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ORIGINAL RESEARCH - CLINICAL

Association Between Clonal Hematopoiesis and Left Ventricular Reverse Remodeling in Nonischemic Dilated Cardiomyopathy

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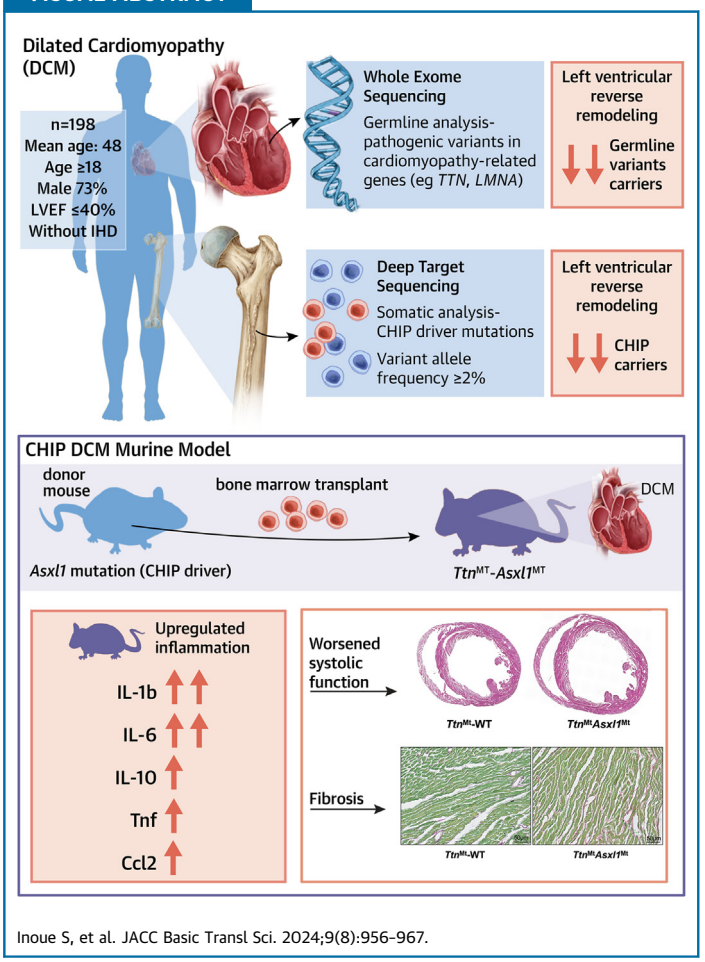
VISUAL ABSTRACT



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HIGHLIGHTS

- **Study investigators analyzed simultaneously germline variants in cardiomyopathy-related genes and somatic mutations in CHIP driver genes from blood samples in nonischemic patients with DCM.**
- **DCM patients with CHIP had a decreased probability of achieving LVRR, serving as a potent surrogate marker for adverse events in DCM. This effect was independent of established risk factors, including germline cardiomyopathy-related gene variants.**
- **CHIP caused by *Asxl1* mutation exacerbated cardiac dysfunction and myocardial fibrosis in DCM murine model with the germline truncating variant in the *Ttn* gene.**

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SUMMARY

Although clonal hematopoiesis of indeterminate potential (CHIP) is an adverse prognostic factor for atherosclerotic disease, its impact on nonischemic dilated cardiomyopathy (DCM) is elusive. The authors performed whole-exome sequencing and deep target sequencing among 198 patients with DCM and detected germline mutations in cardiomyopathy-related genes and somatic mutations in CHIP driver genes. Twenty-five CHIP driver mutations were detected in 22 patients with DCM. Ninety-two patients had cardiomyopathy-related pathogenic mutations. Multivariable analysis revealed that CHIP was an independent risk factor of left ventricular reverse remodeling, irrespective of known prognostic factors. CHIP exacerbated cardiac systolic dysfunction and fibrosis in a DCM murine model. The identification of germline and somatic mutations in patients with DCM predicts clinical prognosis. (JACC Basic Transl Sci 2024;9:956–967)

ABBREVIATIONS AND ACRONYMS

BMT = bone marrow transplantation

CHIP = clonal hematopoiesis of indeterminate potential

CMR = cardiac magnetic resonance

CVD = cardiovascular disease

DCM = dilated cardiomyopathy

HF = heart failure

HFrEF = heart failure with reduced ejection fraction

LV = left ventricular

LVEF = left ventricular ejection fraction

LVRR = left ventricular reverse remodeling

VAF = variant allele frequency

WT = wild-type

Dilated cardiomyopathy (DCM) is a group of heterogeneous myocardial disorders that are characterized by unexplained left ventricular (LV) dilation and dysfunction. DCM is a major causative disease of severe heart failure with reduced ejection fraction (HFrEF) requiring mechanical circulatory support and heart transplantation, particularly in young adults and adolescents. Heritable and acquired factors contribute to the pathogenesis and development of DCM.[1]

The heritable factors represent pathogenic variants in genes related to cardiomyopathy. With the advent of novel technologies such as next-generation sequencing, many genetic studies have been conducted. Approximately 40% of patients with DCM have pathogenic variants, which are associated with a worse clinical prognosis compared with those without pathogenic variants.[1,2] Furthermore, the clinical severity of patients with DCM varies depending on the specific genes involved. Mutations in the *TTN* gene, which encodes titin, are associated with LV reverse remodeling (LVRR) and a lower risk for major adverse cardiac events, whereas the opposite is observed for mutations in the *LMNA* gene.[3] These findings suggest that genetic analysis is crucial to predict the disease phenotype and prognosis of patients with DCM. However, analysis of germline mutations alone cannot explain the whole etiology of patients with DCM, and the identities of secondary factors superimposed on germline mutations are being explored.

Acquired factors consisting of environmental factors and comorbidities are candidate secondary factors. In addition, clonal hematopoiesis caused by somatic mutations in hematopoietic stem cells without evidence of hematological malignancy, referred to as clonal hematopoiesis of indeterminate potential (CHIP),[4] has attracted considerable attention as a cause of cardiovascular disease (CVD). Although CHIP increases the relative risk for hematologic malignancies by 10- to 100-fold, and its prevalence increases with age, the absolute risk remains limited (0.5% per year).[5] However, it has been reported that patients with CHIP are at risk for atherosclerotic CVD, heart failure (HF), and mortality related to CVD. Growing evidence suggests that the

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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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presence of CHIP causes vascular inflammation and is associated with the risk for atherosclerotic CVD.[6] Furthermore, recent studies demonstrated that CHIP increases the incidence of newly diagnosed HF by 25% and is related to the poor prognosis of patients with HFrEF.[7,8] Along with the relevance of CHIP to ischemic heart disease, the impact of CHIP on nonischemic DCM as well as its pathophysiology come into the spotlight. In this study, we investigated the effect of CHIP in nonischemic patients with DCM using a multicenter DCM cohort in Japan and its possible pathophysiology using animal experiments.

