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Clonal Hematopoiesis in Clinical and Experimental Heart Failure With Preserved Ejection Fraction

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BACKGROUND: Clonal hematopoiesis (CH), which results from an array of nonmalignant driver gene mutations, can lead to altered immune cell function and chronic disease, and has been associated with worse outcomes in patients with heart failure (HF) with reduced ejection fraction. However, the role of CH in the prognosis of HF with preserved ejection fraction (HFpEF) has been understudied. This study aimed to characterize CH in patients with HFpEF and elucidate its causal role in a murine model.

METHODS: Using a panel of 20 candidate CH driver genes and a variant allele fraction cutoff of 0.5%, ultradeep error-corrected sequencing identified CH in a cohort of 81 patients with HFpEF (mean age, 71±6 years; ejection fraction, 63±5%) and 36 controls without a diagnosis of HFpEF (mean age, 74±7 years; ejection fraction, 61.5±8%). CH was also evaluated in a replication cohort of 59 individuals with HFpEF.

RESULTS: Compared with controls, there was an enrichment of *TET2*-mediated CH in the HFpEF patient cohort (12% versus 0%, respectively; P=0.02). In the HFpEF cohort, patients with CH exhibited exacerbated diastolic dysfunction in terms of E/e′ (14.9 versus 11.7, respectively; P=0.0096) and E/A (1.69 versus 0.89, respectively; P=0.0206) compared with those without CH. The association of CH with exacerbated diastolic dysfunction was corroborated in a validation cohort of individuals with HFpEF. In accordance, patients with HFpEF, an age ≥70 years, and CH exhibited worse prognosis in terms of 5-year cardiovascular-related hospitalization rate (hazard ratio, 5.06; P=0.042) compared with patients with HFpEF and an age ≥70 years without CH. To investigate the causal role of CH in HFpEF, nonconditioned mice underwent adoptive transfer with *Tet2*–wild-type or *Tet2*-deficient bone marrow and were subsequently subjected to a high-fat diet/L-NAME (Nω-nitro-l-arginine methyl ester) combination treatment to induce features of HFpEF. This model of *Tet2*-CH exacerbated cardiac hypertrophy by heart weight/tibia length and cardiomyocyte size, diastolic dysfunction by E/e′ and left ventricular end-diastolic pressure, and cardiac fibrosis compared with the *Tet2*–wild-type condition.

CONCLUSIONS: CH is associated with worse heart function and prognosis in patients with HFpEF, and a murine experimental model of *Tet2*-mediated CH displays greater features of HFpEF.

Key Words: biomarkers ■ clonal hematopoiesis ■ heart failure ■ prognosis

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Clinical Perspective

What Is New?

- *TET2*-driven clonal hematopoiesis (CH) was enriched in a cohort of patients with heart failure with preserved ejection fraction (HFpEF).
- In patients with HFpEF, CH was associated with worse diastolic heart function and outcome.
- In a murine model of HFpEF, *Tet2*-mediated CH led to higher echocardiographic E/e′, greater left ventricular end-diastolic pressure, and greater cardiac fibrosis.

What Are the Clinical Implications?

- *TET2*-driven CH may represent a novel pathophysiologic mechanism in HFpEF.
- Our findings establish a rationale for measuring CH in patients with HFpEF to predict future outcomes.
- Targeting *TET2*-mediated CH may be beneficial to prevent or treat HFpEF.

Nonstandard Abbreviations and Acronyms

Alberta HEART	Alberta Heart Failure Etiology and Analysis Research Team
BMI	body mass index
BNP	brain natriuretic peptide
CH	clonal hematopoiesis
HF	heart failure
HFD	high-fat diet
HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
HR	hazard ratio
HSC	hematopoietic stem cell
L-NAME	Nω-nitro-l-arginine methyl ester
LV	left ventricular
NT-proBNP	N-terminal pro–B-type natriuretic peptide
SCAN-MP	Screening for Cardiac Amyloidosis With Nuclear Imaging for Minority Populations
VAF	variant allele fraction

Heart failure (HF) is a clinical syndrome of breathlessness or fatigue, or both, caused by impaired cardiac function. HF can be further categorized into HF with reduced ejection fraction (HFrEF) or HF with preserved ejection fraction (HFpEF), which are clinically associated with a predominant systolic dysfunction and diastolic dysfunction, respectively.[1] Despite a similar prevalence of HFpEF and HFrEF, the mortality rate of HFrEF has dropped significantly because of advancements in care, whereas the mortality rate of HFpEF has remained largely uncurbed because of limited US Food and Drug Administration–approved treatments for HFpEF.[2–4] The pathogenesis of HFpEF is poorly understood compared with that of HFrEF, yet recent findings suggest a pronounced role of inflammation, caused by diabetes, hyperlipidemia, or hypertension, in the development of HFpEF.[5] However, these conditions alone are insufficient for the development of HFpEF, suggesting the presence of additional factors that contribute to its pathogenesis. Thus, a better understanding of the mechanisms that contribute to HFpEF is required to address this prevalent yet underserved disease.

Clonal hematopoiesis (CH) is an emerging immunologic phenomenon that has been implicated in different diseases.[6,7] In this process, hematopoietic stem cells (HSCs) incur somatic mutations as a consequence of aging, smoking, or other environmental or biological stresses. In some cases, these mutations occur in driver genes, such as *DNMT3A*, *TET2*, *TP53*, *ASXL1*, or *JAK2*.[8–13] When these driver genes are altered, they provide a selective growth advantage to the HSCs. As a result, the variant cell line outcompetes neighboring cells in the bone marrow niche and undergoes a clonal expansion. Furthermore, these mutations are maintained in the progeny cells of the HSCs, and they can potentially affect the function of leukocytes and promote a chronic inflammatory state.[9,11–13] Recent studies have associated CH with worsened prognosis in HFrEF and in patients with an ischemic pathogenesis of HF.[14–17] However, there is no current literature connecting CH to the prognosis of patients with HFpEF. In this study, we elucidated the significance of CH in populations of patients with HFpEF and controls without a diagnosis of HFpEF, and characterized the effects of *Tet2*-mediated CH in a murine model of HFpEF.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Clinical Data (Alberta HEART)

All participant data were sourced from the Alberta HEART (Heart Failure Etiology and Analysis Research Team) cohort (Understanding and Treating Heart Failure With Preserved Ejection Fraction: Novel Mechanisms, Diagnostics and Potential Therapeutics; URL: https://www.clinicaltrials.gov; Unique identifier: NCT02052804) and described by Ezekowitz et al.[18,19] In brief, the cohort was recruited in Alberta, Canada, from 2010 to 2014 in an outpatient setting from a variety of clinics and the community at large. The cohort has been prospectively

followed for clinical outcomes since inception in 2010 by annual administrative health data abstraction. Standard baseline demographic characteristics, laboratory data, and other medical history data were collected by medical record review and direct contact with the participant during study entry and following visits. Transthoracic echocardiography was performed with the participants at rest in the left lateral decubitus position using commercially available Phillips iE33 ultrasound imaging system (Philips Medical Systems) equipped with an S5-phased or X5-phased array transducer. All images were stored digitally for offline analysis (Xcelera; Philips Medical Systems). Standard apical 4- and 2-chamber views were recorded with care taken to avoid foreshortening. Left ventricular (LV) volumes were measured from the apical 4- and 2-chamber views. LV end-systolic volume and end-diastolic volume were calculated using the Simpson biplane method of discs. LV ejection fraction was derived and expressed as a percentage. Participants received standardized follow-up every 3 months, during which additional information was collected. Median follow-up was 1355 days (25th–75th percentile, 854–1774). Participants were assigned to the control group or HFpEF group through an adjudication process conducted by team members with clinical experience and expertise. The adjudication process required 2 expert clinicians to review each case independently while blinded to each other's adjudication. The control population lacked a diagnosis of HF at any time during the study, were not at risk of developing HF, and did not exhibit any symptomatology of HF at the time of study entry. Given the diverse and ever-changing guidelines and criteria for HFpEF,[20] no strict criteria for HFpEF were used. Instead, the 2 expert clinicians reviewed each case independently and assigned participants to the HFpEF group on the basis of medical history, echocardiography, other radiologic testing, and laboratory information. The data for adjudication were sourced from medical records and testing performed at study entry. Blood samples for ultradeep, error-corrected sequencing were taken at time of study entry.

