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Clonal Hematopoiesis and Risk of Progression of Heart Failure With Reduced Left Ventricular Ejection Fraction

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ABSTRACT

BACKGROUND Clonal hematopoiesis driven by somatic mutations in hematopoietic cells, frequently called clonal hematopoiesis of indeterminate potential (CHIP), has been associated with adverse cardiovascular outcomes in population-based studies and in patients with ischemic heart failure (HF) and reduced left ventricular ejection fraction (LVEF). Yet, the impact of CHIP on HF progression, including nonischemic etiology, is unknown.

OBJECTIVES The purpose of this study was to assess the clinical impact of clonal hematopoiesis on HF progression irrespective of its etiology.

METHODS The study cohort comprised 62 patients with HF and LVEF <45% (age 74 ± 7 years, 74% men, 52% non-ischemic, and LVEF 30 ± 8%). Deep sequencing was used to detect CHIP mutations with a variant allelic fraction >2% in 54 genes. Patients were followed for at least 3.5 years for various adverse events including death, HF-related death, and HF hospitalization.

RESULTS CHIP mutations were detected in 24 (38.7%) patients, without significant differences in all-cause mortality ($p = 0.151$). After adjusting for risk factors, patients with mutations in either DNA methyltransferase 3 alpha *(DNMT3A)* or Tet methylcytosine dioxygenase 2 *(TET2)* exhibited accelerated HF progression in terms of death (hazard ratio [HR]: 2.79; 95% confidence interval [CI]: 1.31 to 5.92; $p = 0.008$), death or HF hospitalization (HR: 3.84; 95% CI: 1.84 to 8.04; $p < 0.001$) and HF-related death or HF hospitalization (HR: 4.41; 95% CI: 2.15 to 9.03; $p < 0.001$). In single gene-specific analyses, somatic mutations in *DNMT3A* or *TET2* retained prognostic significance with regard to HF-related death or HF hospitalization (HR: 4.50; 95% CI: 2.07 to 9.74; $p < 0.001$, for *DNMT3A* mutations; HR: 3.18; 95% CI: 1.52 to 6.66; $p = 0.002$, for *TET2* mutations). This association remained significant irrespective of ischemic/nonischemic etiology.

CONCLUSIONS Somatic mutations that drive clonal hematopoiesis are common among HF patients with reduced LVEF and are associated with accelerated HF progression regardless of etiology. (J Am Coll Cardiol 2021;77:1747–59)
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ABBREVIATIONS AND ACRONYMS

CHIP = clonal hematopoiesis of indeterminate potential

DNMT3A = DNA methyltransferase 3 alpha

HF = heart failure

LVEF = left ventricular ejection fraction

NT-proBNP = N-terminal portion of pro-B-type natriuretic peptide

TET2 = Tet methylcytosine dioxygenase 2

VAF = variant allelic fraction

The accumulation of somatic DNA mutations is a hallmark of aging in many tissues, which over time may become a mosaic of cells with different genotypes due to the clonal expansion of cells that harbor de novo mutations (1). In this context, human sequencing studies have established that normal aging is frequently accompanied by the acquisition of somatic mutations in hematopoietic stem cells that confer a competitive advantage to the mutant cell, leading to clonal hematopoiesis and the development of clones of mutant leukocytes in peripheral blood (2–6). The most commonly mutated genes that drive clonal hematopoiesis are DNA methyltransferase 3 alpha *(DNMT3A)* and Tet methylcytosine dioxygenase 2 *(TET2)*, which encode for epigenetic regulators of gene expression. Although clonal hematopoiesis heightens the risk of developing hematological cancer, typically after the accumulation of multiple mutations, most individuals with mutant blood clones will never develop hematological disorders during their lifespan, which has led to the definition of this condition as clonal hematopoiesis of indeterminate potential (CHIP) (7), also frequently defined as age-related clonal hematopoiesis. Unexpectedly, CHIP in nonsymptomatic individuals has been shown to be predictive of all-cause mortality mainly due to an increased incidence of atherosclerotic conditions (coronary artery disease and ischemic stroke) (3,8,9). Beyond atherosclerosis, experimental studies suggest that some clonal hematopoiesis-related mutations also influence cardiac remodeling and function in certain conditions. Clonal hematopoiesis driven by somatic mutations in *TET2* worsens cardiac function in experimental mouse models of ischemic heart failure (HF) with reduced left ventricular ejection fraction (LVEF) (10) and in aged mice (11). Furthermore, DNA sequencing of bone marrow samples from patients with chronic HF with reduced LVEF of ischemic origin revealed an association between CHIP and adverse outcomes (12,13). However, given that all HF patients in these previous studies had a prior myocardial infarction, the interpretation of these findings may be confounded by the long-lasting effects of myocardial ischemia on the hematopoietic system (14) and the presence of concomitant coronary atherosclerosis in these patients, particularly considering the tight association between CHIP and atherosclerotic cardiovascular disease (3,8,9). The effect of CHIP on HF outcomes among patients with nonischemic etiology remains unexplored. On this basis, the current study was designed to assess the clinical impact of CHIP mutations on HF progression irrespective of its etiology through deep sequencing studies in a cohort of chronic HF that included both ischemic and nonischemic origins.

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METHODS

STUDY DESIGN, POPULATION, AND OUTCOMES. The study population was obtained from a single-center prospective registry of ambulatory patients with chronic HF (15). To assess the impact of CHIP on long-term follow-up, the selected cohort was comprised of consecutive patients older than 60 years, with LVEF below 45%, a prior history of HF hospitalization, and available follow-up of at least 3 years after blood sampling at inclusion. Patients with noncardiac diseases expected to reduce life expectancy were excluded. All patients in this cohort with a history of prior neoplasia were in complete remission and were not receiving any cancer-related treatment at the time of inclusion; hence, they were not excluded from the study. The minimum follow-up of patients who survived to the end of the study was 3.5 years, with a median follow-up of 3.65 years for the entire cohort (interquartile range [IQR]: 2.00 to 5.14 years). HF diagnosis and treatment were established according to standard HF management as recommended by contemporary guidelines, and coronary anatomy was defined by coronariography (16). Ischemic HF etiology was defined as the presence of any epicardial coronary vessel with ≥75% stenosis or any history of prior myocardial infarction or coronary revascularization (either percutaneous transluminal coronary angioplasty or coronary artery bypass grafting). HF patients who did not fulfill these criteria were classified as having nonischemic etiology (Supplemental Methods). Three adverse clinical outcomes were studied: all-cause death, all-cause death combined with hospitalization for HF, and HF-related death combined with hospitalization for HF. Information about outcomes was obtained from medical records, patients' physicians, and relatives. In all cases, the study investigators provided details about the death and/or hospitalization episode, and data were reviewed by an independent adjudication committee using established definitions (Supplemental Methods) (16). Death was further confirmed and was investigated using the National Insurance and Death Records. Total mortality was divided into HF-related death (including sudden cardiac death and refractory pump failure-related death), other cardiovascular disease-related

