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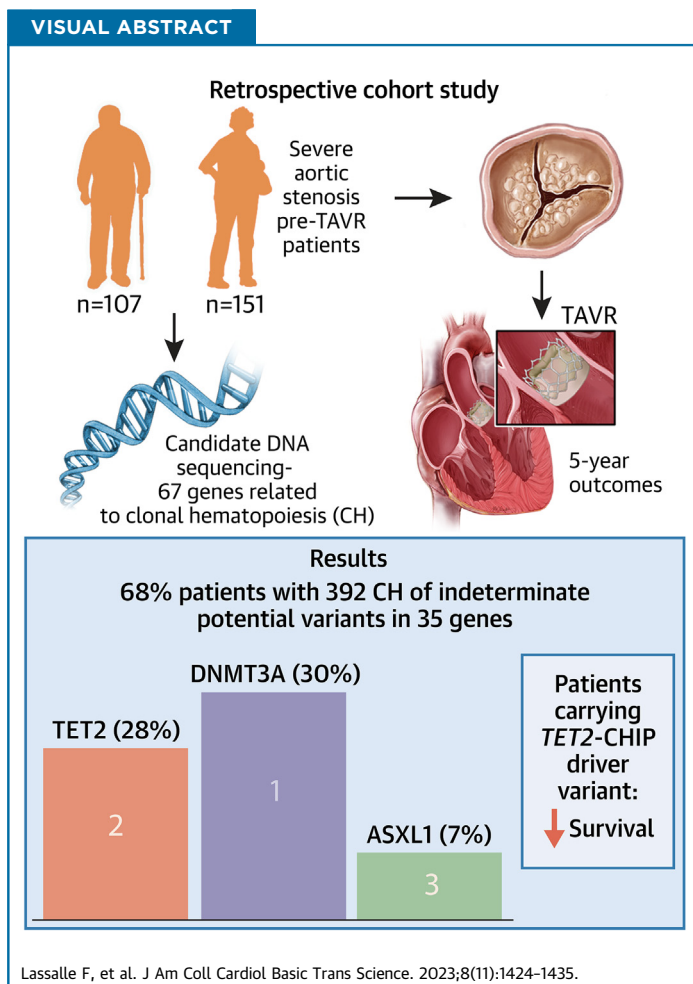
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ORIGINAL RESEARCH - CLINICAL

# Negative Impact of *TET2* Mutations on Long-Term Survival After Transcatheter Aortic Valve Replacement



Fanny Lassalle, PHARM.D, PH.D,<sup>a</sup> Nicolas Duployez, PHARM.D, PH.D,<sup>b</sup> Flavien Vincent, MD, PH.D,<sup>a</sup> Antoine Rauch, MD, PH.D,<sup>a</sup> Tom Denimal, MD,<sup>a</sup> Mickael Rosa, PH.D,<sup>a</sup> Julien Labreuche, BST,<sup>c</sup> David Dombrowicz, PH.D,<sup>a</sup> Bart Staels, PH.D,<sup>a</sup> Claude Preudhomme, PHARM, PH.D,<sup>b</sup> Sophie Susen, MD, PH.D,<sup>a</sup> Eric Van Belle, MD, PH.D,<sup>a</sup> Annabelle Dupont, PHARM.D, PH.D<sup>a</sup>



**HIGHLIGHTS**

- AVS is associated with a high prevalence and large spectrum of CHIP-driver variants.
- Patients carrying *TET2* mutations with low VAF (2%-10%) have a decreased overall 5-year survival after TAVR.
- These findings could provide a novel tool for prognosis assessment in patients with severe AVS undergoing TAVR.
- Future functional studies in relevant preclinical models are needed to investigate the mechanisms by which CHIP-driver variants contribute to AVS and impact the clinical prognosis after TAVR, especially in *TET2*.

From the <sup>a</sup>University of Lille, Inserm, Centre Hospitalier Universitaire Lille, Institut Pasteur de Lille, U1011-EGID, Lille, France; <sup>b</sup>Unité Mixte de Recherche (UMR) 9020-UMR-S 1277-Canther-Cancer Heterogeneity, Plasticity and Resistance to Therapies, Institut de Recherche contre le Cancer de Lille, University of Lille, CNRS, Inserm, Centre Hospitalier Universitaire Lille, Lille, France; and the <sup>c</sup>Department of Biostatistics, Centre Hospitalier Universitaire Lille, Lille, France.

## SUMMARY

Clonal hematopoiesis of indeterminate potential (CHIP) is considered as being a novel age-related risk factor for cardiovascular diseases. By capture-sequencing of a 67-gene panel, we established a large spectrum of CHIP in 258 patients with aortic valve stenosis undergoing transcatheter aortic valve replacement (TAVR) and assessed their association with long-term survival after TAVR. One or several CHIP variants in 35 genes were identified in 68% of the cohort, *DNMT3A* and *TET2* being the 2 most frequently mutated genes. Patients carrying a *TET2*-CHIP-driver variant with low variant allele frequency (2%-10%) had a significant decrease in overall survival 5 years after TAVR. (J Am Coll Cardiol Basic Trans Science 2023;8:1424-1435) © 2023 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## ABBREVIATIONS AND ACRONYMS

**AVS** = aortic valve stenosis  
**CHIP** = clonal hematopoiesis of indeterminate potential  
**CRP** = C-reactive protein  
**IL** = interleukin  
**TAVR** = transcatheter aortic valve replacement  
**TNF** = tumor necrosis factor  
**VAF** = variant allele frequency  
**vWF:Ag** = von Willebrand factor antigen

**A**ortic valve stenosis (AVS) is the most prevalent valve disease in the elderly with an estimated prevalence of 2% to 7% in the population aged 65 years and older worldwide and a significant morbidity and mortality when left untreated.<sup>1</sup> The valve calcification leading to AVS is an active inflammatory process developing through an initial endothelial injury induced by high shear stress and close to atherosclerosis.<sup>2</sup> Over the past couple of years, transcatheter aortic valve replacement (TAVR) has become the recommended technique for patients with severe AVS at high surgical risk as an alternative for surgical valve replacement, and its indications are even being extended to patients at moderate and low surgical risk.<sup>3-5</sup> However, despite a clear improvement in the patient's prognosis and quality of life, our team and others reported that TAVR remains associated with significant bleeding or thrombotic post-procedural complications like strokes or gastrointestinal bleedings,<sup>6-9</sup> which could be associated with systemic inflammation that occurs in more than 50% of patients within 48 hours after TAVR. Some teams tried to predict these TAVR complications by studying circulating biomarkers related to inflammation like C-reactive protein (CRP),<sup>10-12</sup> N-terminal prohormone of B-type natriuretic peptide,<sup>13</sup> platelet- or neutrophil-to-lymphocyte ratio<sup>14,15</sup> or von Willebrand factor (vWF),<sup>8</sup> but even if the results are suggesting that these circulating markers can help to predict TAVR complications, they need to be tested in larger randomized populations. Clonal hematopoiesis of indeterminate potential (CHIP), defined as the presence of an expanded somatic blood-cell clone in persons without other