Data management and regulatory, ethical, and administrative review for Alberta HEART were provided in-kind by the Canadian VIGOUR Centre (Edmonton, Alberta, Canada), and biologic samples were managed by the Canadian BioSample Repository (Edmonton, Alberta, Canada) at the University of Alberta, Canada. Written informed consent was obtained from all participants. All analyses of laboratory studies and echocardiographic measurements were performed on baseline values, and patients with missing data for a given variable were excluded from analysis for that variable.

Validation Clinical Cohort (SCAN-MP)

Participant data were sourced from the SCAN-MP cohort (Screening for Cardiac Amyloidosis With Nuclear Imaging for Minority Populations; URL: https://www.clinicaltrials.gov; Unique identifier: NCT020528) as described by Ruberg et al.[21] In brief, participants were recruited from Columbia University Irving Medical Center, New York, NY; Harlem Hospital, New York, NY; Yale University, New Haven, CT; and Boston Medical Center, MA. All participants self-identified as Black or Hispanic of Caribbean origin. HF was diagnosed by either the modified criteria used by Rich et al.[22] or a score ≥3 on the National Health and Nutrition Examination Survey for HF criteria. Modified criteria used by Rich et al.[22] include a history of acute pulmonary edema or the occurrence of at least 2 of the following that improved with diuretic therapy without another identifiable cause: dyspnea on exertion, paroxysmal nocturnal dyspnea, orthopnea, bilateral lower extremity edema, or exertional fatigue. In addition, patients with HFpEF had an ejection fraction >30%. Only patients with HFpEF who lacked a diagnosis of cardiac amyloidosis were included in the current analysis. At the time of enrollment, medical history, medication inventory, laboratory studies, and transthoracic echocardiography were obtained, and blood samples were collected for assessment of CH.

SCAN-MP was approved by the Western Institutional Review Board in a single institutional review board model (approval no. 20183425). Written informed consent was obtained from all participants. All analyses of laboratory studies and echocardiographic measurements were performed on baseline values, and patients with missing data for a given variable were excluded from analysis for that variable.

Animal Studies: Mice

Wild-type mice (*Tet2*$^{+/+}$), *Tet2*-deficient mice (*Tet2*$^{-/-}$:B6[Cg]-*Tet2*$^{tm1.2Rao}$/J), and Pep Boy mice (B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ) were sourced from Jackson Laboratory (stock 000664, 023359, and 002014, respectively). All strains had a C57BL/6J background. *Tet2*$^{+/-}$ mice were used for breeding, and *Tet2*$^{-/-}$ and *Tet2*$^{+/+}$ male offspring were used for the test group and the control group, respectively. The animal protocols for these experiments were approved by the Institutional Animal Care and Use Committee of the University of Virginia.

Nonmyeloablative Bone Marrow Transplantation

Nonmyeloablative bone marrow transplantation was performed as previously published.[23] In brief, 8- to 12-week-old Pep Boy mice were transplanted with suspensions of bone marrow cells from either Tet2$^{+/+}$ mice or Tet2$^{-/-}$ mice. Unfractionated bone marrow cells (5×10^6 cells/day) were injected by the retro-orbital vein into nonirradiated recipients over 3 consecutive days (total, 1.5×10^7 cells).

HFpEF Model

After nonmyeloablative bone marrow transplantation, mice were randomized to receive either a control diet and water or high-fat diet (HFD; S1850; Bio-serv) and L-NAME (Nω-nitro-l-arginine methyl ester; 1.0 g/L; N5751, Sigma Aldrich) in the drinking water.[24] The drinking water was changed once per week. Throughout the length of the experiment, mice were maintained on a 12-hour light/dark cycle and had unrestricted access to food and water.

Statistical Analyses

Data are presented as mean and SD unless otherwise noted. For continuous data with 1 variable, the Shapiro-Wilk test was performed to evaluate the data distribution. Nonnormally distributed data were analyzed for statistical significance by Mann-Whitney *U* test; normally distributed data were analyzed with a Welch *t* test and a 1-way ANOVA with Dunnett multiple comparison for data with 1 independent variable and 2 groups or >2 groups, respectively. For categorical variables,

Fisher exact tests were performed to test statistical significance. For continuous data with 2 variables, a 2-way ANOVA with post hoc Sidak multiple comparison test was used to test for statistical significance. For multivariable analysis of continuous variables, a multivariable linear model was created adjusting for differences in backgrounds between groups. Data are reported as the coefficient ± standard error. For univariable and multivariable analysis of outcomes, a Cox proportional hazards model was used. Data are reported as hazard ratio (HR) and corresponding 95% CI.