METHODS

STUDY POPULATION. Whole-exome sequencing was performed on patients with DCM, defined as LV ejection fraction (LVEF) ≤40% without an apparent cause of global systolic impairment, including uncontrollable hypertension, congenital heart diseases, and severe primary valvular heart diseases.[9] From 2018 to 2022, patients were enrolled from the University of Tokyo Hospital, Chiba University Hospital, Kyushu University Hospital, Juntendo University Hospital, Tokyo Women's Medical University Hospital, and Niigata University Medical and Dental Hospital. Patients were excluded if they were <18 years of age or had histories of percutaneous coronary intervention, coronary artery bypass graft surgery, or hematological disease. In addition, patients with LV assist device implantation or heart transplantation before genetic analysis were excluded. Whole-exome sequencing and downstream analyses were performed at the University of Tokyo Hospital as a core laboratory. The graphical method is shown in Supplemental Figure 1.

This study was approved by the ethics committee of the University of Tokyo Hospital (approval number G2249) and conformed to the principles of the Declaration of Helsinki. Written consent was obtained from all patients before inclusion.

LIBRARY PREPARATION AND NEXT-GENERATION SEQUENCING. DNA was purified from whole blood using a PAXgene Blood DNA Kit (Qiagen). The quality of purified DNA was confirmed using a Qubit Fluorometer (Invitrogen). For whole-exome sequencing, 50 ng of each sample was used to prepare libraries using a Twist Comprehensive Exome Kit (Twist Bioscience). For deep target sequencing focusing on CHIP driver genes, a Twist Custom Panel (Twist Bioscience) including 54 genes was applied (Supplemental Table 2). Enzymatic fragmentation, end repair, ligation of TWIST unique dual index primers, purification, and hybridization of the capture probe were performed following the protocol provided by Twist Bioscience. Libraries were sequenced using a NovaSeq 6000 (Illumina). Read quality was assessed using FastQC and low-quality reads were excluded using fastp.

VARIANT CALLING AND ANNOTATION. Using Burrows-Wheeler Aligner-MEM,[10] all raw sequence data were mapped to the human reference genome (GRCh38).

To detect pathogenic germline cardiomyopathy-related mutations from whole-exome sequencing data, according to the best practice of the Genome Analysis Toolkit, variants were extracted using Genome Analysis Toolkit HaplotypeCaller[11] with 20-fold minimum coverage and annotated using ANNOVAR.[12] Rare variants with minor allele frequencies of <1% in variant databases, including the East Asian population database in the 1000 Genomes Project[13] and the Tohoku Medical Megabank Organization database,[14] were extracted. Variants predicted to alter protein structure or function, such as nonsynonymous variants, nonsense variants, splice-site variants, in-frame and frameshift deletions, and insertions in cardiomyopathy-related genes were subsequently extracted (Supplemental Table 1). Pathogenic and likely pathogenic variants, classified according to the American College of Medical Genetics and Genomics guidelines,[15] were defined as pathogenic germline mutations. For the variants in *TTN*, we designate variants as pathogenic if they are truncating mutations, including frameshift, nonsense, and canonical splice site mutations, and if their minor allele frequencies are <0.0001.

Somatic mutations were detected using Genome Analysis Toolkit Mutect2, freebayes, and pisces from deep target sequencing data.[16-18] To minimize algorithm-specific overcalling, mutations detected by more than 2 of these 3 algorithms were considered candidate mutations for CHIP. Using Genome Analysis Toolkit Funcotator, the detected somatic mutations were annotated and filtered to exclude germline mutations. Only mutations annotated as "PASS" by Funcotator were used in the downstream analysis. The definition of CHIP in this study is based on the following 4 criteria: 1) inclusion in the prespecified list (Supplemental Table 2) on the basis of previous studies[6,8]; 2) read coverage of 100 or more; 3) variant allele frequency (VAF) of 2% or higher; and 4) for missense mutations, predicted as pathogenic by 3 or more of 4 in silico prediction models (Mutation Taster, SIFT, FATHMM, and Polyphen-2).[19-22] If variants had VAFs ≥45%, they were considered potential germline mutations and were excluded.

FOLLOW-UP AND OUTCOMES. Baseline patient information, medical history, current medication, and electrocardiographic and echocardiographic findings were collected at the time of blood sampling for DNA purification. The primary endpoint was LVRR, defined as an absolute increase of LVEF by ≥10% on 1-year follow-up echocardiography, as previously described.[23] Patients who had undergone LV assist device implantation or heart transplantation before the 1-year follow-up were classified as LVRR negative.

ANIMAL MODELS. All animal experiments were approved by the University of Tokyo ethics committee for animal experiments and strictly adhered to the guidelines for animal experiments of the University of Tokyo (approval number P17-058). All wild-type (WT) C57BL/6 mice were purchased from CLEA Japan. Cd45.1 mice were purchased from Sankyo Laboratory Service. *Asxl1* p.G643Wfs*12 mice were generated in a previous study.[24] To obtain an *Asxl1* mutation on a background of Cd45.1 expression, *Asxl1* p.G643Wfs*12 mice were crossed with Cd45.1 mice, and the F1 progeny (Cd45.1/Cd45.2; $Asxl1^{G643Wfs*12/WT}$) were then crossed with Cd45.1 mice. After obtaining Cd45.1; $Asxl1^{G643Wfs*12/WT}$ mice, they were maintained by being crossed with Cd45.1 mice. The DCM murine model *Ttn* truncating variant (*Ttn* p.F28051Ifs*15) was generated for this study as shown in Supplemental Methods.