hematologic abnormalities, has been recently described as a novel age-related risk factor for cardiovascular diseases.<sup>16,17</sup> The most frequently involved genes are *DNMT3A* and *TET2*, which have not only a role in the regulation of DNA methylation but also in proinflammatory pathways in monocytes and macrophages.<sup>18,19</sup> *TET2* has been specifically studied in animal models of cardiovascular diseases and it has been shown that its loss of function leads to accelerated atherosclerosis development in atherosclerosis-prone mice<sup>17,20</sup> and also to a greater cardiac dysfunction in murine models of heart failure,<sup>21</sup> by altering inflammatory responses. Recently, Mas-Peiro et al<sup>22</sup> studied the incidence of CHIP-driver variants in *DNMT3A* and *TET2* in a cohort of 279 patients with severe AVS undergoing TAVR and they reported that 33.3% of patients showed a CHIP-driver variant in *DNMT3A* and *TET2*, and that the presence of these variants was associated with a decreased overall survival during a median 8-month follow-up period after TAVR. However, this study was limited to the 2 most common CHIP genes and did not deliver a large profile of CHIP-driver genes in an AVS cohort and their clinical association. Moreover, the effect of *TET2* was not separated from *DNMT3A*. In our study, we aimed to describe a large spectrum of CHIP-driver genes in a cohort of patients with severe AVS undergoing TAVR and to associate its presence with long-term survival after TAVR. We also aimed to assess the potential prognostic significance of CHIP-driver variants with a variant allele frequency (VAF)  $\geq 2\%$  and under the threshold of 10%, which is considered as low VAF,<sup>23</sup> and to distinguish the impact of *TET2* from other CHIP-driver genes on prognosis.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the [Author Center](#).

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## METHODS

**PATIENTS.** A retrospective cohort of 266 patients with severe AVS undergoing TAVR at the Lille University Hospital (CHU Lille) between 2011 and 2020 was studied. Patients were included from 3 different protocols registered as clinical trials (NCT02628509, NCT02972008, and NCT03728049). Patient inclusion and exclusion criteria, TAVR procedure, and patient follow-up were identical for the 3 cohorts. All patients provided written informed consent. Clinical and biological data were prospectively collected at the time of admission for TAVR. The molecular analysis was performed retrospectively in October 2021 on a sample before the TAVR procedure. Eight patients were finally excluded because they had a history of hematologic disease. The study was approved by the ethics committee and complies with the Declaration of Helsinki.

**MOLECULAR ANALYSIS.** Genomic DNA was extracted using standard procedures and studied by captured-based high-throughput sequencing. Libraries were prepared according to the Twist NGS target enrichment solution (Twist BioScience) following the manufacturer's instructions with a 67-gene panel (Supplemental Table 1) and subsequently run on NovaSeq (Illumina). Raw sequencing data were analyzed with MuTect2 and Vardict for variant calling and the in-house NGSreport Software (CHU Lille) for data visualization, elimination of sequencing/mapping errors, and retention of variants with high-quality metrics. A depth of coverage of at least 1,500× was obtained for all genes. Variants were retained for final interpretation as they were carried by at least 25 reads. Identified variants were named according to the rules of the Human Genome Variation Society. Variant interpretation was performed considering minor allele frequencies in the public GnomAD database of polymorphisms (variants with minor allele frequency >0.02 in overall population/global ancestry or subcontinental ancestry were excluded), variant allele frequencies, prevalence, and clinical interpretation in our in-house database. Frameshift and nonsense variants were always considered as relevant variants and additional in silico predictions were performed when possible on missense and splicing variants. We determined sequencing data variability and threshold of our high-throughput sequencing technology by serial sequencing of the multiplex NGS Tru-Q DNA 7 (Horizon Diagnostics) and the measured VAF for a variant with an expected VAF of 2% was between 1.1% and 2.9 %.

**LABORATORY MEASUREMENTS.** Blood samples were obtained the day before TAVR and between 24 and 72 hours after TAVR, and were analyzed for a complete blood count and CRP by standard methods as part of a patient's care at the Biology and Pathology Center (CHU Lille, France). Von Willebrand factor antigen (vWF:Ag) was measured using an immunoturbidimetric assay (LIAPHEN vWF:Ag, HYPHEN BioMed) on a CS 2400 analyzer (Sysmex), and von Willebrand factor collagen binding (vWF:CB) using an enzyme-linked immunosorbent assay, as previously described<sup>24</sup> with the use of equine type 1 collagen (Horm, Takeda Pharmaceutical). Interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)α concentrations were assessed using the ELLA Automated Immunoassay System (ProteinSimple).

**STATISTICAL ANALYSES.** Continuous variables are expressed as medians (25th-75th percentiles [Q1-Q3]) and categorical variables as frequencies (percentage). Gaussian distribution was tested through the Shapiro-Wilk normality test. The Mann-Whitney *U* test (continuous variables) and the Fisher's exact test (categorical variables) were used for comparisons between groups. Kaplan-Meier (log-rank test) and univariable Cox regression analyses were used to compare survival among the individual groups after exclusion of patients who died within 30 days after the procedure. The proportional hazard assumptions were assessed by using Schoenfeld residuals plots and the Grambsch and Therneau test, and no strong deviation (all *P* values >0.20) were found. Results are presented with HR and 95% CI. In addition, a multivariable Cox regression was performed to account for the potential effect of confounding variables (age, gender, renal insufficiency, heart failure, coronary and peripheral artery diseases, previous ischemic stroke, and atrial fibrillation). A 2-sided *P* value <0.05 was considered to be statistically significant. Statistical analyses were performed using GraphPad Prism software version 9.3 and SPSS version 24.0 (IBM Corp).