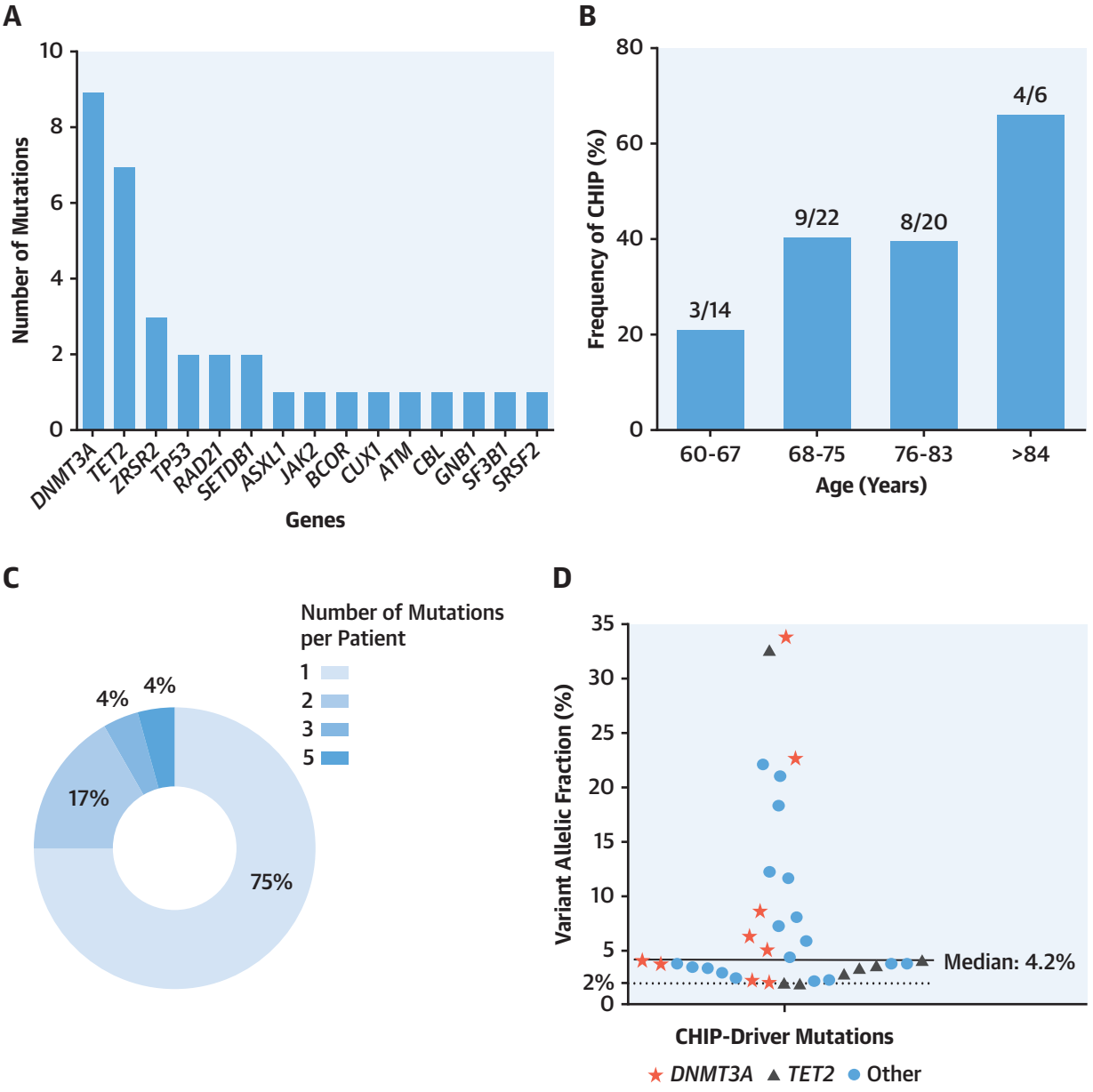
death, and noncardiovascular disease-related death. The study complied with the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from each patient prior to inclusion.

SAMPLE PREPARATION AND NEXT-GENERATION SEQUENCING. DNA was isolated from buffy coat with Maxwell 16 Blood DNA Purification Kit (Promega Corp., Madison, Wisconsin). DNA samples were washed with 2× AMPure XP Beads (Beckman Coulter, Brea, California) and 500 ng of each sample were used for library preparation with Kapa HyperPlus Kit (Roche, Basel, Switzerland). xGen UDI-UMI Adapters (IDT) were used to improve identification of low-frequency variants and prevent sequencing errors. Libraries were size-selected with AMPure XP Beads at 450 to 650 bp. A custom gene panel was designed to detect the presence of somatic mutations in 54 genes previously identified as candidate drivers of clonal hematopoiesis (Supplemental Table 1) (2–6,17). A total of 500 ng of each DNA library were used for panel capture. Twelve samples were pooled for each capture and processed as described in the xGen hybridization capture protocol (IDT), using the Kapa HiFi Polymerase (Roche) for PCR amplification. Quality of DNA samples and libraries were confirmed with Nanodrop Spectrophotometer (Thermo Scientific, Waltham, Massachusetts), Qubit Fluorometer (Invitrogen, Carlsbad, California), and Tapestation 2200 (Agilent, Santa Clara, California). Libraries were sequenced on a HiSeq 4000 (Illumina, San Diego, California). Read quality was assessed using FastQC. Mean base coverage across all samples was 4,184× before UMI family clustering and 4,150× with inclusion of UMIs.

VARIANT CALLING AND ANNOTATION. Raw sequencing reads were mapped to the human genome (GRCh38) using Burrows-Wheeler Alignment-MEM. PCR duplicates were marked based on mapping coordinates, and UMIs and reads from the same fragment were grouped together. Consensus reads for each fragment were obtained and mapped to GRCh38. Putative somatic mutations were identified using Genome Analysis Toolkit (GATK) Mutect2 (18). These putative variants were then filtered to exclude common sequencing artifacts and potentially germline mutations. Among the variants identified as somatic, candidate CHIP driver mutations were identified based on a pre-specified list of variants (Supplemental Table 2), in combination with previous CHIP published data, the Catalogue of Somatic Mutations in Cancer, and in silico pathogenicity predictors. Consistent with the current definition of CHIP (7), only variants with variant allelic fraction (VAF) ≥2% were included in our analyses, unless otherwise noted. Additional methodological details can be found in the Supplemental Methods.