BONE MARROW TRANSPLANTATION AND ECHOCARDIOGRAPHY. Eight- to 10-week-old $Ttn^{F28051Ifs*15/WT}$ Cd45.2 recipients were lethally irradiated with a total dose of 9 Gy. After irradiation, unfractionated bone marrow cells (5×10^6) that were harvested from donor Cd45.1 mice or Cd45.1; $Asxl1^{G643Wfs*12/WT}$ mice and suspended in 0.2 mL phosphate-buffered saline were administered intravenously. Five weeks after bone marrow transplantation (BMT), mice underwent echocardiographic study. Transthoracic echocardiography was performed on conscious mice using a Vevo 2100 imaging system (VisualSonics). To minimize variation in the data, cardiac function was assessed only when the heart rate was 600 to 700 beats/min. M-mode echocardiographic images were obtained from a longitudinal view to measure the size and function of the left ventricle.

STATISTICAL ANALYSIS. On the basis of the results of the genetic analysis, the patients were divided into 2 groups: CHIP carriers and noncarriers. Patients with at least 1 CHIP driver mutation were included as CHIP carriers. All data are summarized as percentages for categorical variables and as mean ± SD or median (Q1-Q3) for continuous variables. Comparisons between groups were performed using chi-square or Fisher exact tests for categorical variables and Student's *t*-test or the Wilcoxon rank sum test for continuous variables. The normality of the data was examined using the Shapiro-Wilk test. Logistic regression analysis was applied to assess the association between genetic status and the prevalence of LVRR, which was the primary endpoint of this study. The results are presented as OR (95% CI) with *P* values. For multivariable logistic regression analysis, CHIP and factors previously reported as predictors of LVRR were adopted as explanatory variables.[23,25-27] Given the limited numbers of patients and events, which predisposed the conventional multivariable logistic regression model to the risk for overfitting, we additionally used an inverse probability weighted model as a sensitivity analysis to adjust for the same baseline confounders of CHIP as the conventional model of LVRR. Inverse probability weights for CHIP were derived from the baseline characteristics that follow on the basis of a logistic regression model predicting LVRR: age, male sex, previous HF, estimated glomerular filtration rate, germline variants in cardiomyopathy-related genes, left bundle branch block, and baseline LVEF. All analyses were conducted using R version 4.2.1 (R Foundation for Statistical Computing). A *P* value <0.05 was considered to indicate statistical significance. For echocardiography in mice, 1-way analysis of variance was performed to compare cardiac function among groups with a post hoc test for multiple pairwise comparisons. All statistical tests and graphical depictions of data are defined within the figure legends for the respective data panels.

RESULTS

BASELINE CHARACTERISTICS. A total of 198 patients met the inclusion criteria; their characteristics are shown in **Table 1**. The mean age was 47.6 ± 14.3 years, and 73% were men. The mean baseline LVEF and end-diastolic dimension were 24.6% ± 8.0% and 65.4 ± 9.5 mm, respectively. At baseline, 72% of the cohort were in NYHA functional class I or II. Ninety-two patients (46%) had pathogenic germline mutations, and *TTN* was the most frequently mutated cardiomyopathy-related gene, followed by *LMNA* and *TNNT2* (**Figure 1A**). All detected pathogenic variants in cardiomyopathy-related genes are listed in Supplemental Table 4. Almost all patients received optimal guideline-directed medical therapy with β-blockers, mineralocorticoid receptor antagonists, angiotensin-converting enzyme inhibitors, or angiotensin receptor neprilysin inhibitors.

TABLE 1 Baseline Characteristics of Patients According to Genetic Analysis

	Total (N = 198)	CHIP Carriers (n = 22)	Noncarriers (n = 176)	*P* Value
Age, y	47.6 ± 14.3	56.9 ± 15.8	46.5 ± 13.8	0.007
BMI, kg/m^2	23.8 ± 5.0	22.7 ± 3.2	23.9 ± 5.2	0.13
HF duration, months	18.0 (2-79)	39.0 (7-97)	14.5 (2-85)	0.56
Germline variants	92 (46)	7 (32)	85 (48)	0.22
Male	144 (73)	14 (64)	130 (74)	0.45
FH	65 (33)	4 (18)	61 (35)	0.19
Smoking	98 (49)	12 (55)	86 (49)	0.78
HTN	41 (21)	6 (27)	35 (20)	0.60
DM	39 (20)	6 (27)	33 (19)	0.51
DL	48 (24)	7 (32)	41 (23)	0.54
Previous cancer	5 (3)	0 (0)	5 (3)	0.94
Previous HF	108 (55)	15 (68)	93 (53)	0.26
Albumin, g/dL	4.0 ± 0.6	3.9 ± 0.6	4.0 ± 0.6	0.49
Hemoglobin, g/dL	13.9 ± 2	13.6 ± 2.0	14.0 ± 2.0	0.37
eGFR, mL/min/1.73 m^2	63.0 ± 20.0	57.3 ± 20.4	63.8 ± 19.6	0.17
BNP, pg/mL	204.7 (36.4-517.2)	416.4 (150.4-634.8)	271.0 (88.5-689.4)	0.70
LVEF, %	24.6 ± 8.0	25.0 ± 8.3	24.6 ± 8.0	0.81
LVDd, mm	65.4 ± 9.5	64.4 ± 6.6	65.5 ± 9.8	0.48
LVDs, mm	58.5 ± 10.2	57.2 ± 7.6	58.6 ± 10.5	0.44
LAD, mm	43.4 ± 9.5	43.3 ± 8.1	43.4 ± 9.7	0.96
AF	47 (24)	4 (18)	43 (24)	0.70
QRS duration, ms	120.1 ± 30.4	129.6 ± 36	120.2 ± 30.5	0.25
LBBB	14 (7)	4 (18)	10 (6)	0.086
ICD	22 (11)	5 (23)	17 (10)	0.14
CRT	49 (25)	8 (36)	41 (23)	0.28
MVA	21 (11)	5 (23)	16 (9)	0.11
NYHA functional class				0.46
I	53 (27)	6 (27)	47 (27)	
II	90 (45)	8 (36)	82 (47)	
III	42 (21)	5 (23)	37 (21)	
IV	13 (7)	3 (14)	10 (6)	
Medication				
β-blockers	187 (94)	21 (95)	166 (94)	1.00
ACEIs/ARBs/ARNIs	183 (92)	21 (95)	162 (92)	0.89
MRAs	141 (71)	14 (64)	127 (72)	0.56
SGLT2 inhibitors	62 (31)	5 (23)	57 (32)	0.50
Diuretic agents	122 (62)	12 (55)	110 (63)	0.62
OAC agents	60 (30)	4 (18)	56 (32)	0.29
AADs	33 (17)	6 (27)	27 (15)	0.27

Values are mean ± SD, n (%), or median (Q1-Q3).