## RESULTS

### HIGH FREQUENCY OF CHIP IN PATIENTS WITH AVS.

The initial cohort included 111 men and 155 women. Eight patients (4 men and 4 women) were then excluded because they had a history of hematologic disease. Therefore, the study cohort was composed of 107 (41%) men and 151 (59%) women with a median age of 84 years (Q1-Q3: 79-87 years). Their characteristics are presented in Table 1. A total of 176 patients (68% of the whole cohort) presented at least

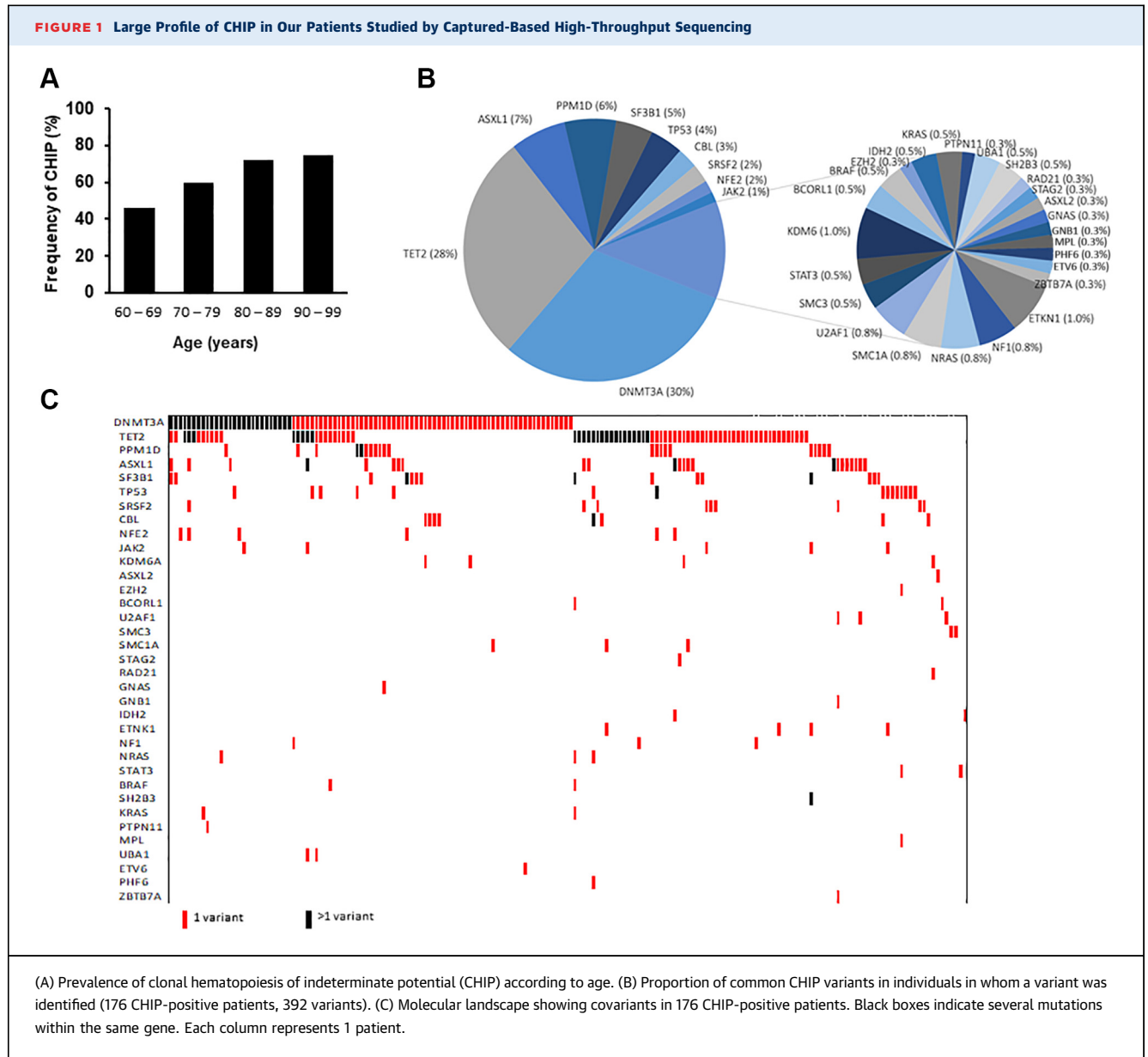
**TABLE 1 Clinical and Biological Characteristics in Patients With and Without CHIP-Driver Mutations**

	All Patients (N = 258)	CHIP-Positive (VAF ≥2%) (n = 176)	CHIP-Negative (n = 82)	P Value
Male	107 (42)	70 (40)	37 (45)	0.42
Age, y	84 (79-87)	84 (80-87)	82 (76-86)	<b>0.009</b>
≤69	13 (5)	6 (3)	7 (9)	0.093
70-79	57 (22)	34 (19)	23 (28)	
80-89	160 (62)	115 (65)	45 (55)	
≥90	28 (11)	21 (12)	7 (9)	
<b>Medical history and CV risk factors</b>				
BMI (kg/m <sup>2</sup> )	27.2 (23.6-30.1)	27.1 (23.5-29.8)	27.6 (23.8-31.6)	0.46
Smoking	32 (12)	21 (12)	11 (13)	0.84
Diabetes	75 (29)	50 (28)	25 (30)	0.77
Arterial hypertension	192 (74)	127 (72)	65 (79)	0.28
Dyslipidemia	123 (48)	80 (45)	43 (52)	0.35
Atrial fibrillation	82 (32)	58 (33)	24 (29)	0.57
Renal insufficiency	113 (44)	79 (45)	34 (41)	0.69
Heart failure	43 (17)	33 (19)	10 (12)	0.21
Coronary artery disease	109 (42)	74 (42)	35 (43)	>0.99
Previous myocardial infarction	22 (9)	19 (11)	3 (4)	0.059
Previous ischemic stroke	42 (17)	25 (14)	17 (21)	0.21
Peripheral artery disease	42 (17)	26 (15)	16 (20)	0.37
Logistic EuroScore	16.9 (12.1-25.1)	17.1 (12.3-27.3)	15.5 (10.4-24.1)	0.23
EuroScore II	3.75 (2.31-6.70)	4.11 (2.60-7.43)	3.25 (1.95-5.84)	0.049
STS score	4.28 (3.11-6.07)	4.30 (3.17-6.05)	3.91 (3.01-6.34)	0.51
CHA2DS2-VASc score	4 (4-5)	4 (4-5)	4 (4-5)	0.84
<b>Biological parameters at admission</b>				
Leucocytes (g/L)	6.5 (5.2-8.0)	6.6 (5.2-8.0)	6.4 (5.4-8.1)	0.96
Neutrophils (g/L)	4.3 (3.4-5.5)	4.3 (3.4-5.2)	4.1 (3.3-5.9)	0.91
Lymphocytes (g/L)	1.3 (0.9-1.7)	1.3 (0.9-1.7)	1.2 (0.9-1.6)	0.68
Monocytes (g/L)	0.5 (0.4-0.7)	0.5 (0.4-0.8)	0.6 (0.4-0.7)	0.68
Platelets (g/L)	220 (170-268)	223 (171-271)	211 (169-257)	0.44
C-reactive protein (mg/L)	1 (1-7)	1 (1-7)	1 (1-7)	0.56
vWF:Ag (U/L)	174 (133-225)	173 (132-224)	180 (133-231)	0.86
Ratio vWF:CB/vWF:Ag	0.74 (0.63-0.85)	0.73 (0.60-0.84)	0.75 (0.65-0.86)	0.63

Values are n (%) or median (25th-75th percentiles).  
 BMI = body mass index; CHIP = clonal hematopoiesis of indeterminate potential; CV = cardiovascular; VAF = variant allele frequency; vWF:Ag = von Willebrand factor antigen; vWF:CB = von Willebrand factor collagen binding.