STATISTICAL ANALYSIS. Based on the identification of CHIP variants, patients were classified as non-CHIP (no detected CHIP mutation), CHIP (at least 1 CHIP mutations in any of the sequenced genes), DNMT3A-CHIP (at least 1 CHIP mutation in *DNMT3A*), TET2-CHIP (at least 1 CHIP mutation in *TET2*), or DNMT3A/TET2-CHIP (at least 1 CHIP mutation in *DNMT3A* or *TET2*). Univariate statistical analyses were performed to investigate potential differences in clinical characteristics among these groups. Medians and IQRs were calculated for continuous variables and number of cases and percentages for categorical ones. Statistical differences were evaluated with Mann-Whitney *U* tests, chi-square tests, and Fisher exact tests, as appropriate. Associations with all-cause death and the composite of death or HF hospitalization were analyzed with Kaplan-Meier plots, log-rank tests, and Cox proportional-hazards regression models. Associations with the composite of HF-related death or hospitalization for HF were analyzed with cumulative incidence curves, Gray's tests and Fine-Gray regression models, considering other causes of death as competing risk. Regression models were adjusted for age, sex, ischemic etiology, LVEF, and serum N-terminal portion of pro-B-type natriuretic peptide (NT-proBNP) levels. In some cases, an extended statistical adjustment was used, which included all the previously listed covariates in addition to other established predictors of adverse HF progression, namely glomerular filtration rate, New York Heart Association functional class, systolic blood pressure, and the number of previous HF hospitalizations. The interaction between CHIP and ischemic HF etiology was investigated by including interaction terms in regression models and by performing separate competing risk analyses in ischemic and non-ischemic HF patients. A post hoc analysis excluding patients with a history of malignancies was performed to corroborate key findings in the absence of this potentially confounding factor. Patients with no detected CHIP mutations with VAF ≥2% (i.e., non-CHIP patients) were used as the control group in all analyses of the association between CHIP and adverse outcomes, including the analyses of the effects of *DNMT3A* and/or *TET2* mutations and mutations in single genes. All analyses were performed using R statistical software version 4.0.2 (R Foundation, Vienna, Austria) and considering 0.05 as the significance level.

FIGURE 1 Number and Distribution of Mutations Associated With CHIP in the Study Cohort



(A) The number of CHIP-related mutations identified with variant allele fraction (VAF) ≥2% in a custom panel of 54 genes. **(B)** Increased frequency of CHIP with age. **(C)** Number of CHIP-related mutations per patient. **(D)** VAF of CHIP mutations detected in the study cohort; a 2% VAF threshold was used to identify CHIP mutations **(dotted line)**. Mutations in *DNMT3A* and *TET2* are indicated by specific symbols. CHIP = clonal hematopoiesis of indeterminate potential; *DNMT3A* = DNA methyltransferase 3 alpha; *TET2* = Tet methylcytosine dioxygenase 2.

RESULTS