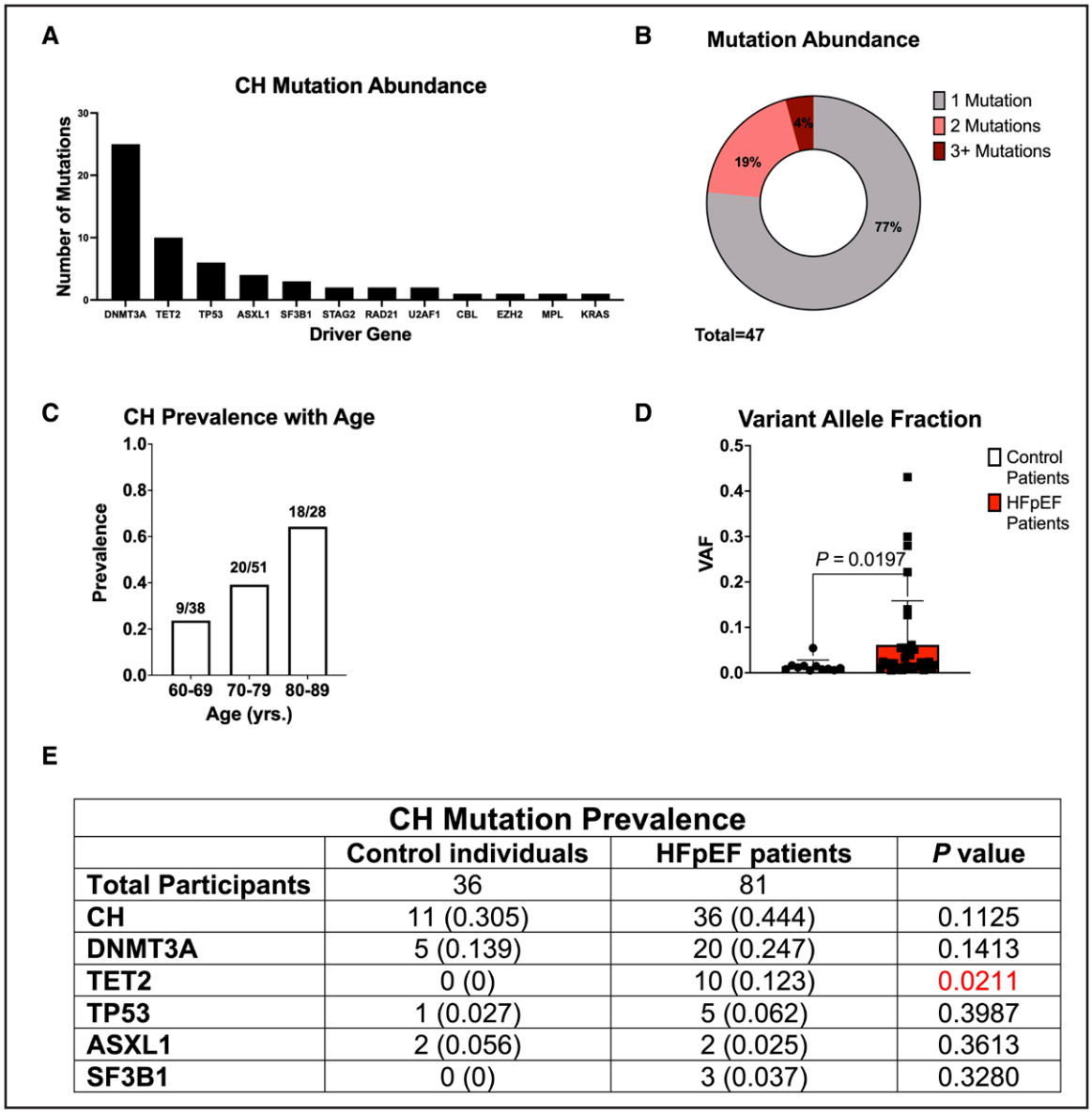
RESULTS

Patients With HFpEF and CH Display Worse Cardiac Diastolic Function and Outcomes

To investigate the significance of CH in HFpEF, peripheral blood samples were sourced from the Alberta HEART cohort.[19] Ultradeep, error-corrected sequencing with a panel of 20 candidate driver genes was then used to identify and quantify CH (Table S1). CH mutations only included frameshift, splicing, stop-gain, or nonsense variants that had the potential to influence protein function. The sequencing methodology was sufficient to resolve mutations with variant allele fractions (VAFs) as low as 0.005. As such, we used this VAF as our threshold for diagnosing CH. The cohort was composed of 81 patients with HFpEF and 36 controls without a diagnosis of HFpEF. As expected, the clinical characteristics of the patients with HFpEF differed significantly from those of the control individuals with respect to hypertension, diabetes, smoking history, and a number of other comorbidities (Table S2). In this cohort, ultradeep, error-corrected sequencing identified mutations in 12 known CH driver genes (Figure 1A). *DNMT3A* and *TET2* were the most commonly mutated genes. Of all the individuals with CH in the cohort, individuals with 1 mutation, 2 mutations, or 3+ mutations accounted for 77%, 19%, and 4%, respectively (Figure 1B). Consistent with previous reports,[7,16] CH prevalence increased with age (Figure 1C). Individuals between the ages of 60 and 69 years displayed a CH mutation prevalence of 24%, whereas 64% of individuals between 80 and 89 years of age displayed CH mutations. As shown in Figure 1D, patients with HFpEF possessed, on average, 4-fold larger CH clones than control individuals (VAF of 0.015 versus 0.061; *P*=0.0197). No *TET2* mutations were identified in control individuals, whereas 10 patients with HFpEF harbored a detectable *TET2* mutation (Figure 1E).

Further analysis characterized the functional significance of CH within the HFpEF population. The backgrounds of patients with HFpEF with CH and patients with HFpEF without CH were similar (Table). However, patients with HFpEF with CH tended to be older (77 versus 72 years; *P*=0.001) and have a lower body mass index (BMI; 28 versus 32 kg/m^2; *P*=0.008) and greater angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker use (87% versus 64%; *P*=0.01) than patients with HFpEF without CH. Patients with HFpEF with CH had worse baseline diastolic function compared with patients with HFpEF without CH, as evidenced by an increase in both E/e′ and E/A and a decrease in deceleration time (Figure 2A through 2C). Patients with HFpEF with CH had elevated levels of the BNP (brain natriuretic peptide) and NT-proBNP (N-terminal pro–B-type natriuretic peptide) biomarkers of HFpEF severity compared with patients with HFpEF without CH at baseline (Figure 2D and 2E). Multivariate analysis was performed to adjust for age, BMI, and angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker use (Table S3). After adjustment, E/e′, E/A, and deceleration time remained statistically significant, and CH became associated with increased left atrial volume index. In addition, many of these differences were maintained at different VAF cutoffs for identifying CH in both univariate and multivariate analyses, which corrected for corresponding differences in background characteristics (Tables S4 and S5). Given the differences in heart function, we then evaluated whether this difference was maintained when only stratifying for the presence or absence of mutations in either of the 2 most common CH driver genes—*DNMT3A* and *TET2*—as has been done previously.[15,16,25] Patients with HFpEF with a *DNMT3A/TET2* mutation and patients with HFpEF without *DNMT3A/TET2* mutation had similar backgrounds (Table S6). However, patients with HFpEF with a *DNMT3A/TET2* mutation tended to be female (70% versus 46%; P=0.0339), have a lower BMI (28 versus 31 kg/m2; P=0.0385), and have a lower prevalence of chronic obstructive pulmonary disease (7% versus 31%; *P*=0.0128). After adjusting for background differences, mutations in either *DNMT3A* or *TET2* were associated with worse diastolic function by E/A and deceleration time (Table S7). Furthermore, if using 0.01 or 0.02 as VAF cutoffs for diagnosing CH, mutations in either *DNMT3A* or *TET2* were associated with worse diastolic function by E/e′.

In a separate validation cohort, peripheral blood mononuclear cells were sourced from the ongoing SCAN-MP clinical trial,[21] which comprised patients with HFpEF from minority populations. Ultradeep, error-corrected sequencing was performed to identify and quantify CH. Patients with HFpEF with CH and patients with HFpEF without CH displayed no differences in background characteristics (Table S8). However, patients with HFpEF with CH exhibited worse diastolic function by both E/e′ (18.4 versus 13.8; *P*=0.003) and e′ alone (5.0 versus 6.2; *P*=0.03) compared with patients with HFpEF without CH (Figure S1A and S1B). Furthermore, this association held if patients with HFpEF were stratified for the presence or absence of mutations in either *DNMT3A* or *TET2*. Despite no difference in background characteristics (Table S9), patients with HFpEF with a *DNMT3A/TET2* mutation exhibited an increased E/e′ (18.7 versus



E

CH Mutation Prevalence			
	Control individuals	HFpEF patients	*P* value
Total Participants	36	81	
CH	11 (0.305)	36 (0.444)	0.1125
DNMT3A	5 (0.139)	20 (0.247)	0.1413
TET2	0 (0)	10 (0.123)	0.0211
TP53	1 (0.027)	5 (0.062)	0.3987
ASXL1	2 (0.056)	2 (0.025)	0.3613
SF3B1	0 (0)	3 (0.037)	0.3280

Figure 1. Characterization of clonal hematopoiesis in the Alberta HEART patient cohort.
A, Abundance of the specified driver gene mutation in the patient cohort (n=58). **B**, Proportion of patients with the specified number of clonal hematopoiesis (CH) mutations (n=47). **C**, CH prevalence as a function of age in the patient cohort (n=117). **D**, Variant allele fraction (VAF) for the largest clone identified in patients with CH in controls and patients with heart failure with preserved ejection fraction (HFpEF). Statistical significance was determined by Mann-Whitney *U* test (n=11 controls and 36 patients with HFpEF). **E**, Prevalence of CH and driver gene mutations in controls and patients with HFpEF. Statistical significance was determined by the Fisher exact test (n=36 controls and 81 patients with HFpEF). Alberta HEART indicates Alberta Heart failure Etiology and Analysis Research Team.

14.3; *P*=0.005) compared with patients with HFpEF without a *DNMT3A/TET2* mutation (Figure S1C).