1 CHIP-driver mutation with a VAF ≥2%, which was the sensitivity reached with the technique (they will be called CHIP-positive). No variant was identified in only 82 patients (CHIP-negative, 32% of the total cohort). The median age of CHIP-positive patients was significantly higher than CHIP-negative patients (84 [Q1-Q3: 80-87] years vs 82 [Q1-Q3: 76-86] years, *P* = 0.009). No difference was found in the prevalence of CHIP between men and women. There were no significant differences in any clinical and biological parameters between CHIP-positive and CHIP-negative patients. As it was previously described in the general population,<sup>25</sup> the prevalence of CHIP increased with age in our AVS cohort, with a prevalence of 46%, 60%, 72%, and 75% of patients in the age groups of ≤69 years, 70-79 years, 80-89 years, and ≥90 years, respectively (Figure 1A).

**HIGH DIVERSITY OF CHIP-DRIVER MUTATIONS IN PATIENTS WITH AVS.** A total of 392 variants in 35 genes were found in 176 patients. A large spectrum of CHIP variants is described in Figures 1B and 1C. The most common genes affected were *DNMT3A* and *TET2*, concerning 30% and 28% of the total variants identified, respectively. Eighty-nine patients carried a variant in *DNMT3A* and 77 patients in *TET2*, including 25 with both genes affected. Other recurrently affected genes were *ASXL1* (n = 23, 7% of total variants), *PPM1D* (n = 21, 6%), *SF3B1* (n = 15, 5%), *TP53* (n = 15, 4%), *SRSF2* (n = 9, 2%), *CBL* (n = 8, 2%), and *NFE2* (n = 6, 2%). The variant V617F in *JAK2* was identified in 5 patients. The median number of variants detected in each patient among those CHIP-positive was 2 (Q1-Q3: 1-3). The median VAF was 3% (Q1-Q3: 2%-8%) (Figure 2). Seventy-one patients



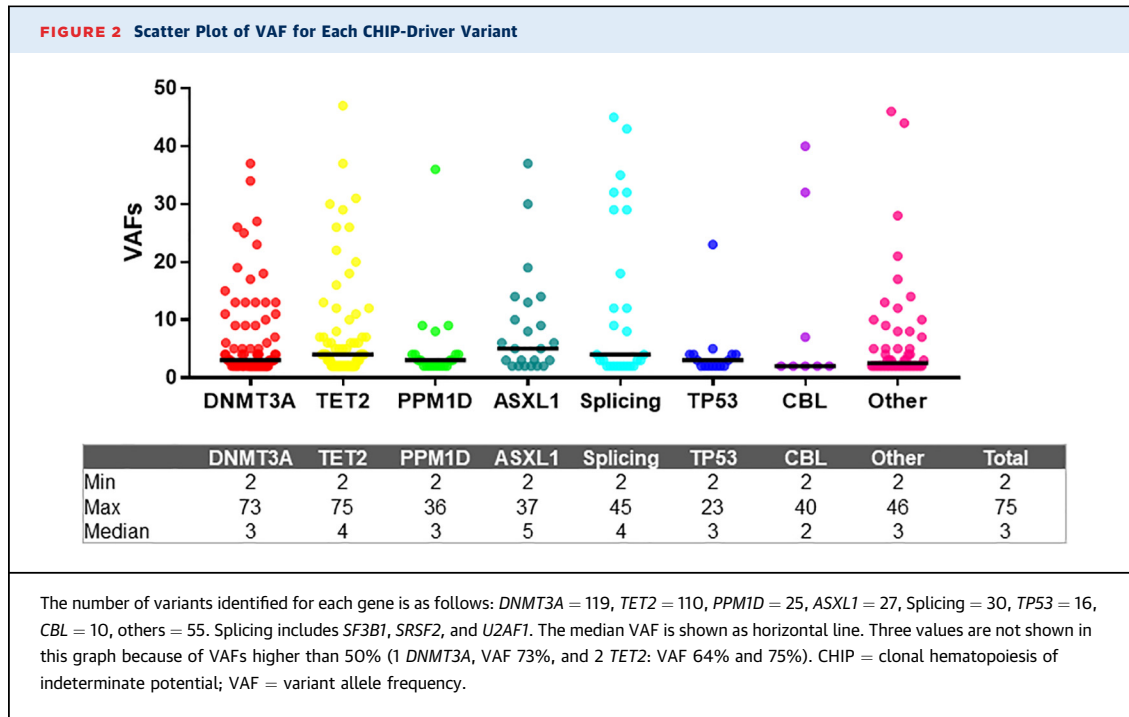
(A) Prevalence of clonal hematopoiesis of indeterminate potential (CHIP) according to age. (B) Proportion of common CHIP variants in individuals in whom a variant was identified (176 CHIP-positive patients, 392 variants). (C) Molecular landscape showing covariants in 176 CHIP-positive patients. Black boxes indicate several mutations within the same gene. Each column represents 1 patient.

(40%) carried only 1 CHIP-driver variant and 105 carried 2 to 10 variants. Seven patients had a VAF >40%, even though they were not known for hematological disease, they were excluded from survival analysis, to eliminate heterozygous single nucleotide polymorphisms. In patients with CHIP, the number of CHIP-driver variants and the median VAF did not significantly vary with a patient's age. Among the 392 variants identified, 262 were single nucleotide substitutions, mainly transitions (63%) over transversions (37%), especially the transition C to T that has been previously described as a mutational signature characteristic of aging also being

