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Myocardial DNA Damage Predicts Heart Failure Outcome in Various Underlying Diseases

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ABSTRACT

BACKGROUND Reliable predictors of treatment efficacy in heart failure have been long awaited. DNA damage has been implicated as a cause of heart failure.

OBJECTIVES The purpose of this study was to investigate the association of DNA damage in myocardial tissue with treatment response and prognosis of heart failure.

METHODS The authors performed immunostaining of DNA damage markers poly(ADP-ribose) (PAR) and γ-H2A.X in endomyocardial biopsy specimens from 175 patients with heart failure with reduced ejection fraction (HFrEF) of various underlying etiologies. They calculated the percentage of nuclei positive for each DNA damage marker (%PAR and %γ-H2A.X). The primary outcome was left ventricular reverse remodeling (LVRR) at 1 year, and the secondary outcome was a composite of cardiovascular death, heart transplantation, and ventricular assist device implantation.

RESULTS Patients who did not achieve LVRR after the optimization of medical therapies presented with significantly higher %PAR and %γ-H2A.X. The ROC analysis demonstrated good performance of both %PAR and %γ-H2A.X for predicting LVRR (AUCs: 0.867 and 0.855, respectively). There was a negative correlation between the mean proportion of DNA damage marker–positive nuclei and the probability of LVRR across different underlying diseases. In addition, patients with higher %PAR or %γ-H2A.X had more long-term clinical events (PAR HR: 1.63 [95% CI: 1.31-2.01]; $P < 0.001$; γ-H2A.X HR: 1.48 [95% CI: 1.27-1.72]; $P < 0.001$).

CONCLUSIONS DNA damage determines the consequences of human heart failure. Assessment of DNA damage is useful to predict treatment efficacy and prognosis of heart failure patients with various underlying etiologies.
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Heart failure is one of the noncommunicable diseases with a growing burden worldwide, and the number of its patients is expected to increase further in the coming decades.[1-3] Despite significant progress in treatment strategies for heart failure, its prognosis is poor, with a 5-year survival rate of <50%.[4,5]

What complicates the entity of heart failure is the great variety of the underlying etiologies, leading to the heterogeneity of treatment response. There are many kinds of diseases that can cause the development of heart failure, including ischemic cardiomyopathy, valvular disease, hypertensive heart disease, atrial fibrillation, cardiac amyloidosis, myocarditis, and dilated cardiomyopathy.[6,7] The heterogeneity of treatment effects also makes clinical decision making more difficult. Researchers, including our group, have argued that early prediction of treatment efficacy can guide clinical decision making[8-11] and that early referral for a ventricular assist device or heart transplantation might be beneficial for patients expected to have a low response rate, especially in the context of increasingly long wait times for heart transplantation.[12-14] Therefore, considerable efforts have been made to identify the predictors of left ventricular reverse remodeling (LVRR), which reflects the treatment response and is closely correlated with prognosis.[8,15-17] Although many LVRR predictors, such as hemodynamic parameters, chamber geometry, late gadolinium enhancement of magnetic resonance imaging, and genetic variants,[9,18-21] have been proposed, most of these were examined in relation to specific underlying diseases without being validated for various other etiologies. Because no common predictor exists for the various etiologies of heart failure, it remains difficult to accurately predict its prognosis and to determine treatment options in advance.

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Many human and other animal studies have suggested that DNA damage plays a critical role in the development of pressure overload–induced heart failure, dilated cardiomyopathy, or aging-related cardiac phenotypes.[22-27] We recently reported, with the use of endomyocardial biopsy specimens of a small number of patients with dilated cardiomyopathy, that patients with a large extent of DNA damage in cardiomyocytes do not respond to drug treatment and that the immunostaining of DNA damage markers might be useful to predict LVRR.[8] These results suggest that DNA damage signatures harbor a signal of irreversibility and could be a promising prognostic indicator of heart failure, at least for dilated cardiomyopathy.[28]

In the present study, we performed immunofluorescence staining of DNA damage markers in endomyocardial biopsy specimens from 175 patients with heart failure with reduced ejection fraction (HFrEF) of various underlying etiologies. We used 2 DNA damage markers: poly(ADP-ribose) (PAR) and γ-H2A.X.[8] PAR is synthesized by PAR polymerase in response to the detection of various types of DNA damage, mediating downstream DNA damage responses, including DNA repair and programmed cell death.[29] γ-H2A.X is formed in response to DNA double-strand breaks via phosphorylation of H2A.X at serine 139.[30] To test whether DNA damage is a common signature of advanced heart failure and can be a prognostic predictor for heart failure of various causes, we conducted a comprehensive analysis of the association between DNA damage and treatment response and long-term outcome across a variety of etiologies.