PREVALENCE OF CHIP AND CLINICAL CHARACTERISTICS. The study cohort was comprised of 62 patients with an established diagnosis of HF (LVEF 29.7 ± 7.8%) and age >60 years (74 ± 7 years, 74% men). Nonischemic etiology was present in 51.6% of patients. CHIP was detected in 24 (38.7%) patients, who carried a total of 34 mutations in 15 genes (Figure 1A). The frequency of CHIP was similar in patients with ischemic HF (40%) and nonischemic HF (37.5%). Consistent with previous findings in population-based cohorts and

TABLE 1 Clinical Characteristics According to the Presence of CHIP Mutations

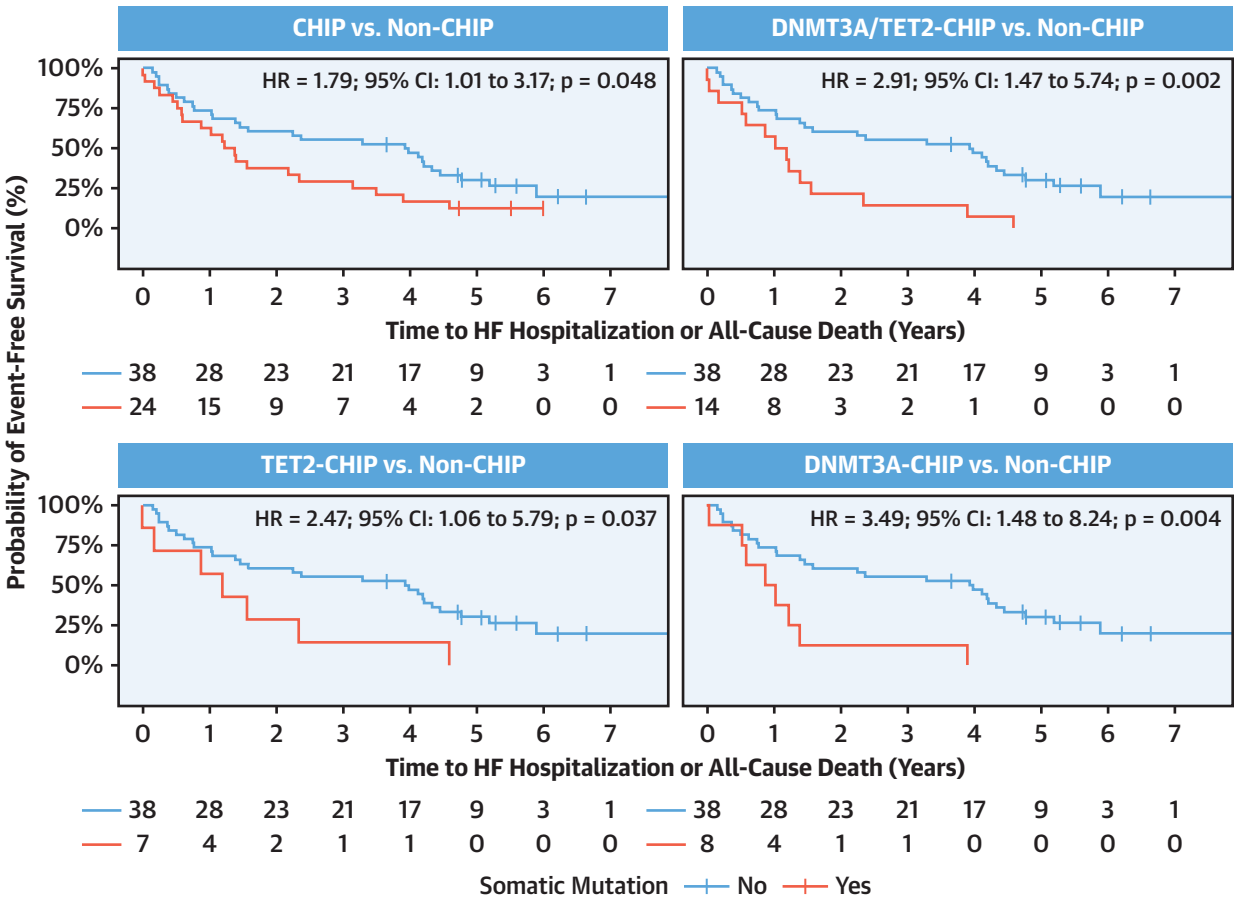
		CHIP >2%		
	Overall (N = 62)	No (n = 38)	Yes (n = 24)	p Value
Age, yrs	74.3 (68.7–79.1)	73.5 (67.3–79.1)	75.4 (70.4–79.2)	0.394
Female	16 (25.8)	12 (31.6)	4 (16.7)	0.313
BMI, kg/m^2	27.5 (25.7–32.1)	27.4 (25.2–31.2)	28.5 (25.9–32.3)	0.329
Hypertension	53 (85.5)	31 (81.6)	22 (91.7)	0.466
Diabetes	38 (61.3)	25 (65.8)	13 (54.2)	0.517
Dyslipidemia	36 (58.1)	24 (63.2)	12 (50.0)	0.448
Smoking	17 (27.4)	9 (23.7)	8 (33.3)	0.591
HF duration, yrs	0.6 (0.0–3.5)	0.6 (0.0–3.0)	0.7 (0.0–4.7)	0.572
Number of prior HF hospitalizations	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.5 (1.0–2.0)	0.911
Ischemic HF etiology	30 (48.4)	18 (47.4)	12 (50.0)	1.000
Prior myocardial infarction	19 (30.6)	12 (31.6)	7 (29.2)	1.000
Prior coronary revascularization	21 (33.9)	12 (31.6)	9 (37.5)	0.838
Prior history of cancer	8 (12.9)	4 (10.5)	4 (16.7)	0.754
NYHA functional class				0.846
I	15 (24.2)	9 (23.7)	6 (25.0)	
II	29 (46.8)	17 (44.7)	12 (50.0)	
III	17 (27.4)	11 (28.9)	6 (25.0)	
IV	1 (1.6)	1 (2.6)	0 (0.0)	
Atrial fibrillation or flutter	28 (45.2)	14 (36.8)	14 (58.3)	0.163
LVEF, %	30.0 (25.4–35.4)	29.8 (25.6–35.4)	31.6 (23.6–35.0)	0.593
LV end-diastolic volume, ml	161.5 (134.7–208.2)	161.0 (135.2–194.8)	168.3 (135.8–211.9)	0.628
LV end-systolic volume, ml	115.7 (91.2–151.8)	114.3 (94.6–134.9)	121.6 (89.8–174.6)	0.549
Heart rate, beats/min	75.5 (70.2–81.8)	77.0 (71.0–83.5)	73.5 (69.8–80.0)	0.244
Systolic blood pressure, mm Hg	124.0 (109.2–140.8)	128.5 (110.2–143.5)	119.5 (106.2–133.8)	0.110
GFR (MDRD), ml/min/1.73 m^2	58.0 (45.0–73.0)	61.1 (49.4–73.0)	48.7 (38.4–74.1)	0.278
Sodium, mmol/l	138.0 (135.0–141.0)	138.0 (135.2–140.8)	137.0 (133.8–141.5)	0.828
NT-proBNP, pg/ml	4,195.5 (2,748.0–10,510.5)	5,353.0 (3,076.5–9,952.0)	3,240.5 (2,278.2–1,3702.2)	0.525
Hemoglobin, g/dl	12.8 (11.0–14.3)	13.1 (11.4–14.4)	12.6 (10.8–13.6)	0.359
Platelets, $\times 10^3$ ul	195.0 (161.0–228.2)	200.5 (175.8–237.2)	186.0 (137.5–223.0)	0.214
Leukocytes, $\times 10^3$ ul	8.9 (7.0–10.7)	9.1 (7.9–10.7)	8.5 (6.5–10.7)	0.329
Lymphocytes, $\times 10^3$ ul	1.5 (1.1–2.0)	1.4 (0.9–2.1)	1.5 (1.2–1.8)	0.544
C-reactive protein, mg/dl	0.8 (0.4–1.8)	0.8 (0.4–1.8)	0.8 (0.4–1.6)	0.937
Interleukin-6, pg/ml	7.1 (3.7–15.8)	6.1 (3.2–11.0)	12.3 (5.4–20.4)	0.058
Interleukin-1β, pg/ml	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.4 (0.4–0.5)	0.470
sST2, ng/ml	27.0 (18.2–46.8)	24.0 (17.0–43.5)	33.0 (21.8–62.0)	0.105
ACE inhibitor or ARB	54 (87.1)	33 (86.8)	21 (87.5)	1.000
Beta-blocker	52 (83.9)	33 (86.8)	19 (79.2)	0.656
MRA	42 (67.7)	28 (73.7)	14 (58.3)	0.327
Digoxin	9 (14.5)	5 (13.2)	4 (16.7)	0.990
Amiodarone	6 (9.7)	2 (5.3)	4 (16.7)	0.299
Anticoagulation	29 (46.8)	15 (39.5)	14 (58.3)	0.235
ICD	12 (19.4)	7 (18.4)	5 (20.8)	1.000
CRT	16 (25.8)	9 (23.7)	7 (29.2)	0.855

