 5 (Ccl5), whereas *DNMT3A* inactivation promoted the expression of CXC chemokine ligand (Cxcl1), CXC chemokine ligand 2 (Cxcl2), IL-6, and Ccl5 in response to lipopolysaccharide as an inflammatory stimulus.[60]

Mechanistic experiments with *ASXL1* have also shown a proinflammatory phenotype associated to macrophages resulting in increased expression of transcripts encoding for IL-1β and IL-6. The increase in cytokines that contributes to cardiac inflammation is consistent with the proposed underlying mechanism that could cause a rapid progression of heart failure.[61]

In another model to study *ASXL1* functions, CHIP mutations were induced and *ASXL1* mutant murine macrophages were shown to exhibit elevated IL-1β expression and increased DNA damage, both of which contribute to enhanced activation of the absent in melanoma 2 (AIM2) inflammasome.[62]

The different patterns in inflammatory gene expression induced by the mutations in the various CHIP driver genes could explain the different impact of different CHIP-driver mutations on clinical outcomes of AVS patients.

Other Mechanistic Hypotheses

A recent review suggested a potential interplay between telomeres and CHIP in AVS. Aging is known for causing telomere shortening and consequent genomic instability and eventually CHIP. CHIP causes a clonal expansion of mutant circulating leukocytes that increases inflammation and leads to an increased shortening of telomers. This process is thought to promote senescence and dysfunction at a valvular level causing AVS.[63] Further studies supporting this hypothesis in the field of atherosclerosis have observed that CHIP-carriers have shorter leukocyte telomer length,[64] that CHIP is more common in individuals with shorter leukocyte telomer length, and that age-dependent telomere length shortening has an impact on the proliferative system.[65] Thus, this potential link between telomere dynamics and CHIP could be helpful to explore future therapies for prevention or treatment of AVS.

Although several mechanistic insights have been gained, future research is still needed to elucidate how CHIP contributes to AVS and its prognosis.

PROSPECTS FOR NOVEL THERAPEUTIC STRATEGIES

Regarding future therapies, a large study including 63 700 patients from 5 randomized trials has shown that established therapies for cardiovascular disease, including treatments targeting the proteins PCSK9 (proprotein convertase subtilisin/kexin type 9), SGLT2 (sodium-glucose cotransporter 2), P2Y12 (purinergic receptor P2Y12), and FXa (Factor Xa), do not seem to be more beneficial in patients harboring a CHIP-driver mutation.[66]

Much attention has been paid to the IL-1ß-NLRP3 inflammasome axis. A post-hoc analysis of the CANTOS (Canakinumab Antiinflammatory Thrombosis Outcomes Study) trial evaluated canakinumab, an anti-IL-1β antibody, as a potential therapeutical target in patients harboring a *TET2*-CHIP-driver mutation with elevated inflammation parameters (C-reactive protein levels >0.20 mg/dL). It was shown that this subset of patients had a reduced risk of major adverse cardiovascular events when treated with this anti-inflammatory agent.[9]

Studies combining both genetic analyses in humans and experiments in mice tested classic agents such as colchicine, a well-known drug targeting general inflammatory pathways, and demonstrated that it attenuates the risk of atherosclerotic disease in *TET2* carriers.[10]

Other anti-inflammatory agents are currently under development. Efforts should focus on developing both general (eg, agents such as colchicine) and more specific (eg, monoclonal antibodies) inflammatory therapies targeting inflammatory pathways. Agents directly targeting the gene abnormality could be better suited to interfere with CHIP-specific mechanisms. Future trials are on their way to evaluate precision medicine strategies tailored to the consequences of specific CHIP-driver mutations in cardiovascular diseases. Although studies on potential personalized treatments for CHIP-carriers in AVS are still lacking, agents targeting well-established inflammatory pathways such as proinflammatory cytokines (eg, IL-1β, IL-6, TNF-α) or the NLRP3 inflammasome complex particularly in *TET2*-carriers may offer a promising line of research. Additionally, recent data suggesting increased fibroblast activation and cardiac fibrosis specifically in *DNMT3A*-carriers may pave the way for future research on anti-fibrotic strategies.