ABBREVIATIONS AND ACRONYMS

BMI = body mass index

HFrEF = heart failure with reduced ejection fraction

LVEF = left ventricular ejection fraction

LVRR = left ventricular reverse remodeling

PAR = poly(ADP-ribose)

METHODS

STUDY POPULATION. We retrospectively recruited consecutive patients with heart failure who underwent endomyocardial biopsy from 2007 to 2021 at either of the 2 participating tertiary hospitals (University of Tokyo Hospital and Nara Medical University Hospital) and had a left ventricular ejection fraction (LVEF) <40% documented with transthoracic echocardiography at the time of biopsy. Diagnosis of heart failure was made according to previous and current guidelines.[4,31] We excluded patients who were included in a previous pilot study[8] and those without formalin-fixed paraffin-embedded biopsy samples. We also excluded patients who had received mechanical support, such as intra-aortic balloon pumping and veno-arterial extracorporeal membrane oxygenation, within 30 days preceding biopsy, who had been treated with a ventricular assist device or heart transplantation before biopsy, and who were lost to follow-up within 12 months. The diagnosis of underlying heart diseases was verified by 3 independent researchers based on previous or current guidelines and published expert consensuses.[4,32-43] In particular, hypertensive heart disease was diagnosed in patients with longstanding poorly controlled hypertension in whom previous or present increased left ventricular wall thickness was confirmed and a family history of cardiomyopathy and other causes of reduced cardiac function, including coronary

artery disease, were excluded.[33,36] Drug-induced cardiotoxicity was diagnosed in patients with previously normal cardiac function that subsequently decreased after exposure to a known cardiotoxic drug, where the absence of coronary artery disease and any other underlying cause, as well as a lack of family history of cardiomyopathy, were confirmed.[37,40] Myocarditis refers to histologically proven myocarditis, including lymphocytic, eosinophilic, and giant cell myocarditis.[34,43] Familial or idiopathic dilated cardiomyopathy was diagnosed in patients who presented with enlarged left ventricles and reduced LVEF without evidence of coronary artery disease and other known causes, with or without a family history of dilated cardiomyopathy and/or known dilated cardiomyopathy-related genetic mutations.[35,36,42] Patients with uncertain underlying etiology were excluded. Given the observational nature of this study, the treatment after biopsy was left to the discretion of each treating physician, including up-titration of medications, treatment of the underlying causes, and mechanical assists.

This study was conducted in accordance with the Declaration of Helsinki, local regulations, and institutional ethical guidelines. The study was approved by the institutional research boards of the University of Tokyo (number 11801) and Nara Medical University (number G107), which waived individual written informed consent with an opt-out policy.

COLLECTION OF CLINICAL DATA. Demographic and clinical data were extracted from medical records. Collected demographic variables included age, sex, body mass index (BMI), underlying heart diseases, duration since the onset of heart failure, family history of heart failure, comorbidities, medications including angiotensin-converting enzyme inhibitors/angiotensin receptor blockers/angiotensin receptor-neprilysin inhibitors, β-blockers, and cardiac implantable electronic devices. Beta-blocker doses were converted and expressed as carvedilol-equivalent doses.[44] Hemodynamic status represented by blood pressure and heart rate on the day of biopsy was recorded. We also reviewed the laboratory data and the electrocardiographic and echocardiographic findings immediately preceding the biopsy. Echocardiographic findings 12 months after the biopsy were collected for determining LVRR.

MEASUREMENTS OF OUTCOMES. This study was designed to investigate the association between the baseline level of DNA damage accumulation in heart tissue and subsequent therapeutic prognosis in patients with HFrEF. To this end, the primary binary outcome was the achievement of LVRR at 1 year. LVRR was defined as an absolute increase in LVEF ≥10% to a final value of >35% as assessed with transthoracic echocardiography.[19,45] Patients who had the composite endpoints as defined below within 1 year were categorized as LVRR-negative. The secondary endpoint was a composite of cardiac death, implantation of ventricular assist device, and heart transplantation analyzed in a time-to-event manner.