predominant in clonal hematopoiesis (Supplemental Figure 1).<sup>26-29</sup>

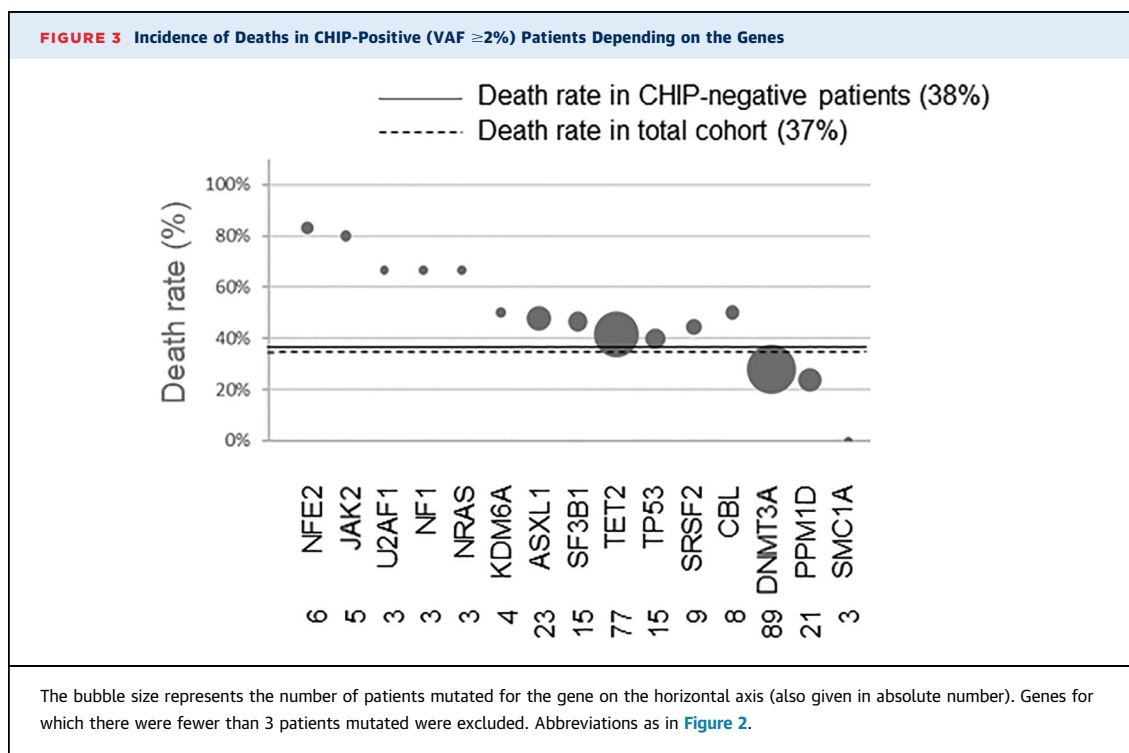
**THE WORSE PROGNOSIS FOR PATIENTS CARRYING A TET2-CHIP-DRIVER VARIANT.** During the 5 years of follow-up after TAVR, 95 patients died (37%), including 64 CHIP-positive and 31 CHIP-negative patients. The death rate of patients with AVS depending on CHIP-driver genes is presented as a bubble chart in Figure 3. We excluded the genes for which there were fewer than 3 patients mutated, as the death rate would not be interpretable. For further analyses, we excluded patients with large VAF (>10%, n = 53) in order to focus on the effect

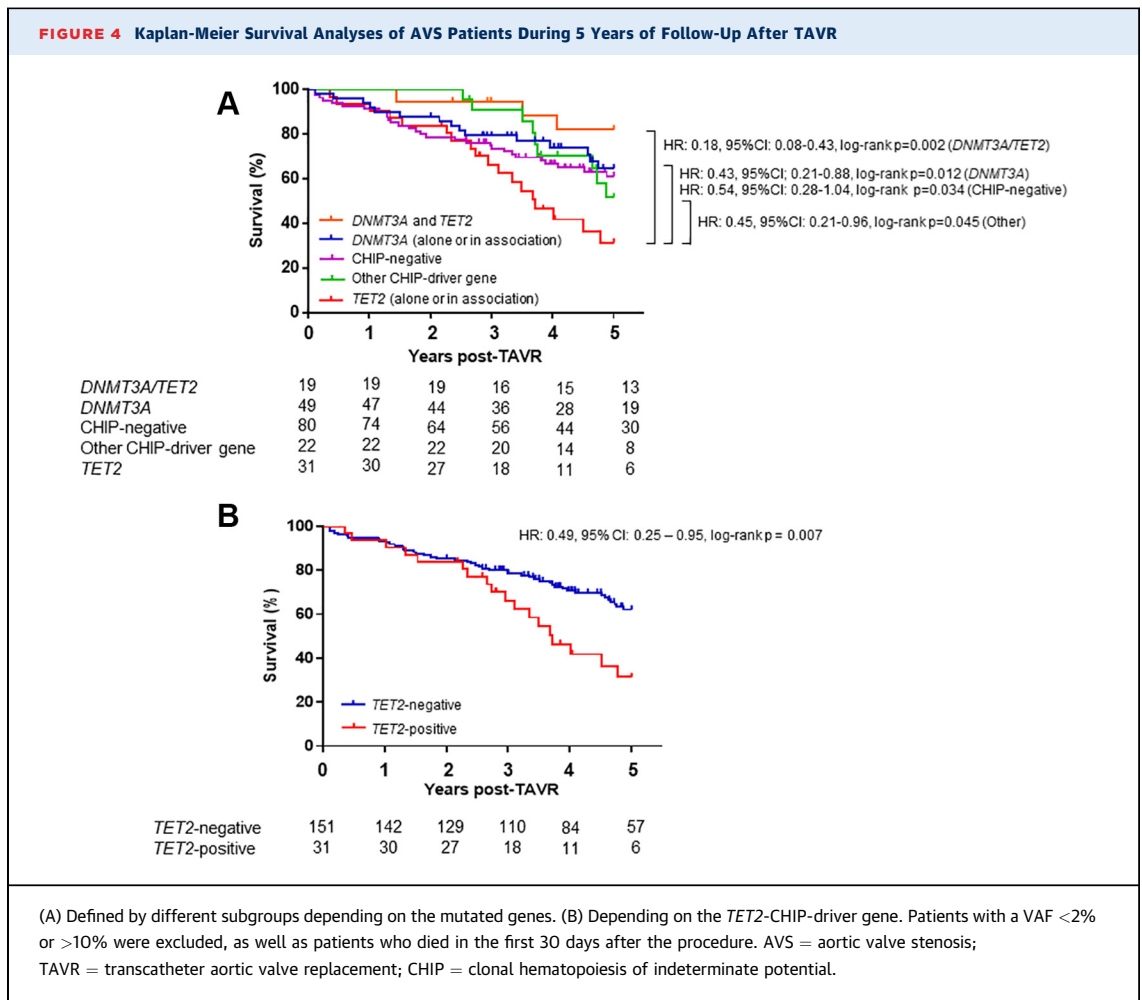




of low VAF (2%-10%). Four patients (2 CHIP-negative, 1 with both *DNMT3A* and *TET2* variants, and 1 with *TET2*-CHIP-driver variant) died within 30 days of TAVR and were also excluded, leading to

a total of 201 patients for survival analysis after TAVR (37% male), including 74 who died. The median time of follow-up was 4.0 years (Q1-Q3: 2.8-5.0 years).