Values are median (interquartile range) or n (%).

ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor antagonist; CRT = cardiac resynchronization therapy; GFR = glomerular filtration rate; HF = heart failure; ICD = implantable cardioverter defibrillator; LVEF = left ventricular ejection fraction; MDRD = modification of diet in renal disease; MRA = mineralocorticoid receptor antagonist; NT-proBNP = N-terminal pro-B-type natriuretic peptide serum levels; NYHA = New York Heart Association; sST2 = soluble ST2.

cardiovascular disease patients (2,3,5,9,12,19), the prevalence of CHIP increased with age (**Figure 1B**). Among patients with CHIP, 18 (75%) had a candidate driver mutation in only 1 gene and 6 individuals showed 2 or more mutations, including 1 patient who presented 5 mutations with no history of malignancy (**Figure 1C**). A list of all candidate driver mutations and their distribution among patients with CHIP is included in Supplemental Table 3. The most frequently mutated genes (**Figure 1A**) were *DNMT3A* (9 mutations, affecting 8 patients, 12.9% of the cohort), and *TET2* (7 mutations, affecting 7 patients,

FIGURE 2 Association of CHIP With Survival Free of HF Hospitalization

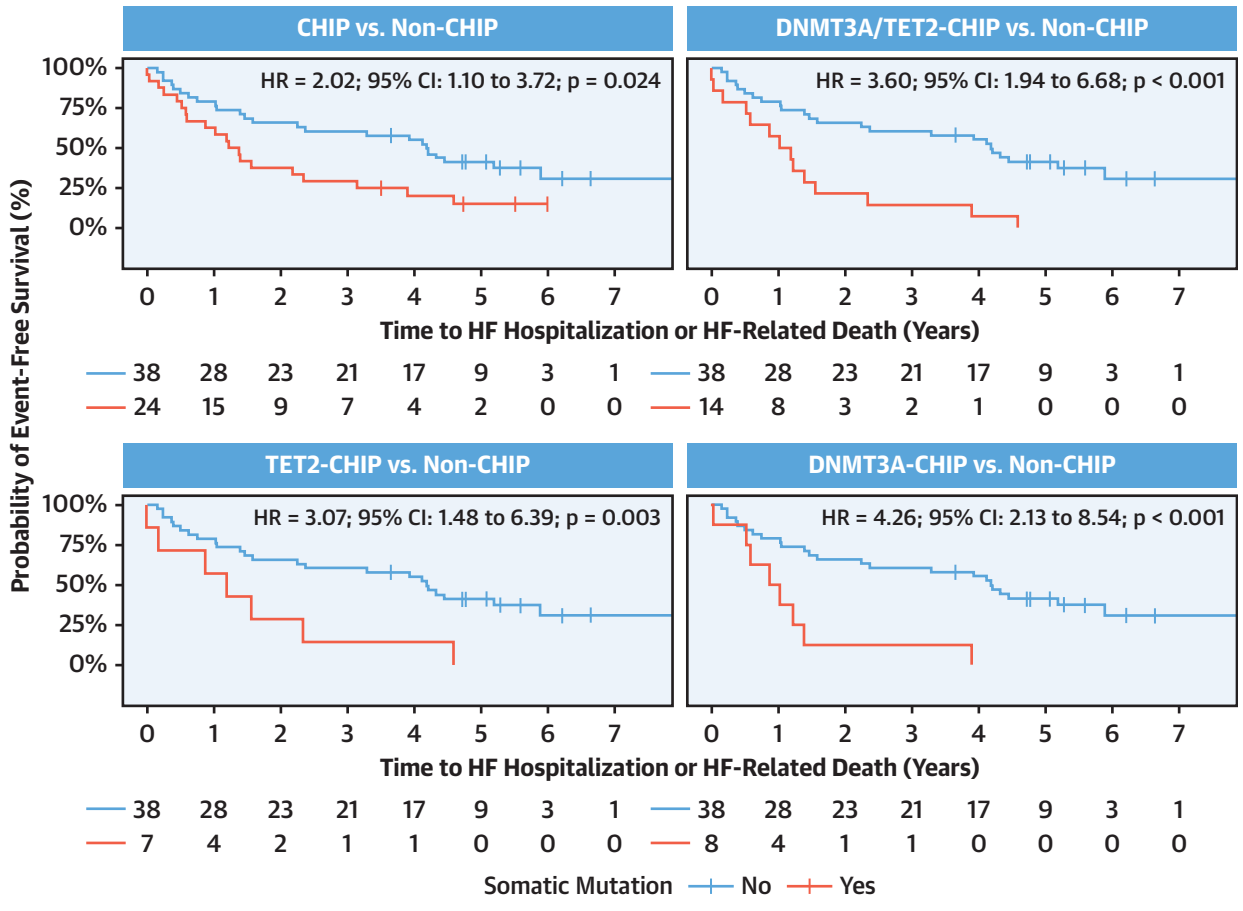


Kaplan-Meier survival curves and unadjusted Cox regression analysis show that patients carrying CHIP mutations had a significantly lower event-free survival and a higher risk of adverse events, compared with patients without CHIP. This impaired prognosis was largely driven by *DNMT3A* mutations and, to a lesser extent, *TET2* mutations. Confidence interval of 95%. Numbers at risk within each group are shown. HF = heart failure; HR = hazard risk; other abbreviations as in **Figure 1**.

11.3%). One patient presented mutations in both *DNMT3A* and *TET2* and an additional patient presented 2 different mutations in *DNMT3A*. Therefore, a total of 14 patients (22.6%) presented *TET2* and/or *DNMT3A* mutations. Schematic representations of the nature and location of the mutations identified in these two genes are shown in Supplemental Figure 1. No other clonal hematopoiesis-related gene was found mutated in >3 patients. Median VAF of CHIP mutations was 4.2% (range: 2% to 34%) (**Figure 1D**), which corresponds to 8.4% of mutant nucleated blood cells at the time of analysis, assuming all mutations are monoalellic. A total of 76% of mutations in patients who exhibited CHIP had a VAF <10%, a threshold used in previous studies to distinguish CHIP with small versus large mutant clones (3,9). Median VAFs of mutations in *DNMT3A*, *TET2,* and other genes were 5.9%, 3.5%, and 4.2%, respectively. In total, 78% of *DNMT3A* mutations and 86% of *TET2* mutations had a VAF <10%, and 72% of mutations in other genes had a VAF below this threshold.

Table 1 summarizes clinical characteristics for the entire cohort, as well as for CHIP mutation carriers and noncarriers. Patients with CHIP did not differ from non-CHIP patients in terms of demographics, risk factors, and clinical characteristics, including left ventricular function parameters and prevalence of ischemic HF etiology. Considering specifically *TET2* and *DNMT3A* mutations, slightly impaired renal function among *TET2* mutation carriers ($p = 0.028$) and lower concentrations of NT-proBNP among *DNMT3A* mutation carriers ($p = 0.042$) were the only significant differences at baseline (Supplemental Table 4). Consistent with previous studies (19,20),

FIGURE 3 **CHIP and Adverse HF Progression**



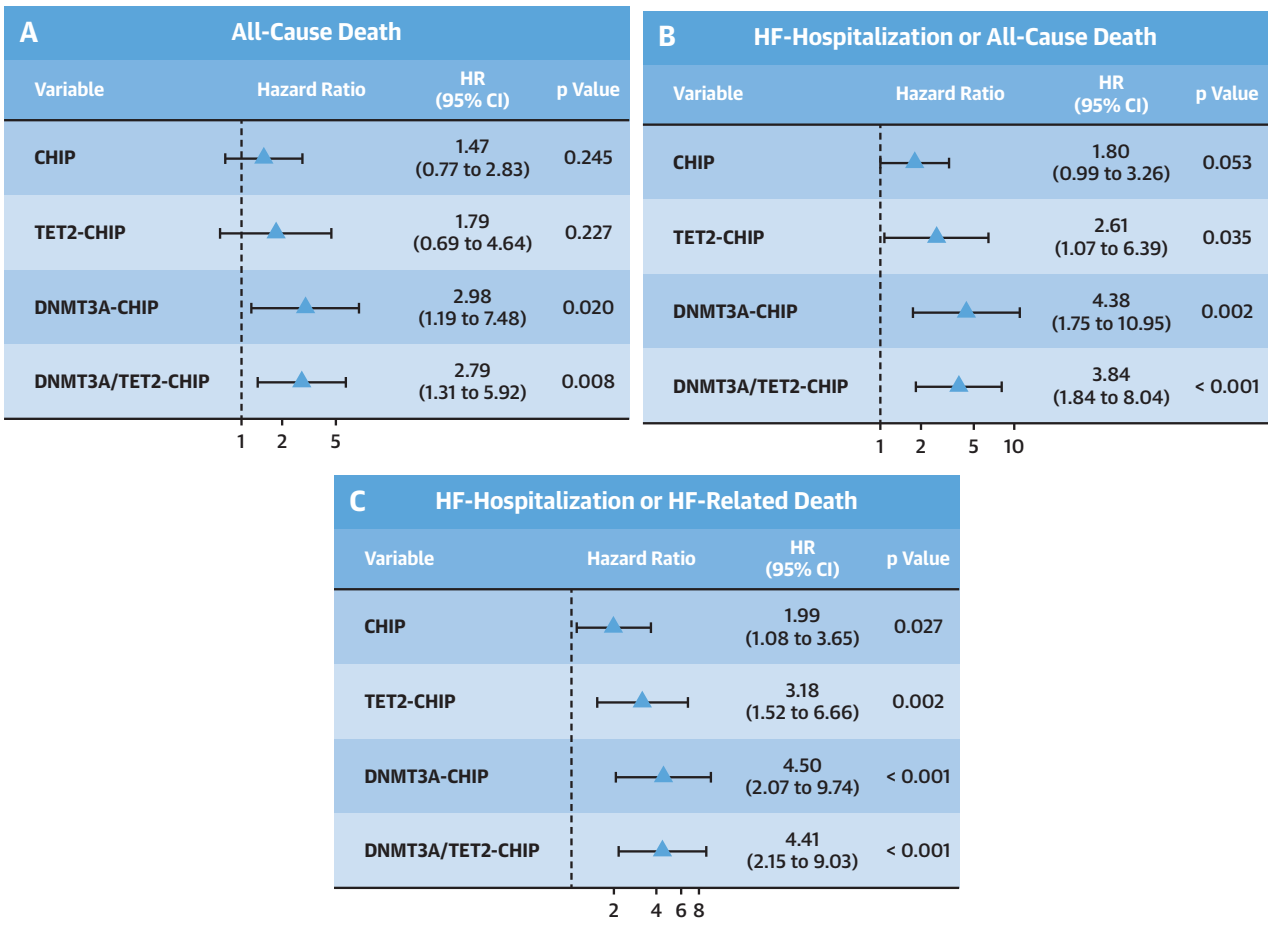
Adverse HF progression was defined as the composite of HF-related death or HF hospitalization. Fine-Gray competing risk analysis shows that patients carrying CHIP mutations had a lower survival free of adverse events directly related to HF progression. In gene-specific analysis, the presence of either *TET2* or *DNMT3A* mutations accelerated HF progression and increased significantly (~3 to 4 times) the probability of HF-related events. Numbers at risk within each group are shown. Abbreviations as in Figures 1 and 2.