FUTURE PERSPECTIVES

CHIP and Progression of Aortic Valve Stenosis

Existing data focus on both clinical and experimental research and on determining the impact on the prognosis of severe AVS. However, further research is needed to assess the effects of CHIP-driver mutations both on the incidence and on the progression of milder stages of aortic stenosis. Efforts should be made to carry out a longitudinal study assessing the valve stenosis and changes in clinical severity at different timepoints in patients with or without CHIP-driver mutations. Additionally, it should be assessed whether VAF increases in CHIP-carriers throughout time and whether severity of AVS worsens with the increase in VAF. The fact that CHIP causes an inflammatory cytokine upregulation could explain, in part, the cause or progression of aortic valve degeneration. Understanding these mechanisms will enable the development of therapies that would allow us to intervene at an earlier stage and contribute to a slower progression of the stenosis.

Other Somatic Mutations in Aortic Valve Stenosis and Their Potential Interactions

Chromosomal alterations have recently emerged in the field of acquired mutations and AVS. Particularly, mosaic loss of Y chromosome (mLOY) in blood cells, the most common acquired somatic mutation in men, has been shown to be associated with a worse clinical long-term outcome, even after successful TAVR.[67] In terms of underlying mechanisms, it was shown that circulating monocytes lacking expression of Y chromosome-encoded genes exhibited a pro-fibrotic gene signature marked by increased sensitivity to transforming growth factor β signaling. Similar results have also been found in murine models of heart failure mimicking the consequences of AVS by transaortic constriction, showing that mLOY induces cardiac fibrosis.[68] Moreover, patients with chronic renal failure as well as patients undergoing coronary angiography were shown to be at increased risk for cardiovascular mortality because of increased markers of diffuse cardiac fibrosis.[69,70] As diffuse cardiac fibrosis is a major determinant of worse outcome in patients with AVS even after successful removal of the stenotic valve by surgery or TAVR,[71] the (undetected) co-occurrence of CHIP and mLOY may have contributed to some of the heterogeneous results addressing the prognostic significance of CHIP in male patients. Indeed, a very recent study in male patients with chronic heart failure demonstrated that 26% of patients simultaneously harbored *DNMT3A/TET2* CHIP-driver mutations and mLOY when using a cut-off value ≥17% and that the co-occurrence of harboring mLOY and *DNMT3A/TET2* mutations significantly contributed to the increased mortality observed in carriers of *DNMT3A/TET2* mutations. Mechanistically, in men with chronic heart failure carrying both *DNMT3A* and mLOY mutations, an upregulation of proinflammatory genes was observed

in monocytes, such as alarmins (S100A8, HMGB2) and interferon-related genes (*IFNGR1*, *TRIM56*, *CD84*).[72] As CHIP and mLOY are both strictly age-dependent and also share some common genetic drivers,[73] future studies will need to assess possible interactions with different types of acquired somatic mutations as well as their prognostic impact.

Conversely, prior studies have found that genes that escape X-chromosome inactivation such as *BMX* and *STS* (encoding for Bmx nonreceptor tyrosine kinase and steroid sulfatase, respectively) contribute, in part, to the regulation of increased myofibroblast activation in female subjects. Additionally, treatment of sex-specific VICs (precursors to profibrotic myofibroblasts) with endothelin-1 and plasminogen activator inhibitor-1, which are factors known to drive myofibroblast activation, confirmed these findings.[74]

Taken together, molecular mechanisms involved in the pathogenesis of aortic valve stenosis seem to be, in part, sex dependent and future therapies may need to focus on sex-specific precision medicine.

In summary, current available literature suggests that CHIP-driver mutations with a VAF ≥2% are prevalent in patients with aortic valve stenosis, and that these mutations have a significant impact on prognosis even after replacement of the stenotic valve. Common and more gene-specific inflammatory pathways seem to explain this effect mechanistically; however, further experimental research is still needed to elucidate this. Future trials will need to focus on precision medicine therapies tailored to each specific CHIP-driver mutation.

ARTICLE INFORMATION

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Disclosures

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