We separated the CHIP-positive patients into different subgroups depending on their mutated genes: *DNMT3A* alone or in association (except *TET2*,  $n = 49$ ), *TET2* alone or in association (except *DNMT3A*,  $n = 31$ ), *DNMT3A* and *TET2* associated ( $n = 19$ ), another CHIP-driver gene ( $n = 22$ ), and CHIP-negative ( $n = 80$ ). Kaplan-Meier survival analysis (Figure 4A) showed that patients carrying a *TET2*-CHIP-driver variant, alone or associated with a variant in another CHIP-driver gene (except *DNMT3A*), had a significantly decreased overall survival 5 years after TAVR in comparison with patients carrying CHIP-driver variants in *DNMT3A* alone or in association (except *TET2*) (HR: 0.43; 95% CI: 0.21-0.88; log-rank  $P = 0.012$ ), in other CHIP-driver genes (HR: 0.45; 95% CI: 0.21-0.96; log-rank  $P = 0.045$ ), with patients carrying both *DNMT3A* and *TET2* CHIP-driver variants (HR: 0.18; 95% CI: 0.08-0.43; log-rank  $P = 0.002$ ), and with CHIP-negative patients (HR:

0.54; 95% CI: 0.28-1.04; log-rank  $P = 0.034$ ). Concerning patients with large VAF (>10% to 40%,  $n = 46$ ), there were no significant differences for overall survival 5 years after TAVR compared with patients with low VAF (2%-10%) ( $P = 0.74$ ) and according to mutated genes ( $P > 0.05$ ) or *TET2* status ( $P = 0.27$ ). This lack of significance may be due to the small number of patients in each group. When we compared the overall survival between *TET2*-positive patients ( $n = 32$ ) and all *TET2*-negative patients (including *DNMT3A*, other CHIP-driver genes and CHIP-negative patients,  $n = 153$ ) after exclusion of patients died within 30 days of TAVR, we observed that patients carrying a *TET2*-CHIP-driver variant with VAF between 2% and 10% had significantly lower survival than patients without a *TET2*-CHIP-driver variant (Figure 4B) (HR: 0.49; 95% CI: 0.25-0.95; log-rank  $P = 0.007$ ). *TET2*-positive patients did not have a higher VAF than *TET2*-negative patients



**TABLE 2 Clinical and Biological Characteristics in Patients Depending on *TET2* Identification**

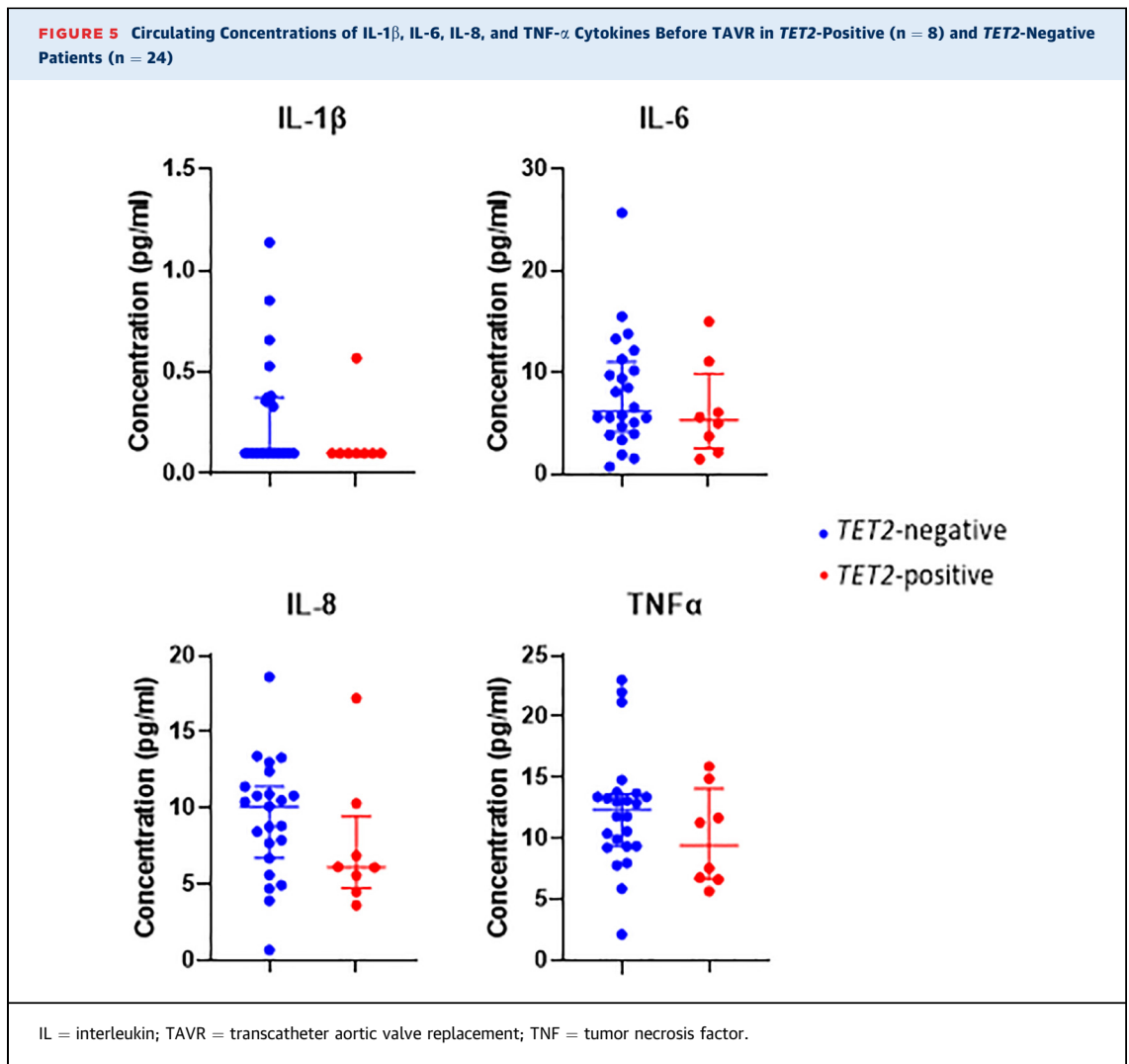
	All Patients (N = 185)	<i>TET2</i> -Positive (n = 32)	<i>TET2</i> -Negative (n = 153)	P Value
Male	72 (39)	14 (44)	58 (38)	0.56
Age, y	84 (79-87)	85 (82-88)	84 (78-86)	0.075
≤69	12 (6)	1 (3)	11 (7)	0.20
70-79	40 (22)	3 (9)	37 (24)	
80-89	114 (62)	24 (75)	90 (59)	
≥90	19 (10)	4 (13)	15 (10)	
Medical history and CV risk factors				
BMI (kg/m <sup>2</sup> )	26.8 (23.3-30.3)	25.9 (21.1-31.2)	27.0 (23.6-30.3)	0.45
Smoking	20 (11)	3 (9)	17 (11)	>0.99
Diabetes	56 (30)	11 (34)	45 (29)	0.67
Arterial hypertension	138 (74)	21 (66)	117 (76)	0.26
Dyslipidemia	90 (48)	13 (41)	77 (50)	0.34
Atrial fibrillation	61 (33)	10 (31)	51 (33)	>0.99
Renal insufficiency	75 (41)	14 (44)	61 (40)	0.70
Heart failure	26 (14)	8 (25)	18 (12)	0.088
Coronary artery disease	77 (42)	16 (50)	61 (40)	0.33
Previous myocardial infarction	14 (8)	4 (13)	10 (7)	0.27
Previous ischemic stroke	32 (17)	3 (9)	29 (19)	0.30
Peripheral artery disease	34 (18)	3 (9)	31 (20)	0.21
Logistic EuroScore	16.9 (12.0-25.2)	17.3 (12.8-30.7)	16.8 (11.6-25.2)	0.50
EuroScore II	3.82 (2.10-6.70)	4.48 (3.10-9.22)	3.75 (2.04-6.69)	0.23
STS Score	4.05 (3.08-6.14)	4.05 (2.80-7.66)	4.04 (3.10-6.08)	0.60
CHA2DS2-VASc score	4 (4-5)	4 (4-5)	4 (4-5)	0.95
Biological parameters at admission				
Leucocytes (g/L)	6.4 (5.3-7.9)	6.7 (5.0-8.4)	6.3 (5.4-7.9)	0.77
Neutrophils (g/L)	4.2 (3.3-5.4)	4.6 (3.3-5.7)	4.2 (3.3-5.4)	0.60
Lymphocytes (g/L)	1.3 (1.0-1.7)	1.4 (0.9-1.6)	1.3 (1.0-1.7)	0.83
Monocytes (g/L)	0.5 (0.4-0.7)	0.5 (0.4-0.8)	0.5 (0.4-0.7)	0.84
Platelets (g/L)	221 (171-266)	229 (197-257)	220 (169-267)	0.54
C-reactive protein (mg/L)	1 (1-6)	1 (1-8)	1 (1-6)	0.87
vWF:Ag (U/L)	171 (132-225)	170 (129-232)	171 (132-224)	0.87
Ratio vWF:CB/vWF:Ag	0.73 (0.63-0.84)	0.72 (0.65-0.87)	0.73 (0.60-0.84)	0.97