IMMUNOHISTOCHEMICAL ANALYSIS. We performed immunofluorescence staining of PAR and γ-H2A.X on formalin-fixed paraffin-embedded biopsy specimens to evaluate the extent of DNA damage in heart tissues. We sliced 4-μm sections from paraffin blocks and placed them on glass slides, which were then deparaffinized and rehydrated. Antigen retrieval was performed by heating the fixed tissue sections in a citrate buffer solution at pH 6.0 (Dako S1699 antigen retrieval solution; Agilent) at 95 °C for 20 minutes with the use of an MI-33 microwave processor (Azumaya Co). Tissue sections mounted on the glass slides were then blocked in 5% normal goat serum (G9023; Sigma-Aldrich) for 60 minutes at room temperature and subsequently incubated with anti-PAR polymer antibody (ab14459, 1:100; Abcam) and anti-γ-H2A.X antibody (number 9718, 1:200; Cell Signaling Technology) overnight at 4 °C. After washing with phosphate-buffered saline solution, samples were stained with secondary antibodies (anti-mouse IgG-Alexa 594 and anti-rabbit IgG-Alexa 647, 1:300; Thermo Fisher Scientific) for 1 hour at room temperature. We counterstained cell membranes and nuclei with wheat germ agglutinin-Alexa 488 (1:200; Thermo Fisher Scientific) and 4,6-diamidino-2-phenylindole (DAPI) (1:1,000; Dojindo Molecular Technologies).

Methods for obtaining and analyzing the images are described in detail in Supplemental Methods and Supplemental Figure 1. For each patient, the proportions of PAR-positive nuclei (%PAR) and γ-H2A.X-positive nuclei (%γ-H2A.X) were calculated based on the analyzed images.

Immunostaining (Z.D.) and collection of clinical data (T.K., K.F., K.O.) were independently performed by different researchers. Acquisition and analysis of immunofluorescence images was conducted jointly by 3 researchers (Z.D., T.K., K.F.) while being blinded from any information of the corresponding patients.

STATISTICAL ANALYSIS. Categoric variables were expressed as n (%) and compared by means of chi-square test. Continuous variables were presented as median (IQR) and compared by means of the Mann-Whitney *U*-test.

We performed ROC analysis to evaluate the performance of %PAR and %γ-H2A.X derived from

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immunostaining of biopsy specimens to predict LVRR. AUC was calculated with the use of logistic regression. The optimal cutoffs were determined to maximize Youden's J-statistics (sensitivity + specificity − 1).[46] With the derived cutoffs of %PAR and %γ-H2A.X, we dichotomized the subjects into PAR-low (< cutoff of %PAR) and PAR-high (≥ cutoff of %PAR), and γ-H2A.X-low and γ-H2A.X-high groups. We applied Kaplan-Meier estimates for the composite endpoint to estimate the survival functions of the dichotomized groups, whose differences were tested with the use of log-rank tests. As a sensitivity analysis for the ROC analysis of %PAR and %γ-H2A.X predicting LVRR at 1 year, we performed a time-dependent ROC analysis as described in Supplemental Methods.

We fitted a logistic regression model for LVRR against baseline characteristics and DNA damage marker information to develop the crude OR. Similarly, a Cox proportional hazards model was fitted to estimate the crude HR. We then applied the inverse probability weighted (IPW) method to adjust for baseline confounders of either %PAR or %γ-H2A.X.[47] Inverse probability weights for either %PAR or %γ-H2A.X were derived from the baseline characteristics as follows based on bivariate analysis results (**Table 1**) and clinical relevance: age, BMI, family history of heart failure, duration since the onset of heart failure, NYHA functional class, systolic blood pressure, left ventricular end-diastolic diameter, the presence of severe mitral regurgitation, B-type natriuretic peptide, use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers/angiotensin receptor-neprilysin inhibitors and β-blockers, and cardiac resynchronization therapy. When calculating propensity scores, any missing value in the baseline characteristics (missing n < 5) was imputed by the group mean regarding the LVRR status. We also conducted conventional multivariate logistic regression and Cox proportional hazards analysis to confirm the robustness of the results.