patients with DNMT3A/TET2-CHIP exhibited higher circulating interleukin (IL)-6 levels (p = 0.045); no differences were observed in circulating levels of other inflammatory markers included in our analysis (Supplemental Table 4).

CHIP MUTATIONS AND ADVERSE HF PROGRESSION. A total of 23 of 38 patients (60.5%) without CHIP and 17 of 24 patients (70.8%) with CHIP died during follow-up. Supplemental Figure 2 shows the survival analysis for CHIP versus non-CHIP patients, which did not differ significantly when considering all CHIP mutations (hazard ratio [HR]: 1.60; 95% confidence interval [CI]: 0.84 to 3.05; p = 0.151). Because most CHIP mutations were found in *DNMT3A* and *TET2*, which have been previously linked to HF progression in experimental models (10,11,21), we focused our analysis on these 2 genes, similar to previous studies (9,12). The combined analysis of mutations in *DNMT3A* and/or *TET2* (DNMT3A/TET2-CHIP) revealed lower survival time in mutation carriers (HR: 2.28; 95% CI: 1.11 to 4.67; p = 0.025). Gene-specific analysis revealed that this association is largely driven by *DNMT3A* mutations (HR: 2.66; 95% CI: 1.11 to 6.38; p = 0.028), which were tightly associated with lower survival, whereas the effect of *TET2* mutations did not reach statistical significance (HR: 1.80; 95% CI: 0.73 to 4.49; p = 0.204) (Supplemental Figure 2).

A total of 38 patients (61.3% of the cohort) were hospitalized due to decompensated HF during follow-up. Considering the composite outcome of all-cause death or HF hospitalization (Figure 2), patients with CHIP exhibited a higher probability of adverse HF

FIGURE 4 Adjusted Risk of Adverse Clinical Outcomes in CHIP Mutation Carriers



Multivariate regression analyses were used to investigate the association between CHIP and all-cause death **(A)**, all-cause death or HF hospitalization **(B)**, and HF-related death or HF hospitalization **(C)**. All models were adjusted for age, sex, ischemic etiology, left ventricular ejection fraction, and N-terminal portion of pro–B-type natriuretic peptide levels. *DNMT3A* mutations were associated with a significantly increased risk of adverse outcomes, including overall mortality, the composite of death or HF hospitalization, and the composite of HF-related death or HF hospitalization. *TET2* mutations showed a strong association with events directly related to HF progression, most notably HF-related death or HF hospitalization. Considering the occurrence of mutations in either *DNMT3A* or *TET2*, the risk of adverse HF progression was >4 times higher compared with patients without CHIP mutations. CI = confidence interval; other abbreviations as in **Figures 1 to 3**.

progression ($p = 0.048$), which was highly significant when considering either *DNMT3A* mutations ($p = 0.004$) or the combination of *DNMT3A* and/or *TET2* mutations ($p = 0.002$), and also reached statistical significance when considering *TET2* mutations exclusively ($p = 0.037$).

Among patients who died, a total of 28 (70%) had a HF-related death and 12 (30%) had a non-HF-related death. Causes of death are detailed in Supplemental Table 5. Competing risk analysis (**Figure 3**) confirmed that CHIP is specifically associated with worse HF progression (composite outcome of HF-related death or HF hospitalization; $p = 0.024$). This association was highly significant when considering *DNMT3A*/*TET2* mutations ($p < 0.001$), *DNMT3A* mutations exclusively ($p < 0.001$), and *TET2* mutations exclusively ($p = 0.003$).

After adjustment for age, sex, ischemic etiology, LVEF, and serum NT-proBNP levels, the presence of CHIP mutations in any of the sequenced genes or specifically in *DNMT3A* or *TET2* remained associated with a higher risk of adverse HF outcomes, most strongly with the composite of HF-related hospitalization or HF-related death (**Figure 4,** Supplemental Table 6). Similar results were obtained when adjusting for additional known predictors of HF progression, including glomerular filtration rate, New York Heart Association functional class, systolic blood

pressure, and the number of previous HF hospitalizations (Supplemental Figure 3, Supplemental Table 7). Importantly, the presence of mutations in either *DNMT3A* or *TET2* remained associated with worse HF progression in both ischemic and nonischemic HF patients in competing risk analyses (Supplemental Figure 4), supporting that the connection between CHIP and adverse HF events is not driven by coronary artery disease. Furthermore, no significant interaction was observed between ischemic HF etiology and the existence of mutations in these genes for any of the HF-related outcomes included in these analyses (Supplemental Table 8).

We further confirmed our findings after the exclusion of 8 patients with a prior history of cancer (described in Supplemental Table 9). The cohort free of a history of malignancies was comprised of 54 patients; 20 patients (37%) presented CHIP mutations and 12 patients (22%) carried mutations in either *TET2* or *DNMT3A* (6 patients in *TET2*, 5 patients in *DNMT3A,* and 1 patient in both genes). In this population, the presence of mutations in either *DNMT3A* or *TET2* mutations was also associated with adverse HF progression, both in unadjusted and adjusted statistical models (Supplemental Figures 5 to 7).

VARIANT ALLELIC FRACTION OF CHIP MUTATIONS AND ADVERSE HF PROGRESSION. We also assessed the potential association between the extent of clonal expansion of *DNMT3A* and *TET2*-mutant hematopoietic cells, estimated based on VAF, and adverse HF outcomes. To do this in a broad range of VAF, we lowered the threshold of CHIP mutation calling to VAF >1%, taking advantage of our deep sequencing strategy. This led to the identification of 7 additional *DNMT3A* mutations and 4 additional *TET2* mutations, affecting 10 patients (Supplemental Table 10). Analysis of the relationship between DNMT3A/TET2-CHIP VAF as a continuous variable and the composite outcome of HF-related death or HF-related hospitalization revealed a highly statistically significant association, both in an unadjusted model and after adjustment for either age and sex or age, sex, ischemic etiology, LVEF, and NT-proBNP levels (**Table 2**). We next investigated the relationship between adverse HF progression and 3 different VAF categories defined based on the current definition of CHIP (7) and the estimated sensitivity of different sequencing strategies to identify CHIP mutations at various VAFs (19): VAF 1% to 2%, VAF 2% to 5%, and VAF >5%. In univariate and multivariate analyses with adjustment for age, sex, ischemic etiology, and several predictors of HF progression, we found that DNMT3A/TET2-CHIP with VAF 2% to 5% and VAF >5% are both significantly associated with the composite of HF-related death or HF hospitalization, whereas no association at all was found for DNMT3A/TET2-CHIP with VAF 1% to 2% (**Table 2,** Supplemental Table 11). These analyses were done considering exclusively the VAF of the most expanded mutation in individuals with more than 1 mutation. Analysis of cumulative VAF (i.e., sum of the VAFs of all mutations in these genes detected in a given patient) led to similar results (Supplemental Table 12).