AAD = antiarrhythmic drug; ACEI = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin receptor blocker; ARNI = angiotensin receptor neprilysin inhibitor; BMI = body mass index; BNP = brain natriuretic peptide; CHIP = clonal hematopoiesis of indeterminate potential; CRT = cardiac resynchronization therapy; DL = dyslipidemia; DM = diabetes mellitus; eGFR = estimated glomerular filtration rate; FH = family history; HF = heart failure; HTN = hypertension; ICD = implantable cardioverter-defibrillator; LAD = left atrial diameter; LBBB = left bundle branch block; LVDd = left ventricular end-diastolic diameter; LVDs = left ventricular end-systolic diameter; LVEF = left ventricular ejection fraction; MRA = mineralocorticoid receptor antagonist; MVA = malignant ventricular arrhythmia; OAC = oral anticoagulant; SGLT2 = sodium glucose cotransporter 2.

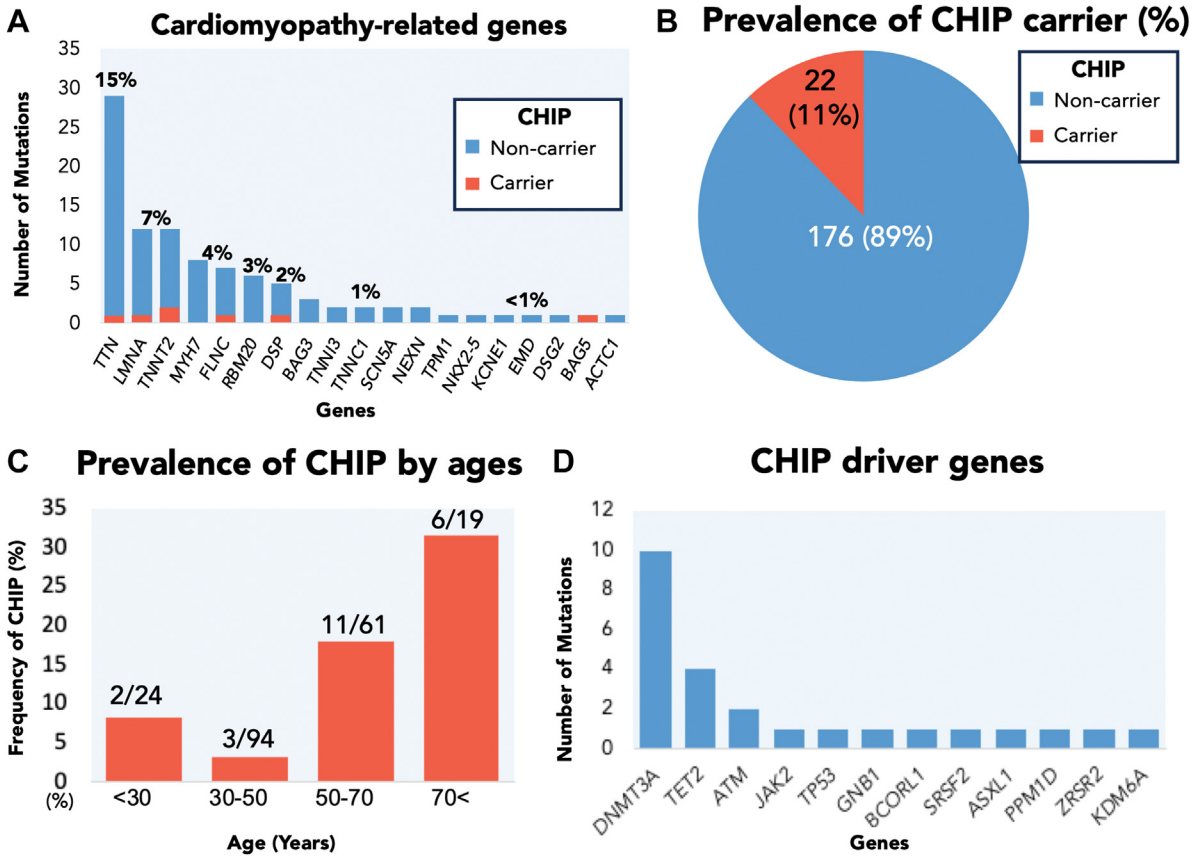
DETECTED SOMATIC MUTATIONS AND THEIR DISTRIBUTION. Twenty-five CHIP driver mutations were detected in 22 patients (11%) (Figure 1B). The mean read coverage of the target area for CHIP was 2,139. As shown in Figure 1C, the prevalence of CHIP increases with age. Mutations that exceeded 10% VAF, defined as a large clonal size in a previous study,[5] were found in 6 patients. *DNMT3A* was the most frequently mutated gene, followed by *TET2* and *ATM* (Figure 1D). A detailed list of all detected CHIP driver mutations and baseline characteristics stratified by CHIP and germline variants is shown in Supplemental Tables 5 and 6.

CHIP carriers were significantly older (mean age 56.9 vs 46.5 years; $P = 0.007$) compared with noncarriers. Both groups had similar LVEFs, durations of HF, medical histories, cardioprotective medications, and cardiac implantable electronic device histories (Table 1). There was no significant difference in smoking and history of cancer therapy, which are risk factors for CHIP, between the groups. Germline variants in cardiomyopathy-related genes were found in 7 of the 22 CHIP carriers, whereas 15 had CHIP mutations but no germline mutations. Of the 7 patients with both CHIP and germline mutation, 2 had mutations in *TNNT2*, and each remaining patient had a mutation in *LMNA*, *FLNC*, *DSP*, *TTN*, and *BAG5*, respectively.

LVRR. LVRR occurred in 88 (44%) of the entire cohort and was more frequent in noncarriers than in CHIP carriers (47.2% vs 22.7%; $P = 0.023$) (Figure 2A). Patients with pathogenic germline mutations had a lower occurrence rate of LVRR than those without germline mutations (33.7% vs 53.8%; $P = 0.005$) (Figure 2B). Univariable logistic regression analysis revealed that CHIP (OR: 0.33; 95% CI: 0.12-0.93; $P = 0.036$) and germline mutations (OR: 0.44; 95% CI: 0.25-0.78; $P = 0.005$) were negative predictors of LVRR. CHIP carriers exhibited a smaller change in LVEF (Figures 2C and 2D). Patients with loss-of-function variants had lowest occurrence rate of LVRR. (Supplemental Figure 2). After adjustment for explanatory variables (age, sex, baseline LVEF, and known prognostic factors of LVRR [estimated glomerular filtration rate, left bundle branch block, and previous HF]), both CHIP (OR: 0.27; 95% CI: 0.09-0.83; $P = 0.022$) and germline mutations (OR: 0.30; 95% CI: 0.15-0.58; $P < 0.001$) remained significant in the multivariable model (Table 2). Inverse probability weighted analysis identified CHIP as an independent predictor of LVRR (OR: 0.32; 95% CI 0.21-0.49; $P < 0.001$), which was consistent with the results of conventional multivariable logistic regression analysis (Supplemental Table 7).