We then viewed each cause of heart failure as a mass with a macro-perspective and explored the association between stained DNA damage markers and LVRR across a wide range of underlying diseases. We calculated the proportion of participants who achieved LVRR in each disease category and their mean %PAR and %γ-H2A.X. We performed weighted linear regressions of mean %PAR and %γ-H2A.X against the proportion of LVRR in each disease category, with the weight being the inverse variance of %PAR or %γ-H2A.X. Disease categories with fewer than 3 subjects

TABLE 1 Baseline Characteristics of Participants

	LVRR− (n = 78)	LVRR+ (n = 97)	*P* Value
Age, y	55.0 (45.0-69.8)	60.0 (47.0-68.0)	0.611
Male	56 (71.8)	70 (72.2)	1.000
BMI, kg/m^2	22.7 (20.6-25.7)	22.8 (20.1-25.6)	0.975
Family history of HF	14 (17.9)	6 (6.2)	0.028
Diagnosis			<0.001
Idiopathic/familial DCM	52 (66.7)	38 (39.2)	
Myocarditis	4 (5.1)	13 (13.4)	
Hypertensive heart disease	0 (0)	13 (13.4)	
Cardiac amyloidosis	5 (6.4)	5 (5.2)	
Tachycardia-induced cardiomyopathy	0 (0)	9 (9.3)	
Doxorubicin cardiotoxicity	5 (6.4)	3 (3.1)	
Cardiac sarcoidosis	3 (3.8)	5 (5.2)	
Dilated-phase HCM	4 (5.1)	1 (1.0)	
Ischemic cardiomyopathy	2 (2.6)	3 (3.1)	
TKI/mAb cardiotoxicity	0 (0)	3 (3.1)	
Valvular diseases	1 (1.3)	2 (2.1)	
Others[a]	2 (2.6)	2 (2.1)	
Comorbidities			
Atrial fibrillation	17 (21.8)	18 (18.6)	0.732
Diabetes	17 (21.8)	27 (27.8)	0.459
End-stage renal disease	3 (3.8)	3 (3.1)	1.000
Duration of HF, d	197 (71-1,089)	75 (26-221)	<0.001
NYHA functional class III or IV	31 (39.7)	49 (50.5)	0.204
Systolic BP, mm Hg	102 (92-124)	107 (95-130)	0.147
Diastolic BP, mm Hg	60 (55-72)	68 (58-78)	0.116
Heart rate, beat/min	78 (69-87)	74 (64-87)	0.186
Electrocardiography			
CLBBB	22 (28.2)	7 (7.2)	<0.001
QRS duration, ms	115 (99-133)	108 (98-122)	0.137
Transthoracic echocardiography			
LVEF, %	27.5 (20.0-35.0)	27.0 (23.0-34.0)	0.934
LVEDD, mm	63.0 (56.0-72.0)	59.0 (53.0-65.0)	0.004
Severe MR	20 (25.6)	10 (10.3)	0.013
Laboratory			
Hb, g/dL	13.6 (12.5-14.7)	13.9 (12.5-14.3)	0.271
eGFR, mL/min/1.73 m^2	62.8 (48.1-72.2)	61.4 (47.6-76.3)	0.974
BNP, pg/mL	324.5 (141.7-866.0)	397.7 (130.8-784.8)	0.679
Na, mEq/L	140.0 (137.0-141.0)	140.0 (138.0-142.0)	0.675
Treatment			
ACEI/ARB/ARNI	51 (65.4)	42 (43.3)	0.006
BB	49 (62.8)	38 (39.2)	0.003
BB dose, carvedilol-equivalent, mg	2.5 (0-10.0)	0 (0-2.5)	0.001
MRA	30 (38.5)	26 (26.8)	0.139
ICD	10 (12.8)	1 (1.0)	0.004
CRT	8 (10.3)	1 (1.0)	0.016

Values are median (IQR) or n (%). [a]1 case each of arrhythmogenic right ventricular cardiomyopathy, peripartum cardiomyopathy, Fabry disease, and takotsubo cardiomyopathy.