We further investigated the effect of CH on prognosis for patients with HFpEF, whose age ranged from 60 to 90 years. Patients with HFpEF with and without CH displayed 5-year cardiovascular-related hospitalization rates of 43.5% and 26.9%, respectively (HR, 2.09; *P*=0.10; Figure S2A). We next examined prognosis by stratifying for an age ≥70 years, at which many of these adverse events become more common. The backgrounds of patients with HFpEF, an age ≥70 years, and CH, and patients with HFpEF and an age ≥70 years and without CH were similar (Table S10), except that patients with HFpEF, an age ≥70 years, and CH tended to be older (80 versus 77 years; *P*=0.02) and have a lower BMI (28 versus 31 kg/m^2; *P*=0.008). Patients with HFpEF, an age ≥70 years, and CH displayed a 5-year cardiovascular-related hospitalization rate of 51%; patients with HFpEF and an age ≥70 years and without CH had a 5-year cardiovascular-related hospitalization rate of 10% (HR, 6.83; *P*=0.0041; Figure 3A and 3B). To eliminate the contribution of age and BMI, a

Table. Clinical Characteristics of Patients With HFpEF With and Without Clonal Hematopoiesis

Characteristics	HFpEF CH⁻	HFpEF CH⁺	*P* value
Total	45	36	
Age, y	72 (7)	77 (7)	0.0012*
Female	21 (0.47)	23 (0.64)	0.0929
Body mass index, kg/m²	32 (6)	28 (5)	0.0083*
Hypertension	38 (0.84)	33 (0.92)	0.2635
Diabetes	19 (0.42)	11 (0.31)	0.1983
Hyperlipidemia	28 (0.62)	23 (0.64)	0.5316
Chronic obstructive pulmonary disease	14 (0.31)	5 (0.14)	0.0585
Chronic kidney disease	7 (0.16)	8 (0.22)	0.3144
History of cancer	0	0	1.0000
History of smoking	24 (0.53)	17 (0.47)	0.3734
Previous myocardial infarction	11 (0.24)	6 (0.17)	0.2830
Previous coronary revascularization	0	0	1.0000
Atrial fibrillation or flutter	22 (0.49)	23 (0.64)	0.1302
Cerebrovascular disease	4 (0.09)	8 (0.22)	0.0866
Peripheral vascular disease	1 (0.02)	2 (0.06)	0.4160
Angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker	39 (0.87)	23 (0.64)	0.0161*
β-Blocker	36 (0.8)	25 (0.69)	0.2015
Diuretic	36 (0.8)	31 (0.86)	0.3375
Calcium channel blocker	21 (0.47)	13 (0.36)	0.2331
Digoxin	3 (0.07)	4 (0.11)	0.3753
Antiarrhythmic	6 (0.13)	7 (0.19)	0.3283
Anticoagulation	21 (0.47)	21 (0.59)	0.2061
Antiplatelet	4 (0.09)	1 (0.03)	0.2570

Continuous variables are expressed as mean (standard deviation); categorical variables are described by absolute count (frequency).
*Statistically significant.

Cox proportional hazards model for HFpEF patient outcomes was performed. After adjusting for both age and BMI, patients with HFpEF, an age ≥70 years, and CH maintained a statistically significant increase in 5-year cardiovascular-related hospitalization rate compared with patients with HFpEF and an age ≥70 years and without CH, suggesting worse prognosis (HR, 5.06; *P*=0.042). The difference in cardiovascular-related hospitalization rate is maintained at VAF thresholds for CH of ≥0.005, ≥0.01, and ≥0.02 in both univariate and multivariate analyses, which corrected for corresponding differences in background characteristics (Tables S11 and S12).

Given the difference in prognosis for patients with HFpEF and an age ≥70 years, we then evaluated whether this difference was maintained at the age threshold when only stratifying for the presence or absence of mutations in either *DNMT3A* or *TET2*. Indeed, patients with HFpEF, an age ≥70 years, and a *DNMT3A/TET2* mutation had a 5-year cardiovascular-related hospitalization rate of 64%, whereas patients with HFpEF and an age ≥70 years and without a *DNMT3A/TET2* mutation had a 5-year cardiovascular-related hospitalization rate of 13% (HR, 5.25; *P*=0.006; Figure 3A and 3C). Patients with HFpEF, an age ≥70 years, and a *DNMT3A/TET2* mutation had a similar background to patients with HFpEF and an age ≥70 years and without a *DNMT3A/TET2* mutation; however, patients with HFpEF, an age ≥70 years, and a *DNMT3A/TET2* mutation tended to be female (71% versus 44%; *P*=0.04; Table S13). After adjusting for sex by Cox proportional hazards model, this difference in cardiovascular-related hospitalizations was maintained (HR, 4.40; *P*=0.017; Figure 3A and 3C). This difference in cardiovascular-related hospitalization rate was maintained at VAF cutoffs of ≥0.005, ≥0.01, and ≥0.02 in a univariate analysis and maintained at VAF cutoffs of ≥0.005 and ≥0.01 in a multivariate analysis, which corrected for corresponding differences in background characteristics (Table S14). To understand how individual mutations in either *DNMT3A* or *TET2* modify prognosis, patients with HFpEF were stratified for the presence or absence of either of these mutants. In multivariate analyses adjusting for differences in backgrounds of patients with HFpEF and an age ≥70 years (Table S15), *DNMT3A*-mediated CH was associated with a significant increase in cardiovascular-related hospitalization rate (HR, 5.39; *P*=0.018; Figure 3A and 3D), and *TET2*-mediated CH was associated with a trending increase in cardiovascular-related hospitalization rate (HR, 2.92; *P*=0.076; Figure 3A and 3E). In contrast with the findings with echocardiographic measures and cardiovascular-related hospitalization data, analyses of all-cause mortality did not reveal associations with CH within the entire cohort (Figure S2B and S2C) or subgroups (Table S16), which may be attributable to the high degree of patient survival in the Alberta HEART cohort.

A Murine Model of HFpEF Accelerates *TET2*-Mediated Hematopoietic Cell Expansion

Because of its enrichment in the HFpEF patient cohort, *TET2* was chosen for further mechanistic studies in mice. To model a more physiologically relevant state of CH, a small number of CD45.2 *Tet2* wild-type (*Tet2*$^{+/+}$) or *Tet2*-deficient (*Tet2*$^{-/-}$) bone marrow cells were adoptively transferred to CD45.1 Pep Boy mice and allowed to expand for 1 month. Mice were then either continued on control diet and water or placed on an HFD and L-NAME drinking water combination treatment to induce features of HFpEF.[24] Serial measurements were performed at baseline, 5 weeks, and 12 weeks after treatment induction (Figure 4A).