Values are n (%) or median (25th-75th percentiles). In this table, patients presenting both *DNMT3A* and *TET2* variants were excluded from the "*TET2*-positive" column, as well as patients with a VAF <2% or >10% from the total cohort. "*TET2*-negative" includes patients with a CHIP-driver variant identified in another gene than *TET2* and CHIP-negative patients.  
 Abbreviations as in Table 1.

nor were older (Table 2). No sex-based differences were present. Moreover, there were no significant differences between *TET2*-positive and *TET2*-negative patients in any clinical or biological parameters, including inflammatory circulating parameters such as leucocytes, neutrophils, monocytes, CRP, vWF:Ag (Table 2), and IL-1 $\beta$ , IL-6, IL-8, and TNF $\alpha$  evaluated in representative subgroups of patients (Figure 5). Finally, carrying a *TET2* variant remained independently associated with decreased overall survival 5 years after TAVR (HR: 0.627; 95% CI: 0.397-0.999; *P* = 0.045) (Table 3) after multivariable Cox regression analysis with age, gender, history of renal insufficiency, heart failure, atrial fibrillation, coronary artery disease, peripheral artery disease, and ischemic stroke.

## DISCUSSION

This is the first study to investigate a large spectrum of CHIP-driver variants in patients with AVS undergoing TAVR. We demonstrated that somatic variants driving clonal hematopoiesis are very frequent in patients with AVS, with a prevalence of 68% of CHIP-positive patients in our cohort of 258 patients. This striking rate is significantly higher compared with data from the general population in the literature.<sup>25,30</sup> We identified 392 variants in a total of 35 hematopoietic genes. In accordance with previous studies, *DNMT3A* and *TET2* were the 2 most frequently mutated genes in the cohort, and the prevalence of CHIP increased with age. We proved that other CHIP-driver genes like *PPM1D*, *ASXL1*,



*TP53*, *SF3B1*, *CBL*, *SRSF2*, *NFE2*, and *JAK2* were also frequently associated with severe AVS. As most studies use a VAF of 2% to define CHIP,<sup>22,31</sup> we used this threshold, even if, on one hand, this threshold is

arbitrary and still discussed due to the improvement of techniques sensitivities,<sup>32</sup> and on the other hand, some new evidence that even CHIP-driver variants with very low VAF (0.5%-2.0%) have a clinical significance.<sup>28</sup> In addition, in our study, the measured VAF for a variant with an expected VAF of 2% will be between 1.1% and 2.9%. Even if we just focus on *DNMT3A* and *TET2*, we found a higher percentage of CHIP-positive patients than Mas-Peiro et al,<sup>22</sup> with 141 patients on 258 (54.7%) against 33.3% in their cohort.

Although most of the studies pool patients harboring a variant in *DNMT3A* and/or *TET2* to evaluate the prognosis, we aimed to distinguish their association with long-term survival one from the other and from the other 33 genes identified in our study. Indeed, these 2 genes and their loss-of-function variants have divergent effects on hematopoietic stem cells,<sup>33</sup> DNA methylation, and inflammatory

**TABLE 3** Multivariable Cox Regression Analysis for Long-Term Survival After TAVR

	P Value	HR	95% CI
TET2 presence (VAF $\geq$ 2%)	0.045	0.63	0.39-0.99
Age	0.086	1.02	0.99-1.05
Gender	0.89	0.97	0.67-1.40
Renal insufficiency	0.63	0.90	0.62-1.33
Heart failure	0.99	0.99	0.57-1.71
Coronary artery disease	0.98	1.00	0.70-1.43
Peripheral artery disease	0.70	1.10	0.66-1.82
Previous ischemic stroke	0.80	1.06	0.67-1.66
Atrial fibrillation	0.42	0.86	0.59-1.24

TAVR = transcatheter aortic valve replacement; VAF = variant allele frequency.

pathways,<sup>18</sup> so they should be considered separately. In addition, *DNMT3A* is involved in multipotent lineage, whereas *TET2* is predominantly restricted to myeloid lineages.<sup>18</sup> We observed by univariate analysis that patients carrying a *TET2*-CHIP-driver variant at low VAF ( $\geq 2\%$  and  $\leq 10\%$ ) had a worse prognosis with significantly decreased overall survival during long-term follow-up after TAVR, which was also evident in multivariable analysis by adjusting for known risk factors for mortality after TAVR. This is not the first study showing a lower impact of *DNMT3A* compared with *TET2* in cardiovascular diseases. In their meta-analysis of 5 cohorts including 56,597 patients with incident heart failure, Yu et al<sup>34</sup> showed that *ASXL1*, *TET2*, and *JAK2* driver-variants were each associated with an increased risk of heart failure, whereas *DNMT3A* was not. Interestingly, we observed that patients carrying both *DNMT3A* and *TET2* CHIP-driver variants have an increased overall survival after TAVR in comparison with patients carrying *TET2* alone, suggesting a protective role of *DNMT3A* on survival after TAVR in patients with the *TET2* variant. The mechanisms involved in this unconventional interplay remain to be described.