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; ARNI = angiotensin receptor-neprilysin inhibitor; BB = beta-blocker; BMI = body mass index; BNP = B-type natriuretic peptide; BP = blood pressure; CRT = cardiac resynchronization therapy; DCM = dilated cardiomyopathy; CLBBB = complete left bundle branch block; eGFR = estimated glomerular filtration rate; Hb = hemoglobin; HCM = hypertrophic cardiomyopathy; HF = heart failure; ICD = implantable cardioverter-defibrillator; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; LVRR = left ventricular reverse remodeling; mAb = monoclonal antibody; MR = mitral regurgitation; MRA = mineralocorticoid receptor antagonist; TKI = tyrosine kinase inhibitor.

were not included in the model. Given the skewed distribution of %PAR and %γ-H2A.X, to confirm the robustness of the results, we fitted another weighted linear regression model with the use of log-transformed (%PAR+1) and log-transformed (%γ-H2A.X+1) instead of %PAR and %γ-H2A.X.

The consistency of %PAR and %γ-H2A.X was confirmed by computing Pearson's correlation coefficient and by calculating the individual intraclass correlation coefficient for consistency with a two-way mixed-effects model.[48]

All data analyses and visualization were carried out with the use of R version 4.0.3 (R Foundation). For all tests, a probability value of $P < 0.05$ was considered to be statistically significant.

RESULTS

BASELINE CHARACTERISTICS OF THE PATIENTS. We initially screened 248 patients with HFrEF who underwent endomyocardial biopsy at the 2 participating facilities from 2007 to 2021. We excluded the following patients: 58 who were included in a previous pilot study,[8] 2 without preserved tissue specimens, 2 on mechanical support in the preceding 30 days or who had previous heart transplantation, 10 who were lost to follow-up within 1 year, and 1 with uncertain diagnosis of the underlying disease. We eventually included 175 patients in this study. The baseline characteristics of the included patients with or without LVRR at 1 year after biopsy and optimizing drug treatment are summarized in **Table 1**.

The patients had a mean age of 56.1 ± 16.1 years and were predominantly men (126 [72.0%]). They had a wide spectrum of underlying heart diseases: Approximately one-half were idiopathic/familial dilated cardiomyopathy, followed by myocarditis, hypertensive heart disease, cardiac amyloidosis, tachycardia-induced cardiomyopathy, cardiac sarcoidosis, doxorubicin-induced cardiomyopathy, and ischemic cardiomyopathy. Among the 17 patients diagnosed with myocarditis, 11 were lymphocytic, 5 were eosinophilic, and 1 was giant cell myocarditis. Patients who achieved LVRR tended to have a higher blood pressure at baseline. The LVEF was similar between patients with and without LVRR, whereas LVRR was associated with a smaller left ventricular end-diastolic diameter and less prevalence of severe mitral regurgitation at baseline.

IMMUNOSTAINING OF DNA DAMAGE MARKERS AND OUTCOMES. Of the 175 participants, 97 (55.4%) achieved LVRR, defined as a ≥10% increase in LVEF to a final value of LVEF >35%, as assessed with transthoracic echocardiography, ie, the primary outcome of this study (**Table 1**). The multidisciplinary treatment at the time of LVRR assessment was similar between both groups regarding drug therapy (Supplemental Table 1).

The representative images of PAR and γ-H2A.X staining of heart tissues from patients with and without LVRR at 1 year are shown in **Figure 1** and Supplemental Figure 2. The number of total nuclei counted in each sample was similar between the LVRR-negative and LVRR-positive groups: medians 976 (IQR: 556-1,732) and 1,127 (IQR: 812-1,634), respectively ($P = 0.140$) (**Figure 2A**). Patients who achieved LVRR had significantly less PAR-positive nuclei (%PAR) and γ-H2A.X-positive nuclei (%γ-H2A.X) than those who did not: %PAR: medians 2.2% (IQR: 1.0%-4.0%) vs 10.7% (IQR: 5.5%-18.5%), respectively ($P < 0.001$); %γ-H2A.X: medians 4.6% (IQR: 1.9%-9.3%) vs 22.1% (IQR: 11.1%-40.0%), respectively ($P < 0.001$) (**Figure 2B**).