CHIP EXACERBATES THE CARDIAC FUNCTION OF MILD DCM MICE WITH *TTN* TRUNCATING VARIANT. Given the multiple variables that can influence the prognosis of patients with DCM, we assessed whether CHIP exacerbates the disease phenotype of DCM in a

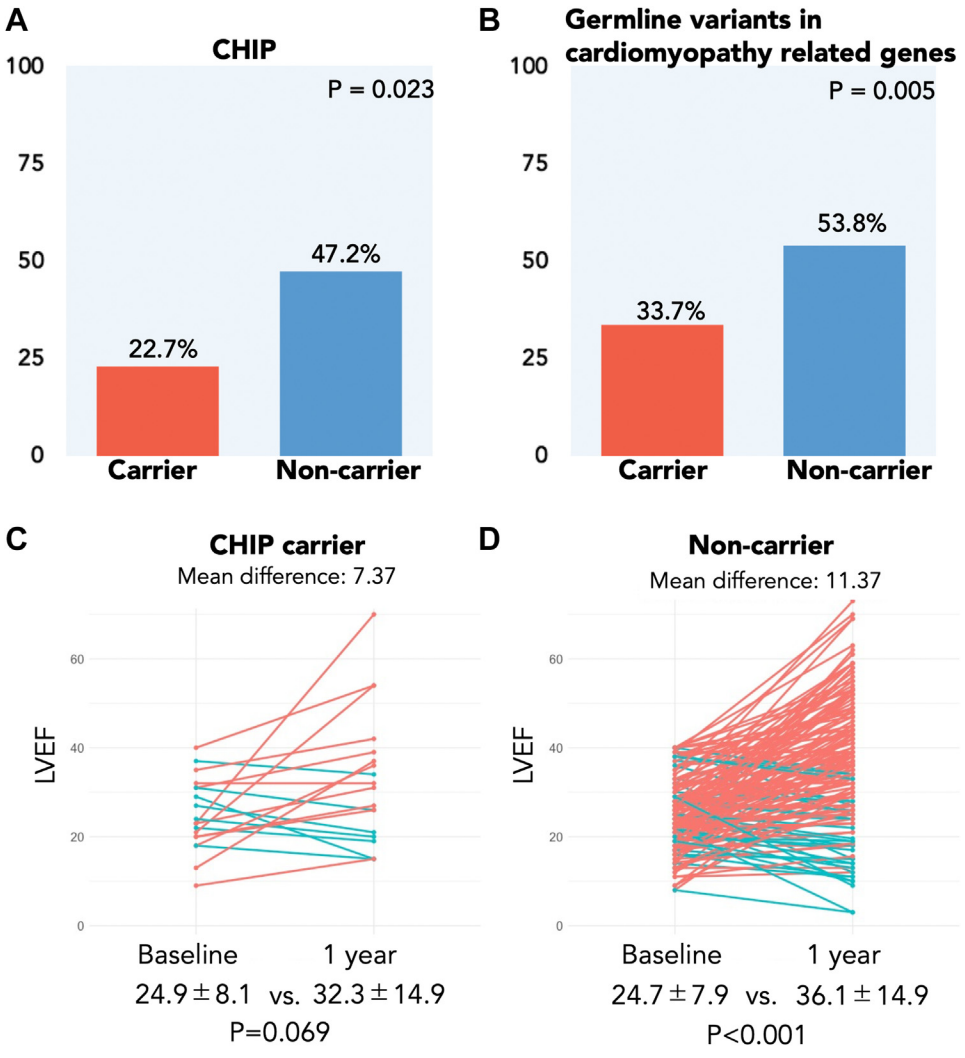
FIGURE 1 Summary of Genetic Analysis for Germline and Somatic Mutations



(A) *TTN* was the most commonly mutated cardiomyopathy-related gene. (B) Twenty-five clonal hematopoiesis of indeterminate potential (CHIP) driver mutations were identified in 22 patients (11%). (C) The prevalence of CHIP increases with age. (D) *DNMT3A* was the most frequent mutated CHIP driver gene.

murine experimental model (**Figure 3A**). We generated mice with heterozygous mutation of *Ttn* p.F28051Ifs*15 (Ttn^{Mt}) on the basis of the genotype of human familial patients with DCM at our facility, and Ttn^{Mt} mice showed mild cardiac dysfunction. We transplanted bone marrow cells from either *Asxl1* p.G643Wfs*12/WT ($Asxl1^{Mt}$) or littermate WT mice. Similar to previous studies of CHIP,[28] transplanted CD45.1 + $Asxl1^{Mt}$ cells showed increased chimerism in Ly6C-high monocytes, neutrophils, and T cells compared with WT controls (**Figure 3B**, Supplemental Figure 3A). For peripheral blood, there were no detectable changes in myeloid populations (**Figure 3C**). Consistent with the clinical paradigm of clonal hematopoiesis, BMT with $Asxl1^{Mt}$ donor cells did not lead to alterations in levels of peripheral blood counts (Supplemental Figure 3B). Ttn^{Mt} mice having undergone BMT from $Asxl1^{Mt}$ donor (Ttn^{Mt}-$Asxl1^{Mt}$) showed significant enlargement of LV diameter and lower LV systolic function compared with Ttn^{Mt} mice having undergone BMT from WT donor (Ttn^{Mt}-WT) (**Figure 3D**, Supplemental Figures 3C and 3D). Histologic analysis showed increased interstitial fibrosis in the hearts of Ttn^{Mt}-$Asxl1^{Mt}$ mice compared with other groups (**Figures 3E and 3F**). Flow cytometry analysis of cardiac immune cells from Ttn^{Mt}-WT and Ttn^{Mt}-$Asxl1^{Mt}$ at 8 weeks after BMT showed increased chimerism of donor-derived ($CD45.1^{+}$) macrophages in Ttn^{Mt}-$Asxl1^{Mt}$, especially in the subset of proinflammatory $Ccr2^{+}$, $MHCII^{+}$ macrophages (**Figure 3G**, Supplemental Figure 3E). Consistent with the flow cytometry analysis, histologic analysis also showed increased macrophage infiltration in the hearts of Ttn^{Mt}-$Asxl1^{Mt}$ mice compared with other groups (Supplemental Figures 3F to 3H). Reverse transcriptase quantitative