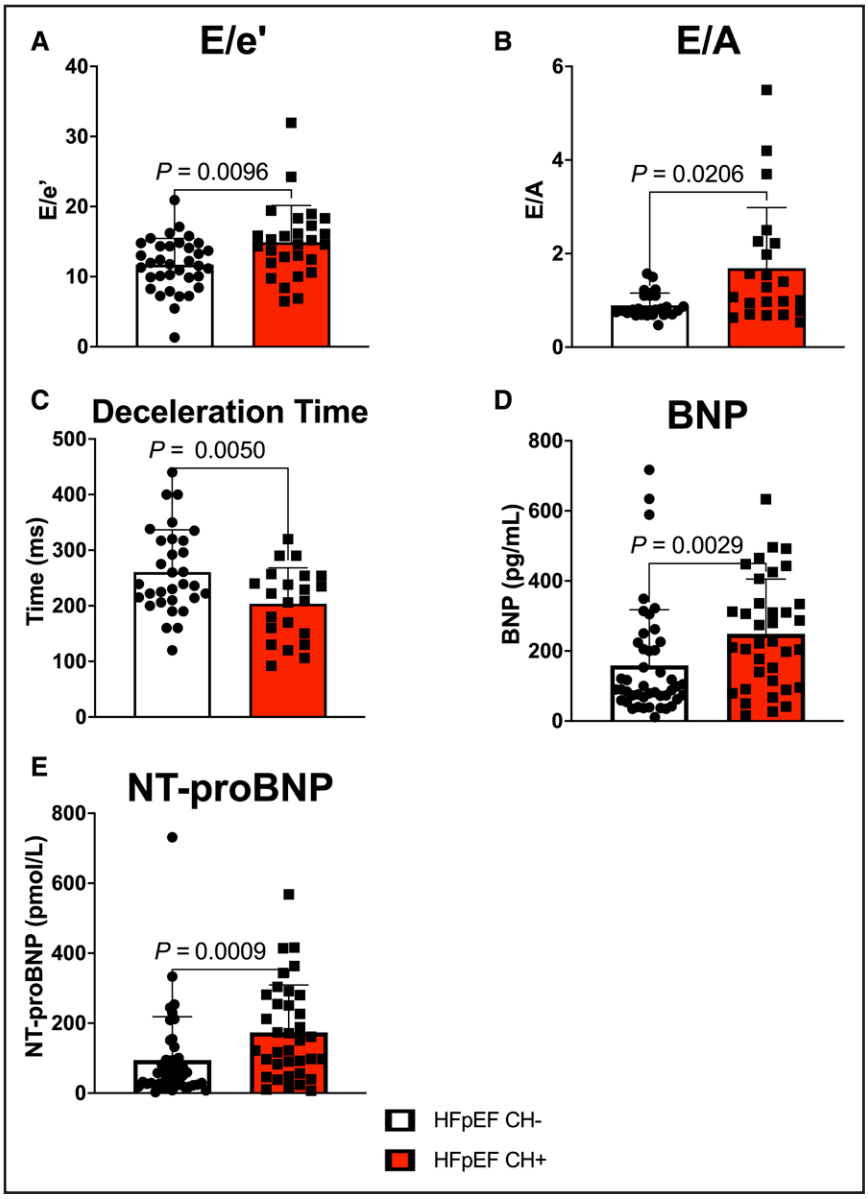


Figure 2. Patients with heart failure with preserved ejection fraction with clonal hematopoiesis exhibit worse diastolic dysfunction and possess elevated levels of heart failure biomarkers at baseline.
A, Echocardiographic analysis of E/e′ in patients with heart failure with preserved ejection fraction (HFpEF) with and without clonal hematopoiesis (CH). Statistical significance was determined by Mann-Whitney *U* test (n=35 HFpEF CH– and 27 HFpEF CH+). **B**, Echocardiographic analysis of E/A in patients with HFpEF with and without CH. Statistical significance was determined by Mann-Whitney *U* test (n=27 HFpEF CH– and 22 HFpEF CH+). **C**, Echocardiographic analysis of deceleration time in patients with HFpEF with and without CH. Statistical significance was determined by Welch *t* test (n=31 HFpEF CH– and 22 HFpEF CH+). **D**, Plasma BNP (brain natriuretic peptide) levels for patients with HFpEF with and without CH. Statistical significance was determined by Mann-Whitney *U* test (n=45 HFpEF CH– and 36 HFpEF CH+). **E**, Plasma NT-proBNP (N-terminal pro–B-type natriuretic peptide) levels for patients with HFpEF with and without CH. Statistical significance was determined by Mann-Whitney *U* test (n=45 HFpEF CH– and 36 HFpEF CH+).

To evaluate clonal expansion in this model, flow cytometry of peripheral blood was performed. As published previously,[23] donor-derived *Tet2*$^{-/-}$ cells expanded more rapidly throughout all white blood cell lineages compared with donor-derived *Tet2*$^{+/+}$ cells, with a bias toward the myeloid and B-cell populations (Figure 4B through 4G). HFD/L-NAME enhanced *Tet2*-mediated cell expansion (Figure 4B, 4C, 4E, and 4G). In accordance, flow cytometry of bone marrow revealed expansion of donor-derived *Tet2*$^{-/-}$ cells in the long-term and short-term HSC pools compared with donor-derived *Tet2*$^{+/+}$ cells (Figure 5A through 5F). Furthermore, in parallel with peripheral blood chimerism, donor chimerisms of HSC pools were significantly higher for *Tet2*$^{-/-}$ cells subjected to the HFD/L-NAME regimen compared with *Tet2*$^{-/-}$ cells exposed to the control conditions. HFD/L-NAME induced leukocytosis by 3 months, with increases in the absolute number of neutrophils, monocytes, and leukocytes for the *Tet2*$^{-/-}$ group (Figure S3). There were no differences in platelet counts or hemoglobin concentration throughout the conditions.

TET2-Mediated CH Exacerbates HF in a Murine Model of HFpEF

To induce HFpEF, a combination of an obesogenic diet and a hypertensive drug was used. The HFD/L-NAME combination increased weight gain compared with respective mice on control diet and water (Figure 6A). Furthermore, the *Tet2*$^{-/-}$ group exhibited increased weight gain compared with the *Tet2*$^{+/+}$ group on the HFD/L-NAME treatment. In addition, the HFD/L-NAME combination treatment led to increased systolic blood pressure compared with the respective control group (Figure 6B).

To test whether *Tet2*-mediated CH modifies cardiac function in this model of HFpEF, serial echocardiographic

A

Correlation of different CH mutations with patient outcomes		
	CV-related Hospitalization	
	HR (Univariable)	HR (Multivariable)
CH	6.83 (1.50-31.05, *P* = 0.013)	5.06 (1.06-24.15, *P* =0.042)
DNMT3A/TET2	5.25 (1.60-17.25, *P* = 0.006)	4.40 (1.30-14.94, *P* =0.017)
DNMT3A	3.55 (1.19-10.59, *P* = 0.023)	5.39 (1.34-21.71, *P* =0.018)
TET2	2.92 (0.90-9.54, *P* = 0.076)	2.92 (0.90-9.54, *P* = 0.076)

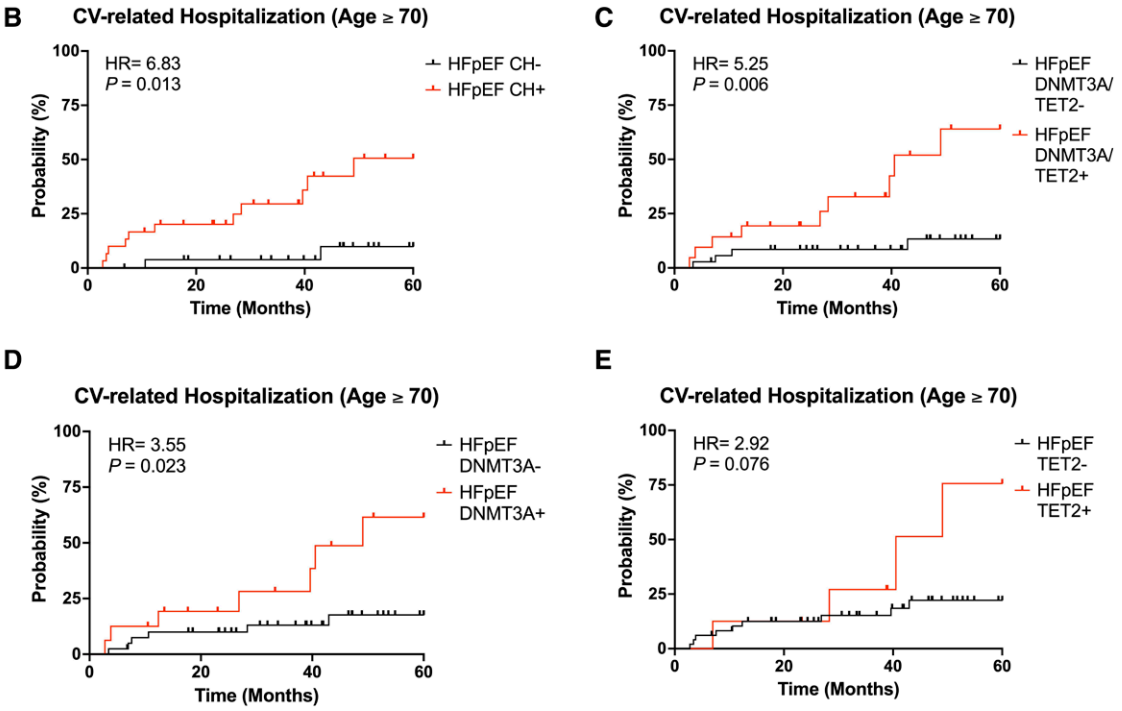


Figure 3. Patients with heart failure with preserved ejection with mutations in clonal hematopoiesis driver genes exhibit worse long-term prognosis.