The ROC analysis demonstrated good performance of both %PAR and %γ-H2A.X for predicting LVRR: AUCs: 0.867 and 0.855, respectively (**Figure 2C**). The performance was similar between %PAR and %γ-H2A.X (DeLong's test: $P = 0.652$). Optimal cutoffs were determined as 6.26% for %PAR (sensitivity 0.91, specificity 0.72) and 13.50% for %γ-H2A.X (sensitivity 0.89, specificity 0.71) by maximizing Youden's J-statistics. Time-dependent ROC analysis as a sensitivity analysis also demonstrated good performance for both markers (AUCs: 0.874 for %PAR and 0.858 for %γ-H2A.X) and developed cutoffs similar to the conventional ROC analysis (Supplemental Figure 3).

When treated as a continuous variable, logistic regression revealed ORs of 0.06 (95% CI: 0.02-0.15; $P < 0.001$) per 10% increase in %PAR and 0.39 (95% CI 0.28-0.54; $P < 0.001$) per 10% increase in %γ-H2A.X for developing LVRR (**Table 2**). Other predictors for LVRR included absence of family history of heart failure, smaller left ventricular end-diastolic diameter, an absence of severe mitral regurgitation, shorter duration from the onset of heart failure, and less extensive treatment when biopsy was performed (**Table 2**). We performed an IPW analysis, adjusting for the baseline confounding variables. Inverse probability weights were calculated for each of %PAR and %γ-H2A.X, which successfully restored the balance of covariates, as indicated by the crude and weighted Pearson's correlation coefficients between %PAR and covariates and those between %γ-H2A.X and covariates (Supplemental Figure 4).[49] IPW analysis identified %PAR and %γ-H2A.X as independent predictors for LVRR (**Table 2**), which was consistent with the results of conventional multivariate logistic regression analysis (Supplemental Table 2).

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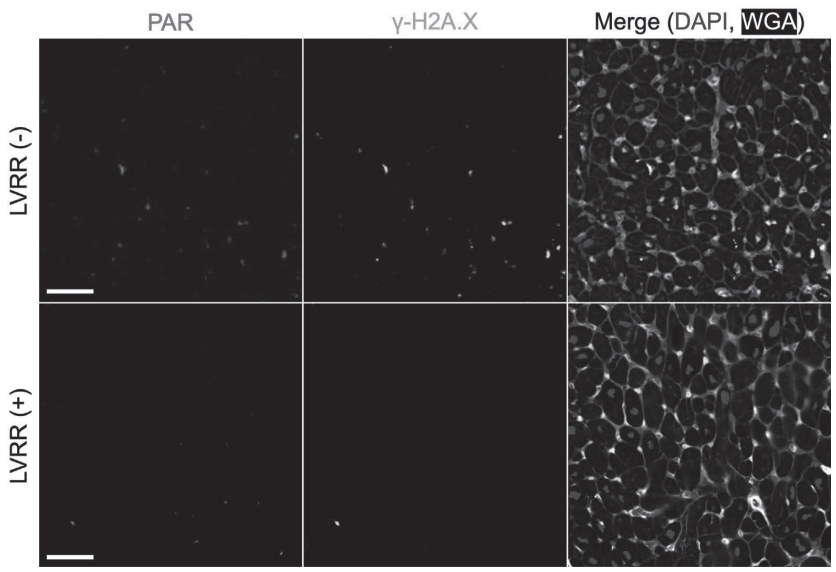
FIGURE 1 Immunohistochemical Analysis of PAR and γ-H2A.X in Endomyocardial Biopsy Specimens



Representative immunofluorescence staining images of one patient each from those who achieved LVRR at 1 year and those who did not. PAR (red) and γ-H2A.X (green) stained images are presented, and merged with DAPI (blue) indicating nuclei and WGA (white) indicating cell membrane. Scale bar = 50 μm. High-power field images are presented in Supplemental Figure 2. DAPI = 4,6-diamidino-2-phenylindole; LVRR = left ventricular reverse remodeling; PAR = poly (ADP-ribose); WGA = wheat germ agglutinin.