*TET2* is a key enzyme that catalyzes the demethylation of methylcytosine in DNA. There are a number of studies that showed that *TET2* deficiency in murine models leads to an enhanced inflammation, particularly with the increased secretion of IL-1 $\beta$  mediated by NLRP3 (NLR family pyrin domain containing 3) inflammasome and IL-6 in macrophages.<sup>21,35</sup> AVS results from an active inflammatory process whose mechanisms are close to atherosclerosis. IL-1 $\beta$  has a predominant role in valve calcification by promoting cell proliferation and expression of matrix metalloproteinases, as was shown by Kaden et al.<sup>36</sup>

Our study emphasizes the fact that IL-1 $\beta$  and the inflammasome could be novel therapeutic targets in AVS and for the improvement of survival after TAVR for patients who are carrying *TET2* somatic variants. The large clinical trial CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) has proved the effectiveness of canakinumab, a monoclonal antibody that inhibits IL-1 $\beta$ , in reducing the risk of cardiovascular events, particularly in patients with elevated markers of inflammation.<sup>37</sup> Colchicine has also been proven to lead to a significantly lower risk of ischemic cardiovascular events in patients with a previous myocardial infarction.<sup>38</sup> Finally, a very recent study has shown that blocking the NLRP3

inflammasome reduces aortic valve calcification and stenosis by preventing the shift of macrophages toward the M1 phenotype and downregulating IL-1 $\beta$  and IL-6 expression.<sup>39</sup>

**STUDY LIMITATIONS.** With the use of fluorescence-activated cell sorting analysis, Mas-Peiro et al<sup>22</sup> observed in patients with AVS and *TET2* CHIP-driver mutation increased levels of circulating nonclassical monocytes (CD14dimCD16 $^{++}$ ). As nonclassical monocytes are known to secrete high levels of inflammatory cytokines,<sup>40</sup> these data support the notion that an inflammatory mechanism may be critical to poor outcomes in TAVR.<sup>41</sup> Results of this study are strengthened by another study of the same group that observed while using single-cell RNA sequencing higher expression of mediators of inflammation, IL-1 $\beta$ , IL-6 receptor, and NLRP3 inflammasome complex, and CD163, a receptor involved in macrophage activation syndrome, in circulating monocytes from 8 patients with severe AVS and *DNMT3A* or *TET2* mutations compared with age-matched healthy control participants without *DNMT3A* and *TET2* mutations.<sup>42</sup> This study does not differentiate patients with *DNMT3A* mutations and patients with *TET2* mutations. However, in our study, we did not find any differences in IL-1 $\beta$ , IL-6, IL-8, and TNF $\alpha$  circulating concentrations between *TET2*-positive and *TET2*-negative patients, possibly due to the relatively small size of the analyzed cohort. However, even if *TET2* depletion results in an overproduction of IL-1 $\beta$ , IL-6, and other inflammatory cytokines at the cellular level,<sup>18</sup> studies reported contradictory results for circulating levels in patients with cardiovascular diseases. Some studies reported modest elevated CRP and/or inflammatory cytokine circulating levels in *TET2*-positive patients compared with *TET2*-negative patients,<sup>43,44</sup> whereas other studies, including large cohorts, have observed no differences.<sup>45,46</sup> Moreover, we measured inflammatory markers in patients with AVS at baseline. Acute systemic inflammation, frequently occurring after TAVR, could exacerbate proinflammatory cytokine expression in *TET2*-positive patients. Future studies are necessary to test this hypothesis. Answering this question could be important to strengthen the idea of using IL-1 and NLRP3 inflammasome blockers in TAVR context. Functional studies in relevant pre-clinical models are still needed to unravel the exact mechanisms by which CHIP-driver variants contribute to AVS and impact the clinical prognosis after TAVR, especially in *TET2*. Our findings need to

be validated in larger studies of consecutive patients before being used for decision making in the management of patients with severe AVS undergoing TAVR.

## CONCLUSIONS

In this study, we have shown that AVS is associated with a high prevalence and a large spectrum of CHIP-driver variants, the most common being in *DNMT3A* and *TET2*. These results are in line with previous studies describing CHIP as a novel age-related risk factor for cardiovascular diseases. Acquired variants in *TET2* with low VAF (2%-10%) are the most associated with decreased long-term survival after TAVR in comparison with other CHIP-driver genes, including *DNMT3A*. This finding could provide a novel tool for prognosis assessment in patients with severe AVS treated by TAVR.

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**ADDRESS FOR CORRESPONDENCE:** Dr Annabelle Dupont, Unite Mixte de Recherche, Inserm 1011, Boulevard du Pr Jules Leclercq, 59037 Lille Cedex, France. E-mail: [annabelle.dupont@univ-lille.fr](mailto:annabelle.dupont@univ-lille.fr).

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## PERSPECTIVES

**COMPETENCY IN MEDICAL KNOWLEDGE:** AVS is an elderly pathology mediated by inflammation. CHIP has been recently described as a novel age-related risk factor for cardiovascular diseases. This study describes in a cohort of 258 patients a large profile and a high prevalence of CHIP-driver genes in AVS. Moreover, patients carrying *TET2* mutations with low VAF (2%-10%) have a decreased overall survival at 5 years after TAVR. These findings contribute to the understanding of the pathogenesis of AVS and might help decision making in the management of patients with AVS undergoing TAVR.

**TRANSLATIONAL OUTLOOK:** Additional functional studies in relevant preclinical models are still needed to unravel the exact mechanisms by which CHIP-driver variants contribute to AVS and impact the clinical prognosis after TAVR, especially in *TET2*. Our findings need to be validated in larger studies of consecutive patients before being used for decision making in the management of patients with severe AVS undergoing TAVR. There are a number of studies that showed that *TET2* deficiency in murine models leads to an enhanced inflammation, particularly via IL-1 $\beta$  and NLRP3 inflammasome. Our study emphasizes the fact that IL-1 $\beta$  and the inflammasome could be novel therapeutic targets in AVS and for the improvement of survival after TAVR for patients who are carrying *TET2* somatic variants.

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**KEY WORDS** aortic valve stenosis, clonal hematopoiesis, survival, transcatheter aortic valve replacement

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**APPENDIX** For supplemental figures and tables, please see the online version of this paper.