A, Univariate and multivariate analysis of cardiovascular (CV)–related hospitalization for patients with heart failure with preserved ejection fraction (HFpEF) and age ≥70 years on the basis of clonal hematopoiesis (CH) status, *DNMT3A/TET2*-driven CH status, *DNMT3A*-driven CH status, and *TET2*-driven CH status. Statistical significance and hazard ratio (HR) were determined by Cox proportional hazards model. **B**, Five-year cardiovascular-related hospitalization on the basis of clonal hematopoiesis status for patients with HFpEF and an age ≥70 years. Statistical significance and HR were determined by Cox proportional hazards model (n=27 HFpEF CH– and 30 HFpEF CH+). **C**, Five-year cardiovascular-related hospitalization for patients with HFpEF with and without *DNMT3A/TET2*-driven CH and with an age ≥70 years. Statistical significance and HR were determined by Cox proportional hazards model (n=36 HFpEF DNMT3A/TET2– and 21 HFpEF DNMT3A/TET2+). **D**, Five-year cardiovascular-related hospitalization for patients with HFpEF with and without *DNMT3A*-driven CH and with an age ≥70 years. Statistical significance and HR were determined by Cox proportional hazards model (n=41 HFpEF DNMT3A– and 16 HFpEF DNMT3A+). **E**, Five-year cardiovascular-related hospitalization for patients with HFpEF with and without *TET2*-driven CH and with an age ≥70 years. Statistical significance and HR were determined by Cox proportional hazards model (n=49 HFpEF TET2– and 8 HFpEF TET2+).

analysis was performed on these mice. The HFD/L-NAME combination induced an increase in LV filling pressures as estimated by an increase in the Doppler-derived E/e′ ratio and measured directly by LV catheterization, collectively indicating worse diastolic heart function (Figure 6C and 6D). Moreover, the adoptive transplantation of *Tet2*$^{-/-}$ bone marrow exacerbated diastolic dysfunction compared with mice receiving *Tet2*$^{+/+}$ bone marrow. The *Tet2*$^{-/-}$ group on the HFD/L-NAME treatment also displayed worse cardiac hypertrophy and fibrosis compared with the *Tet2*$^{+/+}$ group with the same dietary treatment (Figure 6E through 6G).

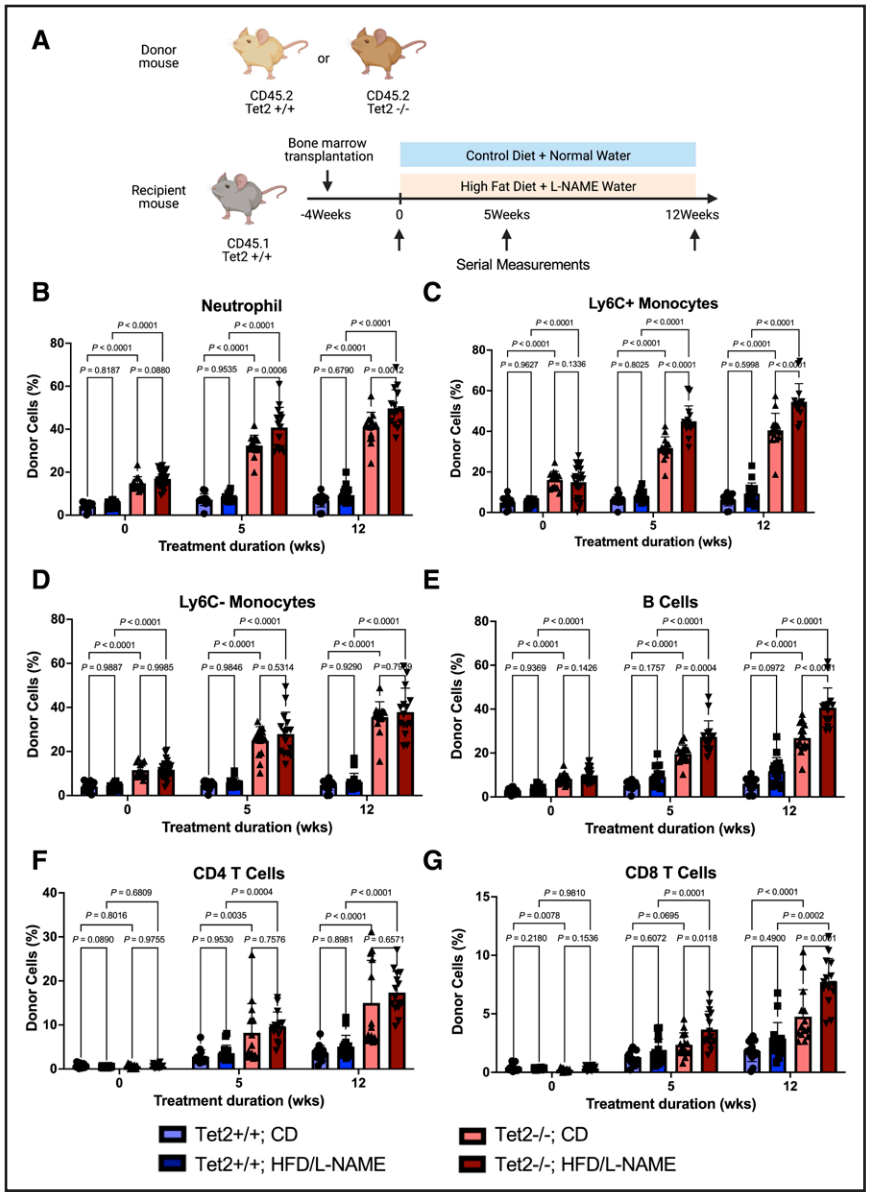


Figure 4. HFD/L-NAME treatment accelerates expansion of Tet2-deficient cells in peripheral blood. **A**, Schematic of the experimental design. CD45.2 Tet2-sufficient or Tet2-deficient bone marrow was adoptively transferred to CD45.1 Pep Boy mice. One month after bone marrow transplantation, mice were started on either high-fat diet (HFD)/L-NAME (Nω-nitro-l-arginine methyl ester) combination treatment or continued on control diet and water. Serial measurements were taken at baseline, 5 weeks, and 12 weeks. Created with BioRender (BioRender.com). **B** through **G**, Flow cytometric quantification of donor cell chimerism for neutrophils, Ly6C+ monocytes, Ly6C− monocytes, B cells, CD4 T cells, and CD8 T cells in the peripheral blood at baseline and after 5 weeks and 12 weeks of treatment. Statistical significance was determined by 2-way ANOVA with post hoc Sidak multiple comparison test (n=13–26 per group).

DISCUSSION

CH is increasingly recognized as a key risk factor for cardiovascular disease. The accessibility of human peripheral blood samples and declining cost of ultradeep error-corrected sequencing have streamlined the analysis of CH in clinical cohorts. In the past few years, numerous studies have been published on the associations between CH and cardiovascular disease[7] and its additive effect with traditional cardiovascular disease risk factors.[26] CH is associated with increased risk of incident HF and increased cardiovascular disease morbidity and mortality in patients with HFrEF.[14–17] Despite these advances, past studies were not stratified for etiology or were constrained to patients with HFrEF.[16] Thus, the current study focused on the role of CH in HFpEF.

In this study, we investigated the significance of CH in controls and patients with HFpEF of the cardiometabolic phenogroup, as evidenced by high BMI and diabetes prevalence. We found an enrichment of patients with *TET2* mutations in the HFpEF cohort versus control individuals. In an analysis of all 20 CH driver genes assayed, there was a significant increase in variant clone size in the patients with HFpEF versus the control group. However, because of differences between the patients with HFpEF and controls, it is unclear whether these findings are being driven specifically by the disease state of HFpEF or by one or more of its comorbidities or covariates. Thus, focusing on patients with HFpEF, analyses revealed that the presence of CH was associated with worse echocardiographic metrics of heart function. The increased E/e′ and E/A and decreased deceleration time collectively indicate worse diastolic