Patients were followed for a median of 1,213 days (IQR: 757-1,797 days). No patient was censored before the assessment of LVRR. The composite endpoint was reached in 28 participants (16.0%): 12 cardiac deaths and 16 implantations of ventricular assist device (within whom 2 received heart transplantation subsequently). Patients with lower %PAR or %γ-H2A.X, as dichotomized by the optimal cutoffs, had a better long-term outcome (log-rank $P < 0.001$ for each) (**Figures 3A and 3B**). We further divided the patients with higher %PAR into 2 subgroups by the median % PAR (13.34%) within this group and similarly divided the patients with higher %γ-H2A.X (median 31.78%), which successfully differentiated the patients at higher risk from those with intermediate risk (Supplemental Figure 5). The Cox proportional hazards model demonstrated HRs of 1.71 (95% CI: 1.36-2.16; $P < 0.001$) per 10% increase in %PAR and 1.42 (95% CI: 1.20-1.67; $P < 0.001$) per 10% increase in %γ-H2A.X, which remained significant in further IPW analysis (**Table 3**). Multivariate Cox proportional hazards analysis also identified both markers as independent predictors of the composite endpoint, along with lower BMI and higher NYHA functional class; B-type natriuretic peptide level was of marginal significance (Supplemental Table 3).

DNA DAMAGE MARKERS AND LVRR ACROSS DIFFERENT UNDERLYING ETIOLOGIES. To clarify the association between the stained DNA damage markers and LVRR across the wide spectrum of underlying etiologies, we calculated the proportion of participants who achieved LVRR at 1 year in each disease category, as well as their mean %PAR and %γ-H2A.X. Patients with hypertensive heart disease and tachycardia-induced cardiomyopathy showed the highest percentage of achieving LVRR and the lowest mean %PAR and %γ-H2A.X. Conversely, dilated-phase hypertrophic cardiomyopathy and doxorubicin-induced cardiotoxicity were the categories with the lowest LVRR achievement rate and the highest mean %PAR and %γ-H2A.X (**Table 4**). Interestingly, we found strong negative correlations between mean %PAR (Pearson's correlation

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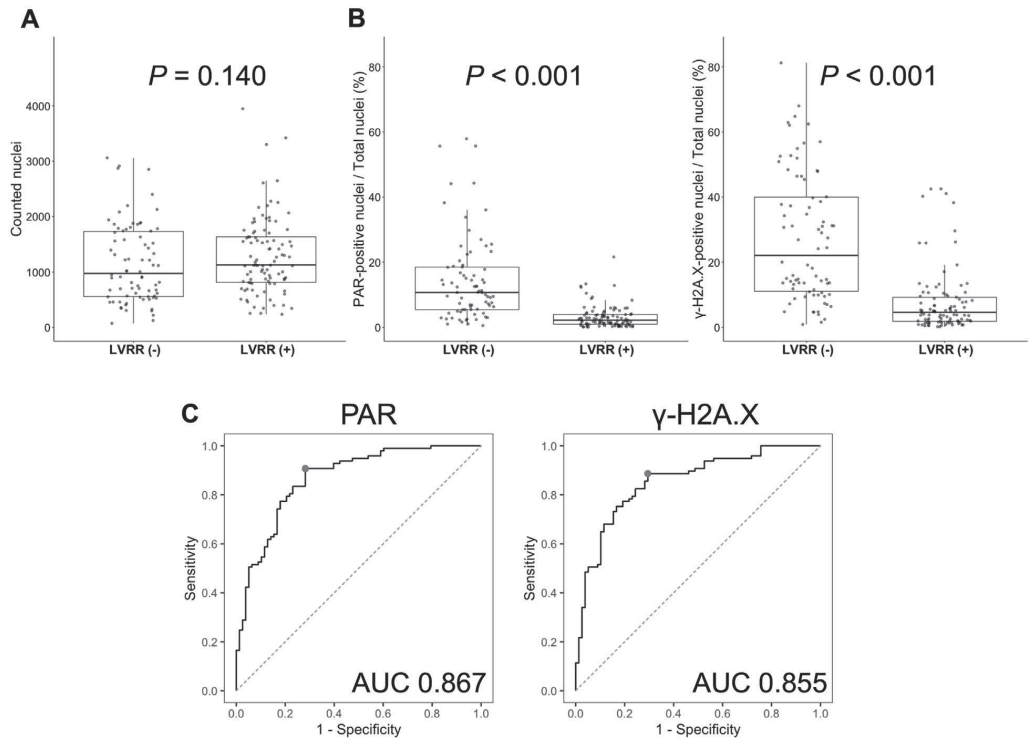
FIGURE 2 Comparisons of DNA Damage Markers Between Patients With and Without LVRR