FIGURE 2 Prevalence of LVRR



(A) Left ventricular reverse remodeling (LVRR) was present in 88 (44%) of the entire cohort and was more frequent in noncarriers than in clonal hematopoiesis of indeterminate potential (CHIP) carriers (47.2% vs 22.7%; $P = 0.023$). (B) Patients with pathogenic germline mutation had a lower occurrence rate of LVRR than patients without germline mutations (33.7% vs 53.8%; $P = 0.005$). (C,D) Absolute changes in left ventricular ejection fraction (LVEF) for 175 patients, excluding the 23 individuals (including 3 CHIP carriers) who underwent left ventricular assist device implantation within 1 year. Patients with improvement of LVEF are depicted in red, and others are shown in blue. Although no differences in left ventricular systolic function were observed for 1 year in the CHIP carrier group (24.9% ± 8.1% vs 32.3% ± 14.9%; $P = 0.069$), the CHIP noncarrier group exhibited a significant improvement in LVEF (24.7% ± 7.9% vs 36.1% ± 14.9%; $P < 0.001$). The mean absolute change in LVEF between the 2 groups, assessed using the Wilcoxon signed rank test, was comparable (7.37% vs 11.37%; $P = 0.27$).

polymerase chain reaction analysis of sorted donor-derived (CD45.1^{+}) macrophages revealed a significant increase in the mRNA expression of various proinflammatory cytokines and chemokines in *Ttn*^{Mt}-*Asxl1*^{Mt} (Figure 3H). Finally, quantitative polymerase chain reaction of bone marrow-derived macrophages generated from *Asxl1*^{Mt} mice also showed increased expression of various proinflammatory cytokines after the stimulation by lipopolysaccharide (Figure 3I).

DISCUSSION

We simultaneously examined germline and somatic variants in DCM and found that among 198 patients with DCM, 92 had pathogenic germline mutations in cardiomyopathy-related genes, and 22 were CHIP carriers. Fifteen of these 22 patients had CHIP without germline variants, suggesting that CHIP is profoundly relevant to DCM. We also found that pathogenic

germline variants in cardiomyopathy-related genes and CHIP were independent negative predictors of LVRR in DCM. CHIP exacerbated cardiac systolic dysfunction and fibrosis in the DCM murine model.

Previous large-scale studies of CHIP screening in individuals without hematological disorders showed that the prevalence of CHIP increases with age. CHIP was detected in >10% of patients older than 65 years but was detected in only 1% of patients younger than 50 years.[6,29] As patients younger than 60 years were excluded from the previous study of CHIP in HFrEF,[8] the burden of CHIP in young adults and adolescents has not been well understood. In the present study (mean age 47.6 years), CHIP was much more frequently observed in patients with DCM—about 10% even in patients younger than 70 years—and the prevalence of CHIP was higher in each age category than that reported in the previous studies.[5,6] This indicates that there might be an undetermined relationship between CHIP and DCM. The expansion of CHIP involves changes in hematopoietic stem cells and their surrounding environment; the degeneration of hematopoietic stem cells is due primarily to aging but is influenced by the surrounding environments such as bone marrow and surrounding microvessels, which are changed by comorbidities including atherosclerotic CVD.[30] Myocardial infarction has been reported to dysregulate the bone marrow vascular niche, suggesting that the disease causing HF itself may provide an environment conducive to the development of CHIP.[31] It remains to be clarified whether DCM itself accelerates the acquisition of CHIP.

Inherited DCM typically affects young patients and is the major causative disease of severe HFrEF and sudden cardiac death.[32] Although formulating therapeutic strategies on the basis of the prediction of major adverse cardiac events at the time of diagnosis is essential in DCM, it remains challenging to provide an accurate prediction. LVRR was reported to occur in about one-third of patients with DCM, and patients with LVRR had a significantly favorable long-term prognosis.[27] Reported predictors of LVRR include sex,[33] duration of HFrEF,[26] left bundle branch block,[34] and late gadolinium enhancement on cardiac magnetic resonance (CMR) imaging.[35] Moreover, genetic analysis has revealed a strong association between genotype and LVRR.[23] Although the genetic profile is critical to stratify the long-term risk, some individuals without pathogenic germline mutations have severe clinical courses, suggesting that there are unidentified factors for the pathogenesis and progression of DCM. Recently, Sikking et al[36] reported that clonal hematopoiesis, even with a VAF lower than 2%, worsens the prognosis in DCM with large sample size, but they did not include germline mutations as explanatory variables in their multivariable analysis. In this study, we conducted an analysis of germline variants in all cases and demonstrated their independent effect on LV systolic dysfunction. In addition, animal models were used to demonstrate the additive effects of germline mutations associated with CHIP and DCM, confirming the conceptual findings.