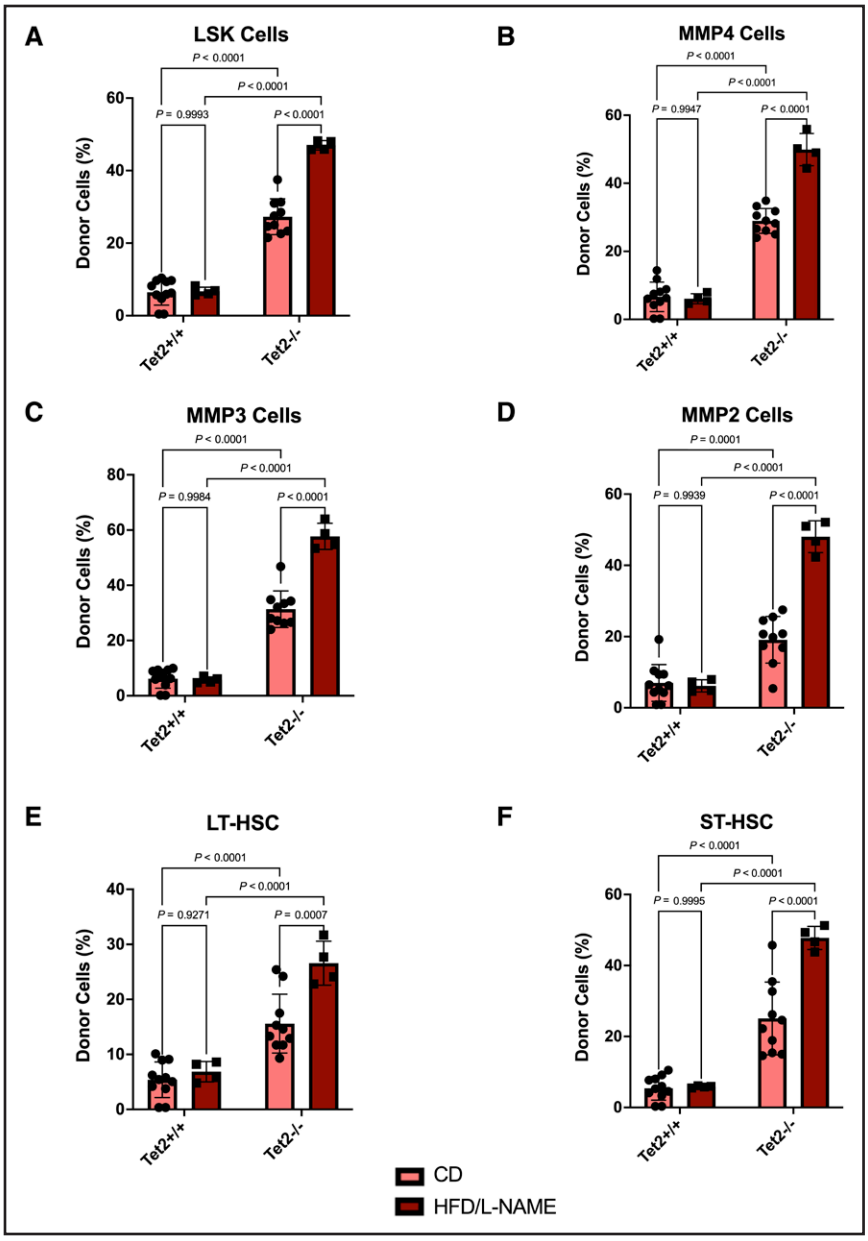


Figure 5. HFD/L-NAME treatment promotes expansion of *Tet2*-deficient cells in the hematopoietic stem and progenitor cells.
A through **F**, Flow cytometric quantification of donor cell chimerism in LSK ($Lin^{-}Sca1{+}c\text{-}Kit^{+}$) cells, multipotent progenitor 4 (MMP4) cells, multipotent progenitor 3 (MMP3) cells, multipotent progenitor 2 (MMP2) cells, long-term hematopoietic stem cells (LT-HSC), and short-term hematopoietic stem cells (ST-HSC) of the bone marrow after 12 weeks of treatment. Statistical significance was determined by 2-way ANOVA with post hoc Sidak multiple comparison test (n=4–11 per group). HFD indicates high-fat diet; and L-NAME, Nω-nitro-l-arginine methyl ester.

heart function in patients with HFpEF and CH compared with those without CH. The increased left atrial volume index after multivariable adjustment suggests structural remodeling of the heart in patients with HFpEF and CH compared with patients with HFpEF and without CH. In accordance, these findings are associated with worse long-term prognosis as evidenced by increased 5-year cardiovascular-related hospitalization rate. Consistent with previous literature,[15,16,25] we also stratified for the presence or absence of *DNMT3A/TET2* mutations and found similar results. The association between CH and worse prognosis appears to become more pronounced with age. Patients with HFpEF and an age ≥70 years had an over 4-fold greater odds of experiencing a cardiovascular disease–related hospitalization if they possessed a *DNMT3A* or *TET2* mutation. This age-dependent association of CH with cardiovascular-related hospitalization may be attributed to the greater prevalence of comorbidities and hospitalization in patients of advancing age. Furthermore, the significance of these findings is maintained when explicitly adjusting for covariates in a multivariable model (Table S16). In sum, the presence of CH appears to be an important determinant of the functional status and morbidity of HFpEF.

Our study expands upon previous clinical findings connecting CH and HF. Studies have reported that *DNMT3A*- or *TET2*-mediated CH is associated with worse prognosis in patients with chronic ischemic HF and after ST-segment–elevation myocardial infarction.[15,25] It has also been reported that patients with *DNMT3A*- or *TET2*-mediated CH exhibit adverse HF progression irrespective of ischemic or nonischemic pathogenesis.[16] Yu et al[17]

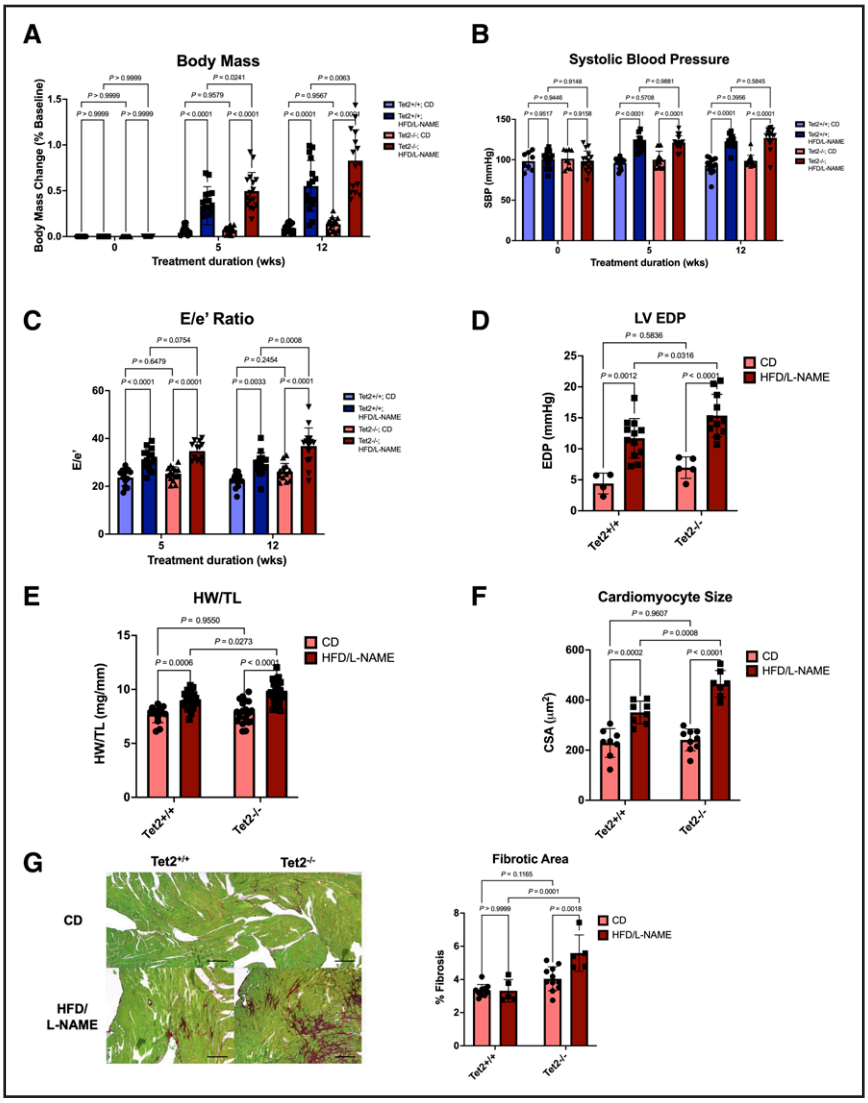


Figure 6. Tet2-mediated clonal hematopoiesis exacerbates cardiomyopathy in a model of heart failure with preserved ejection fraction.