DISCUSSION

This study evaluated the relationship between clonal hematopoiesis driver mutations and the long-term evolution of HF with reduced LVEF. Our results confirm previous reports of adverse outcomes in ischemic HF patients carrying mutations in *DNMT3A* and *TET2*, and, importantly, extend these findings to HF patients with nonischemic etiologies and to the progression of HF from a clinical perspective (**Central Illustration**).

CLONAL HEMATOPOIESIS AND ADVERSE HF PROGRESSION. Clonal hematopoiesis driven by somatic mutations is emerging as a new cardiovascular risk modifier and a potential mechanistic link between cardiovascular disease and aging (22–24). Within the context of HF, previous clinical evidence supporting the pathophysiological relevance of clonal hematopoiesis result exclusively from the study of ischemic HF patients. An analysis of bone marrow samples from patients with chronic ischemic HF who had undergone autologous bone marrow treatment for acute myocardial infarction revealed an association between mutations in *DNMT3A* and/or *TET2* and greater risk for death and death combined with HF hospitalization, independently of age (12). The same investigators reported a dose-response association between the number of CHIP mutations or its cumulative clone size and clinical outcome (13). Our current study largely validates and expands these previous findings in an independent cohort and, to our knowledge, provides the first evidence supporting the possibility that somatic mutations driving clonal hematopoiesis accelerate clinical progression of HF in the absence of ischemic heart disease. We found an association between DNMT3A/TET2-CHIP and all-cause death or death combined with HF hospitalization. Yet, notably, this association became more relevant when analyzing specifically HF-related death and hospitalization, which distinctively supports the clinical impact of clonal hematopoiesis on HF progression, beyond its known connection to

TABLE 2 VAF of *TET2* and *DNMT3A* Mutations and Risk of Adverse HF Events

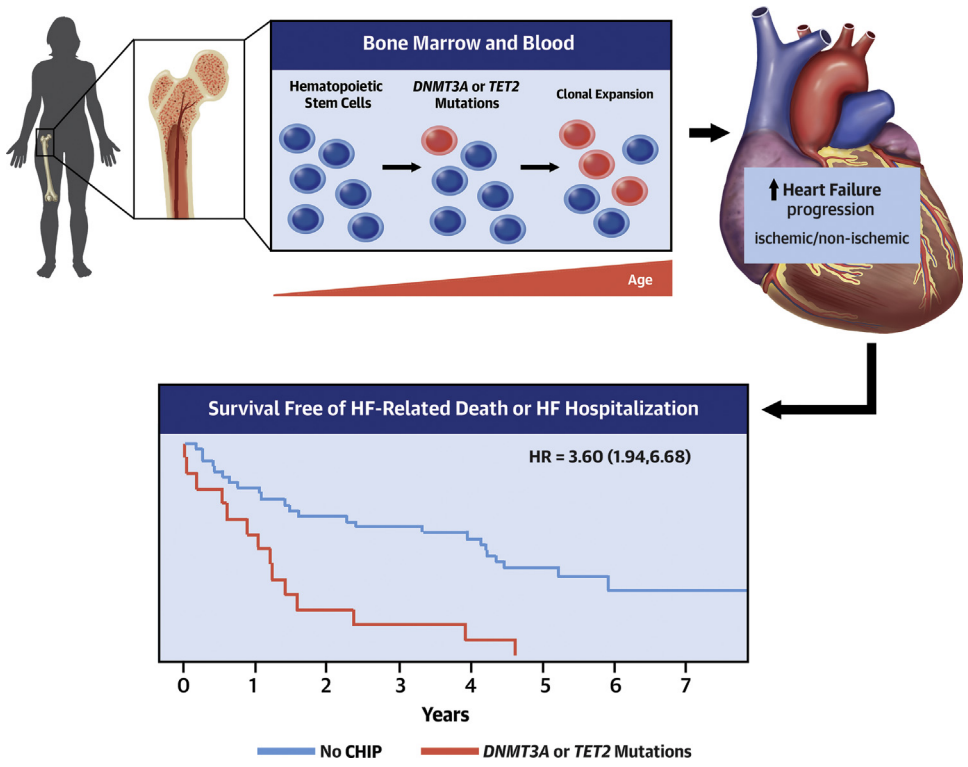
	All-Cause Death		HF Hospitalization or All-Cause Death		HF Hospitalization or HF-Related Death	
	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
Continuous VAF						
Univariate						
VAF, per %	1.02 (0.98–1.07)	0.285	1.06 (1.01–1.10)	0.016	1.07 (1.02–1.11)	0.001
Adjusted, model 1						
VAF, per %	1.02 (0.97–1.06)	0.433	1.05 (1.01–1.10)	0.021	1.06 (1.02–1.10)	0.002
Adjusted, model 2						
VAF, per %	1.01 (0.97–1.06)	0.619	1.04 (0.99–1.09)	0.084	1.06 (1.01–1.10)	0.015
Categorized VAF						
Univariate						
Non-CHIP, reference	–	–	–	–	–	–
1% ≤ VAF <2%	0.40 (0.09–1.71)	0.216	0.58 (0.20–1.68)	0.313	0.89 (0.34–2.29)	0.807
2% ≤ VAF <5%	1.97 (0.86–4.50)	0.106	2.34 (1.07–5.15)	0.034	3.10 (1.49–6.44)	0.002
VAF ≥5%	1.60 (0.54–4.72)	0.392	2.72 (1.00–7.40)	0.050	3.59 (1.57–8.23)	0.003
Adjusted, model 1						
Non-CHIP, reference	–	–	–	–	–	–
1% ≤ VAF <2%	0.39 (0.09–1.67)	0.202	0.58 (0.20–1.70)	0.323	0.89 (0.35–2.30)	0.815
2% ≤ VAF <5%	2.64 (1.11–6.30)	0.028	2.44 (1.10–5.42)	0.028	3.30 (1.46–7.48)	0.004
VAF ≥5%	1.43 (0.48–4.24)	0.523	2.51 (0.89–7.07)	0.082	3.20 (1.38–7.40)	0.007
Adjusted, model 2						
Non-CHIP, reference	–	–	–	–	–	–
1% ≤ VAF <2%	0.36 (0.08–1.66)	0.192	0.44 (0.14–1.40)	0.165	0.84 (0.32–2.20)	0.728
2% ≤ VAF <5%	2.86 (1.15–7.09)	0.023	2.90 (1.24–6.81)	0.014	3.83 (1.60–9.19)	0.003
VAF ≥5%	1.41 (0.46–4.33)	0.550	2.89 (1.00–8.39)	0.050	3.51 (1.46–8.47)	0.005

Cox regression models were used to test the association between VAF of DNMT3A/TET2 mutations and all-cause death or the composite of all-cause death or heart failure (HF) hospitalization. Fine-Gray regression models were used to test the association with the composite of HF-related death or HF hospitalization with other causes of death as competing risk. Regression models were adjusted for age and sex (model 1) or age, sex, ischemic etiology, LVEF and serum NT-proBNP levels (model 2).