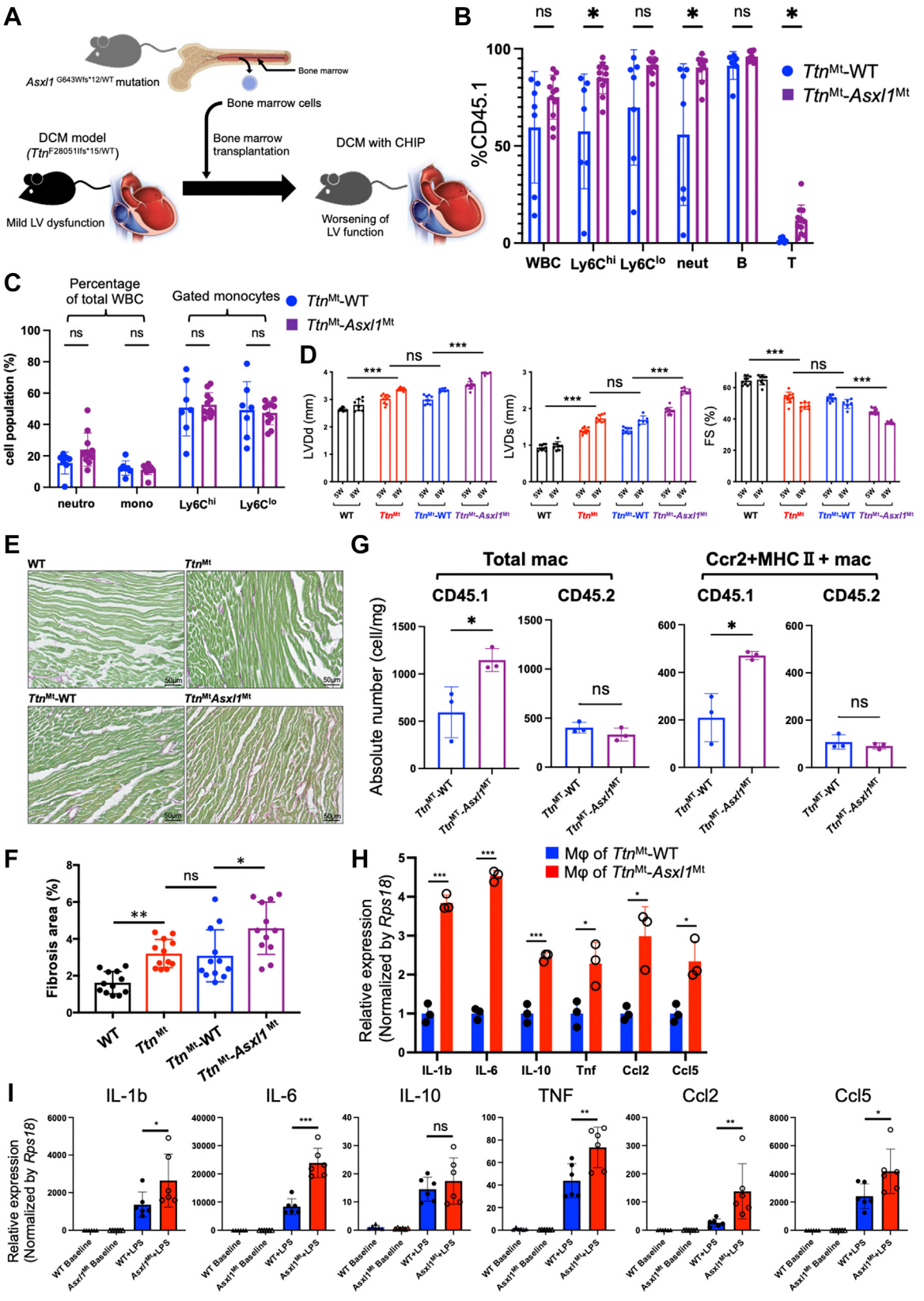
TABLE 2 Logistic Regression Analysis for Left Ventricular Reverse Remodeling

	Univariable Analysis		Multivariable Analysis	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
CHIP	0.33 (0.12-0.93)	0.036	0.27 (0.09-0.83)	0.022
Germline variants	0.44 (0.25-0.78)	0.005	0.30 (0.15-0.58)	<0.001
Male	0.60 (0.32-1.12)	0.11	0.44 (0.21-0.90)	0.025
Age	1.00 (0.98-1.02)	0.973	1.02 (0.99-1.04)	0.26
FH	0.63 (0.34-1.16)	0.138		
Smoking	0.63 (0.36-1.11)	0.113		
HTN	1.81 (0.90-3.62)	0.094		
DM	1.09 (0.54-2.20)	0.811		
DL	1.20 (0.63-2.31)	0.578		
Previous HF	0.37 (0.20-0.65)	0.001	0.29 (0.15-0.57)	<0.001
Previous cancer	0.83 (0.14-5.08)	0.84		
LBBB	0.93 (0.31-2.80)	0.901	0.74 (0.20-2.66)	0.65
AF	0.50 (0.25-1.00)	0.05		
eGFR	1.01 (1.00-1.03)	0.084	1.02 (1.00-1.04)	0.079
Baseline LVEF	0.99 (0.96-1.02)	0.56	0.98 (0.94-1.02)	0.24
ICD	0.33 (0.12-0.93)	0.036		
CRT	0.20 (0.09-0.44)	<0.001		
MVA	0.05 (0.01-0.39)	0.004		
Diuretic agents	0.64 (0.36-1.13)	0.126		
OAC agents	0.77 (0.42-1.43)	0.407		
AADs	0.28 (0.11-0.68)	0.005		

Abbreviations as in Table 1.

DNMT3A, which encodes DNA methyltransferase 3A, an enzyme that modulates gene transcription by catalyzing DNA methylation,[37] and *TET2*, which encodes ten-eleven translocation methylcytosine dioxygenase 2, a regulator of interleukin-1β expression through histone modulation,[28] were the 2 most frequent CHIP driver genes identified in this study. This is consistent with previous studies of CHIP in CVD.[8] In a mouse model, inactivation of *DNMT3A* and *TET2* promotes inflammation via the dysregulation of specific cytokines and chemokines, resulting in cardiorenal fibrosis.[38] Furthermore, *PPM1D* and *TP53* are associated with the DNA damage repair pathway and are driver genes of clonal hematopoiesis, especially in the cancer treatment setting.[39,40] In a mouse model of nonischemic HF, *Ppm1d* overexpression and *Trp53* deficiency promoted neutrophil-mediated inflammation, and the accumulation of DNA damage in

FIGURE 3 CHIP Exacerbates Cardiac Dysfunction of Mild DCM Mice With *Ttn* Truncating Variant



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immune cells resulted in myocardial fibrosis.[41,42] Interestingly, the development of CHIP through 2 different pathways—epigenetic modulation and DNA damage repair—leads to the common phenomenon of cardiac inflammation. Many basic and clinical studies have suggested that chronic inflammation plays a critical role in the development of HFrEF.[43] Myocardial fibrosis detected as late gadolinium enhancement on CMR imaging was a negative predictor of LVRR and clinical outcome in DCM,[35] suggesting that cardiac fibrosis is negatively associated with cardiac reversibility. It has been reported that the presence of late gadolinium enhancement in CMR imaging and genotype could predispose patients to end-stage HFrEF and fatal arrhythmias.[44] In addition to these predispositions, CHIP could induce extra inflammation followed by fibrosis and deteriorate the reversibility of cardiac contractility. In this study, as CMR images were available in a limited number of patients, it was challenging to determine a causal relationship between CHIP and late gadolinium enhancement in CMR. Late gadolinium enhancement was observed in 4 of 5 patients derived from CHIP carriers, and 3 of them did not have germline variants in cardiomyopathy-related genes (Supplemental Figure 4). The quantity and distribution of late gadolinium enhancement were inconsistent among these patients, which was attributed to the small sample size. Further well-designed studies are necessary to address this.