A, Normalized change in body mass relative to baseline body mass measured at the initiation of treatment. Measurements were performed at baseline and after 5 and 12 weeks of treatment. Statistical significance was determined by 2-way ANOVA with post hoc Sidak multiple comparison test (n=14–17 per group). **B**, Systolic blood pressure measured by tail-cuff plethysmography at baseline and after 5 and 12 weeks of treatment. Statistical significance was determined by 2-way ANOVA with post hoc Sidak multiple comparison test (n=8–17 per group). **C**, Serial echocardiographic analysis after 5 and 12 weeks of treatment. Statistical significance was determined by 2-way ANOVA with post hoc Sidak multiple comparison test (n=13–16 per group). **D**, Left ventricular end diastolic pressure (LV EDP) was obtained by left ventricle catheterization after 12 weeks of treatment. Statistical significance was determined by 2-way ANOVA with post hoc Sidak multiple comparison test (n=4–12 per group). **E**, Heart weight normalized to tibia length (HW/TL) after 12 weeks of treatment. Statistical significance was determined by 2-way ANOVA with post hoc Sidak multiple comparison test (n=13–28 per group). **F**, Quantification of cardiomyocyte cross-sectional area from wheat germ agglutinin (WGA)–stained hearts after 12 weeks of treatment. Statistical significance was determined by 2-way ANOVA with post hoc Sidak multiple comparison test (n=7–9 per group). **G**, Representative images and analysis of picrosirius red/fast green staining of the hearts after 12 weeks of treatment. Scale bars are included in the lower right corner and correspond to 200 μm. Statistical significance was determined by 2-way ANOVA with post hoc Sidak multiple comparison test (n=5–11 per group). HFD indicates high-fat diet; and L-NAME, Nω-nitro-l-arginine methyl ester.

found that CH, particularly with mutations in the *ASXL1*, *TET2*, and *JAK2* driver genes, was associated with incident HF in an analysis of 5 large biobanks. More recently, the study by Shi et al.[27] focused specifically on the role of CH in incident HFpEF. This study reported that CH, using a VAF threshold of 2%, was associated with incident HFpEF in individuals younger than 65 years, but this association was not found when the entire cohort was analyzed. Our data suggest that the association between CH and HFpEF can extend to patients older than 65 years, and that VAFs as low as 0.5% may be predictive of this condition. The significance of this finding is bolstered by the fact that small clones become almost ubiquitous with advanced age.[28] We found that lower VAF cutoffs tended to better stratify patients in terms of prognosis, as evidenced by the higher HR with lower VAF cutoffs

(Table S12). This may be because these small clones can expand over the course of follow-up and consequently exert a greater biological effect with time. Thus, traditional DNA sequencing approaches, which are limited by their sequencing depth and therefore VAF detection limit, are not able to detect these clones, which appear to harbor notable prognostic significance. As a consequence, although previous work has documented a pronounced effect of CH on prognosis,[6,15,16,25,29,30] these data may be an underestimation of the true effect of CH.

To examine HFpEF in an experimental model, we used a combination of an obesogenic diet and the hypertensive drug L-NAME, as previously described.[24] This model was chosen as it reproduces aspects of the metabolic syndrome and consequent features of HFpEF that can be observed in these patients and further mirrors the cardiometabolic phenogroup largely investigated in our clinical cohort. Furthermore, it does not exploit genetic perturbations that are generally not observed in patients. As such, it was deemed the more physiologically realistic and applicable model for our murine studies. In addition, the nonirradiated adoptive transfer model of CH was used to model the spontaneous development and expansion of *Tet2*-deficient clones. This model avoids dynamic changes induced in both the hematopoietic niche and cardiovascular system, and appetite loss as a consequence of irradiation.[31–33] Together, we reasoned that this combined model more faithfully produces features of HFpEF and *TET2*-mediated CH for mechanistic studies.

As previously published in the adoptive transfer model,[23] *Tet2*-deficient hematopoietic cells were found to expand more rapidly than *Tet2*-sufficient clones. However, the HFD/L-NAME treatment accelerated expansion of *Tet2*-deficient donor cells in both peripheral blood and HSC lineages. These data mirrored the increased VAF of CH clones observed in patients with HFpEF compared with controls. This accelerated expansion may be partially attributed to increased hematopoiesis. Compared with the $Tet2^{-/-}$ group on control conditions, the $Tet2^{-/-}$ group on HFD/L-NAME treatment exhibited leukocytosis with elevation of several white blood cell lineages. Furthermore, hypertension has been demonstrated to increase hematopoiesis in murine and human models.[34] Thus, these differences may reconcile the lack of accelerated *Tet2*-deficient clonal expansion observed when mice are subjected to a HFD alone, atherogenic stimuli, or other models of HF.[10,35,36] Moreover, under the conditions of experimental HFpEF, the increased chimerism may exacerbate the sequelae of *Tet2*-mediated CH.

In this murine model, *Tet2*-mediated CH exacerbates several features of HFpEF. The $Tet2^{-/-}$ group manifested increased E/e′, LV end-diastolic pressure, heart weight/tibia length, and cardiac fibrosis compared with the $Tet2^{+/+}$ group on the HFD/L-NAME treatment, suggesting worse diastolic function, cardiac hypertrophy, and cardiac fibrosis. This again parallels our clinical data, which showed that patients with HFpEF and CH have significantly worse diastolic function. Our data suggest that *Tet2*-mediated CH directly exacerbates HF in a murine model of HFpEF and reproduces many findings observed in a human cohort of HFpEF

This study extends upon previous work in elucidating a causal connection between *TET2*-mediated CH and cardiovascular disease.[7] Our laboratory has shown that Tet2 deficiency in HSCs spontaneously leads to HFrEF in aging mice.[23] In addition, Tet2 deficiency in HSCs exacerbates HF in ischemic models of HFrEF such as myocardial infarction and nonischemic models of HFrEF such as pressure overload and angiotensin II infusion.[9,10] Collectively, *Tet2*-mediated CH exacerbates HFpEF and HFrEF in murine models of disease.

We acknowledge several limitations of the current study. First, for our clinical studies, the sample size is modest, and all patients were recruited from the same geographic location. However, in an analysis of a small cohort from SCAN-MP, we were able to corroborate that CH was associated with diastolic dysfunction. In the future, larger multicenter studies are required to corroborate these findings and potentially reveal novel associations whose effect sizes were too small to uncover in our analyses. Second, we acknowledge that HFpEF is a diverse disease state with myriad phenogroups.[37,38] Our cohort was composed predominately of the cardiometabolic phenogroup, in which patients typically have obesity and diabetes. As such, the role of CH in other HFpEF phenotypes remains unclear. Third, it will be informative to discern whether CH has an effect on cardiovascular-related mortality rate through the analysis of larger cohorts or a longer follow-up. Regarding this point, cohorts of Canadian patients with HFpEF, such as the one examined herein, may exhibit reduced morbidity and mortality because of the predominance of White participants enrolled in the study.[39,40] Fourth, the adoptive transfer of Tet2-deficient HSCs in experimental studies will generate larger homozygous clones than the heterozygous clones typically observed in humans. However, this is common for almost all experimental models, which use exaggerated stimuli to model disease in a temporally and fiscally feasible manner. In addition, it has been demonstrated that similar to Tet2 deficiency, Tet2 heterozygosity promotes clonal expansion and cardiac dysfunction, albeit to a lesser extent.[10,23] Fifth, we only used 1 murine model of HFpEF. However, many other models of HFpEF do not reliably reproduce many of the clinical phenotypes of HFpEF.[41] Furthermore, models that resemble the clinical phenotype tend to use a combination of an obesogenic diet, hypertensive drug, and aging. Because the other robust models of HFpEF are largely redundant with our own, they were not examined in our study. Sixth, male mice were used exclusively for this study because female mice are more resistant to HFD-induced metabolic disturbances and inflammation.[42]

TET2-mediated CH is enriched among patients with HFpEF, and patients with HFpEF with CH display worse heart function and prognosis, particularly in individuals with an age ≥70 years. Experimental models of HFpEF and *Tet2*-mediated CH suggest this relationship to be causal and may serve as a platform to elucidate further the pathophysiology and possible treatments for HFpEF exacerbated by CH.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Expanded Methods
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