CHIP = clonal hematopoiesis of indeterminate potential; *DNMT3A* = DNA methyltransferase 3 alpha; *TET2* = Tet methylcytosine dioxygenase 2; VAF = variant allelic fraction; other abbreviations as in Table 1.

atherosclerotic conditions. Importantly, CHIP remained associated with worse HF progression after adjusting for age, sex, ischemic/nonischemic etiology, and various well-established predictors of adverse HF outcomes. This association was observed when CHIP was defined based on the detection of a candidate driver mutation in any of the analyzed genes, but gene-specific analysis revealed significant associations between HF progression and mutations in either *DNMT3A* or *TET2*. The clinical impact of *DNMT3A* mutations was particularly large and was apparently greater than that of *TET2* mutations. This may be related to the bigger clone size observed for *DNMT3A* mutations in our study, particularly considering that we found a positive association between VAF and HF progression. Alternatively, it may reflect a more potent contribution of *DNMT3A* mutations to the pathophysiology of HF. There are limited data available on the role of *DNMT3A* in this context. Hematopoietic DNMT3A deficiency has been associated with worse cardiac function after angiotensin II infusion in mice, but this result needs to be interpreted cautiously, as it was obtained using a lentiviral vector-mediated CRISPR/Cas9 approach and its potential off-target effects were not investigated (21). Thus, understanding the mechanisms that underlie the association between somatic *DNMT3A* mutations and HF progression will require further investigation. Conversely, the direct causal contribution of *TET2* mutation-driven clonal hematopoiesis to cardiac dysfunction is strongly supported by preclinical studies using TET2-deficient mice and a variety of experimental approaches. Hematopoietic TET2 loss of function has been found to worsen cardiac function in an ischemic HF model of coronary ligation (10) and, importantly, also in nonischemic models, such as those associated with cardiac hypertrophy secondary to aging, transverse aortic banding, or angiotensin II infusion (10,11,21). Supporting the conclusions of these preclinical studies, our current study is consistent with the possibility that somatic mutations that drive clonal hematopoiesis contribute to HF progression, independently of its ischemic or nonischemic etiology.

CENTRAL ILLUSTRATION **Clonal Hematopoiesis of Indeterminate Potential and Accelerated Progression of HF With Reduced Left Ventricular EF**



Pascual-Figal, D.A. et al. J Am Coll Cardiol. 2021;77(14):1747–59.

Aging is associated with a higher frequency of somatic mutations that drive clonal hematopoiesis, particularly mutations in *DNMT3A* and *TET2*, which, in the presence of HF with reduced LVEF, result in accelerated progression of the disease in terms of HF-related mortality and acute HF decompensations leading to hospitalization. *DNMT3A* = DNA methyltransferase 3 alpha; *TET2* = Tet methylcytosine dioxygenase 2.

Previous studies in population-based cohorts, cardiovascular disease patients, and animal models suggest an overactivation of the pro-inflammatory NLRP3/IL-1β/IL-6 pathway in carriers of CHIP mutations in *DNMT3A* and *TET2* (9,10,19–21,25–28). Consistent with these previous studies, we found higher circulating levels of IL-6 in HF patients with DNMT3A/TET2-CHIP. Considering the known connection between the IL-1β/IL-6 pro-inflammatory axis and HF (29–31), future studies in large cohorts are warranted to investigate whether this pathway is indeed overactivated in HF patients with mutations in *DNMT3A* or *TET2* and whether targeting these proinflammatory mediators may be of particular preventive/therapeutic interest in these patients.

MUTANT CLONE SIZE AND HF PROGRESSION. Our analysis of the association between HF outcomes and the VAF of DNMT3A/TET2 mutations revealed that there is a significant dose-response association between mutant clone size and HF progression. These data support the possibility of a causal and direct contribution of *DNMT3A* and *TET2* mutations to adverse clinical outcomes in HF patients, which will require validation in larger cohorts. This analysis also provides valuable information on the VAF thresholds required for the identification of clinically relevant CHIP. The established definition of CHIP requires that candidate driver mutations are present with a VAF >2% (7). However, high-sensitivity sequencing has revealed that CHIP mutations at lower VAF are an almost ubiquitous phenomenon (6,17,32). Thus, it is of utmost importance to define the mutant clone size cut-off that associates with heightened cardiovascular risk. Although previous studies have suggested a dose-dependent effect of VAF on cardiovascular outcomes, they used sequencing depths that may not reliably detect CHIP variants in the VAF 2% to 5%

range (3,8,9) or lacked statistical adjustment for potentially confounding variables (12). In this context, our high coverage, error-corrected sequencing strategy provides further insight into the association between the VAF of CHIP mutations and adverse HF outcomes. After statistical adjustment for multiple risk covariates, we found that DNMT3A/TET2-CHIP with VAF >2% is associated with HF-related mortality and HF hospitalization, whereas no association at all was found for DNMT3A/TET2-CHIP with VAF 1% to 2%. These results suggest that the 2% VAF cut-off currently established for the identification of CHIP is clinically relevant in the context of HF outcomes. However, future studies with larger sample size will be required to validate these findings and evaluate whether relevant VAF thresholds differ in ischemic and nonischemic HF or among the various CHIP candidate driver genes.

STUDY STRENGTHS AND LIMITATIONS. The strengths of the present study include the accurate clinical characterization of patients with ischemic and nonischemic HF etiologies, the long-term follow-up, the analysis of outcomes specifically related to HF progression, and the use of a highly sensitive sequencing strategy to identify clonal hematopoiesis-related mutations. Conversely, this study is limited mainly by its modest sample size, which needs to be considered when interpreting our findings. Nevertheless, our results corroborate and expand previous data from ischemic HF patients and lend support to the hypothesis that somatic mutations that drive clonal hematopoiesis also have a clinically meaningful effect on the progression of nonischemic HF. Additional studies with larger sample sizes will be required to examine further this hypothesis and deepen our understanding of the relationship between CHIP and HF outcomes.

CONCLUSIONS

Our study supports the clinical relevance of age-related clonal hematopoiesis, particularly when driven by mutations in *DNMT3A* or *TET2*, in the progression of ischemic and nonischemic HF with reduced LVEF. As we gain a greater appreciation of the role of clonal hematopoiesis as a link between aging and cardiovascular disease, understanding the effects and prognostic value of specific somatic mutations should pave the way to new precision medicine therapeutic and preventive strategies.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Somatic mutations that drive clonal hematopoiesis play pivotal roles in the progression of HF with reduced LVEF, irrespective of etiology.

TRANSLATIONAL OUTLOOK: Understanding the impact and prognostic value of clonal hematopoiesis-related mutations may facilitate development of preventive and therapeutic strategies for individuals with or at risk of developing HF with reduced LVEF.

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APPENDIX For an expanded Methods section as well as supplemental tables and figures, please see the online version of this paper.