As noted earlier, CHIP increases the incidence of HF[7] and is also involved in the exacerbation of HFrEF,[8] as seen in the present results. Furthermore, it has been recently reported that many individuals who harbor pathogenic germline mutations show no cardiac phenotypes,[45] suggesting that the other second-hit factors are necessary for the induction of HFrEF. For example, a recent study showed that anthracyclines induces protracted LV dysfunction in mice with the *Ttn* truncating variant, which do not demonstrate any significant phenotype of HF.[46] Recently, Min et al[47] reported that *Asxl1*-mediated clonal hematopoiesis exacerbated LV dysfunction in a murine myocardial infarction model and an angiotensin II-induced pressure overload model through elevated cytokine and chemokine production. *ASXL1* somatic variants have been reported not only to increase the risk for developing HF in cohorts of healthy participants but also to be more associated with reduced LVEF than variants in other genes.[7] Therefore, we examined the effects of *Asxl1*-mediated clonal hematopoiesis on LV function using a mild DCM murine model harboring *Ttn* mutation in the present study. Ttn^{Mt}-$Asxl1^{Mt}$ mice showed significantly lower LV systolic function compared with Ttn^{Mt}-WT mice, suggesting that CHIP plays a significant role in the onset and progression of HFrEF as a second-hit factor by up-regulating inflammation and fibrosis. We validated the increased expression of proinflammatory cytokines in *Asxl1*-mutated macrophages in both in vitro and in vivo experiments. $Ccr2^{+}MHCII^{+}$ cardiac-resident macrophages are derived from monocytes and are known to be a proinflammatory subtype that would worsen HF.[48,49] Cardiac immune cells from Ttn^{Mt}-$Asxl1^{Mt}$ mice had increased chimerism only in the donor $CD45.1^{+}$ macrophages, especially in the $Ccr2^{+}MHCII^{+}$ subset, which suggests that *Asxl1*-mutated monocytes are more likely to be recruited to the heart and differentiate into proinflammatory subtype macrophages. Although further studies are needed to clarify the causal link between CHIP and DCM, analysis of germline mutations and CHIP provides a more precise stratification of progressive risk in DCM.

FIGURE 3 Continued

(A) Experimental design for testing the effect of CHIP on the dilated cardiomyopathy (DCM) murine model. (B) The chimerism of transplanted CD45.1 cells was analyzed using flow cytometry at 5 weeks after bone marrow transplantation (BMT) (n = 7 for $Ttn^{F28051Ifs*15/WT}$ with BMT of wild-type [WT] donor [Ttn^{Mt}-WT]; n = 11 for $Ttn^{F28051Ifs*15/WT}$ with BMT of $Asxl1^{G643Wfs*12/WT}$ donor [Ttn^{Mt}-$Asxl1^{Mt}$]) Data are shown as mean and SD. (C) Flow cytometry analysis of peripheral blood from Ttn^{Mt}-WT (n = 7) and Ttn^{Mt}-$Asxl1^{Mt}$ (n = 9) shows that there were no detectable changes in myeloid populations. (D) Echocardiographic assessment of the heart in each group at 5 and 8 weeks after BMT (n = 10 and n = 8 for WT; n = 10 and n = 8 for $Ttn^{F28051Ifs*15/WT}$ [Ttn^{Mt}]; n = 9 and n = 6 for Ttn^{Mt}-WT; n = 12 and n = 8 for Ttn^{Mt}-$Asxl1^{Mt}$). Data are shown as mean and SD. (E,F) Histochemical detection of collagen fibers by Sirius red/fast green dye staining in each group at 5 weeks after BMT. Results of quantitative analysis of fibrosis area are also shown (n = 12 for each group). Data are shown as mean and SD. (G) Flow cytometry analysis of cardiac immune cells from Ttn^{Mt}-WT (n = 3) and Ttn^{Mt}-$Asxl1^{Mt}$ (n = 3) showed increased chimerism of donor-derived ($CD45.1^{+}$) macrophages in Ttn^{Mt}-$Asxl1^{Mt}$, especially in the subset of proinflammatory $Ccr2^{+}$, $MHCII^{+}$ macrophages. (H) Messenger RNA (mRNA) expression levels of various proinflammatory cytokines and chemokines in the sorted donor-derived ($CD45.1^{+}$) cardiac macrophages in Ttn^{Mt}-WT and Ttn^{Mt}-$Asxl1^{Mt}$ at 8 weeks after BMT. (I) mRNA expression levels of various proinflammatory cytokines and chemokines in the bone marrow–derived macrophages generated from WT or $Asxl1^{Mt}$ mice after stimulation by lipopolysaccharide. Statistical significance was evaluated using multiple unpaired Student's *t*-tests with Holm-Šidák post hoc test (B,C,G), 2-way analysis of variance (ANOVA) with the Bonferroni post hoc test (D), 1-way ANOVA with the Tukey post hoc test (E,F), or unpaired Student's *t*-tests (H,I). $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. Source data are provided as a Source Data file. B = B cells; $Ly6C^{hi}$ = Ly6C-high monocytes; $Ly6C^{lo}$ = Ly6C low monocytes; neut = neutrophils; T = T cells.

STUDY LIMITATIONS. First, this was an observational and retrospective study with a small sample size. Furthermore, there was no validation cohort, so caution must be exercised in interpretation. Second, all study participants were East Asian, and there may be some ethnicity-specific characteristics. Third, whether other clonal hematopoietic-related genes, such as Dnmt3a and Tet2, also exacerbate HF in DCM needs to be determined in future studies.

CONCLUSIONS

Pathogenic germline mutations and CHIP are independent negative predictors of LVRR in patients with DCM. Therefore, the assessment of both germline and somatic mutations in DCM is useful to predict clinical prognosis.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: CHIP is an adverse prognostic factor of DCM independent of traditional risk factors, including germline variants in cardiomyopathy-related genes.

TRANSLATIONAL OUTLOOK: Considering that the progression of DCM is associated with CHIP, further basic research will lead to a more precise stratification and delineate causal relationship between them.

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