(A) Comparisons of the total number of counted nuclei. (B) The proportions of nuclei stained with PAR and γ-H2A.X, respectively, between patients with LVRR at 1 year and those without. (C) Characteristic ROCs of the proportions of PAR-positive nuclei (%PAR) and γ-H2A.X–positive nuclei (%γ-H2A.X) to predict 1-year LVRR. The AUC for %PAR predicting 1-year LVRR was 0.867 (95% CI: 0.813-0.920). The AUC for %γ-H2A.X predicting 1-year LVRR was 0.855 (95% CI: 0.799-0.911). The red dots represent the optimal cutoffs derived to maximize Youden's J-statistics. Abbreviations as in Figure 1.

coefficient: −0.84; $P = 0.001$) and mean %γ-H2A.X (Pearson's correlation coefficient −0.85; $P = 0.001$) and the proportion of LVRR across various underlying diseases (**Figures 3C and 3D**). Analyses using log-transformed %PAR and %γ-H2A.X confirmed the robustness of these correlations (Supplemental Table 4, Supplemental Figure 6). In addition, we noticed a significant correlation between %PAR and %γ-H2A.X in each tissue specimen, as indicated by a Pearson's correlation coefficient of 0.70 ($P < 0.001$) (Supplemental Figure 7A). The intraclass correlation coefficient was 0.617 (95% CI: 0.517-0.701; $P < 0.001$). The correlation was consistent across different underlying diseases (Supplemental Figures 7B-7J).

DISCUSSION

This study demonstrates that quantification of DNA damage as represented by %PAR and %γ-H2A.X immunostaining of endomyocardial biopsy tissue has high performance in predicting both LVRR and long-term clinical events in patients with HFrEF due to various underlying diseases. Importantly, we were able to illustrate a continuous relationship between the amount of DNA damage and the probability of LVRR across different etiologies (**Central Illustration**).

Heart transplantation is considered the ultimate treatment for patients with advanced heart failure who do not respond well to other multidisciplinary

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therapies. Increasing numbers of patients are waiting for heart transplants, and longer wait times are reported in many countries.[12-14,50] Accurate prediction of the treatment response as represented by LVRR and long-term prognosis potentially help to identify the patients who would benefit more from an early referral for heart transplantation or a ventricular assist device. It has been previously argued that blood pressure, chamber geometry of the left ventricle, presence of severe mitral regurgitation, and late gadolinium enhancement in cardiac magnetic resonance were negatively associated with LVRR in patients with dilated cardiomyopathy.[19,51] However, few studies investigated the prognostic predictors that could be generally applied to patients with HFrEF with its wide spectrum of etiologies. The present study established a single prognostic indicator for HFrEF with a good performance across a wide spectrum of underlying causes.

We demonstrated that DNA damage marker positivity in myocardial tissue was predictive of both LVRR and the composite endpoint, independently from the duration of heart failure (**Tables 2 and 3**, Supplemental Tables 2 and 3). The associations between disease duration and outcomes were attenuated when adjusted for other factors including DNA damage markers (Supplemental Tables 2 and 3), suggesting that disease duration is associated with outcomes via its contribution to the accumulation of DNA damage. Assessment of DNA damage markers is of prognostic value even in cases where disease duration is unknown. Furthermore, the accumulation of large amounts of DNA damage, as represented by high positivity of PAR and γ-H2A.X staining, reflects prolonged or severe internal or external stress on the myocardium, where the occurrence of DNA damage exceeds its repair. Long-term mechanical unloading with the use of a ventricular assist device was demonstrated to be able to reduce DNA damage marker staining.[27] Although this reversibility raises concerns that a single-point assessment for prognosis prediction may not be perfectly accurate, it is important to note that this effect has not been reported in patients undergoing medical therapy alone. Collectively, given the direct involvement of DNA damage in the pathogenesis of heart failure, the evaluation of DNA damage demonstrated its potential to guide treatment and thus may serve as a molecular marker in precision medicine for heart failure.

Accumulating evidence shows that DNA damage plays a causative role in the development of heart failure. We recently reported that accumulation of single-strand breaks in cardiomyocytes causes pressure overload-induced heart failure in mice.[23] In both murine pressure overload-induced heart failure and human dilated cardiomyopathy, DNA damage-induced activation of p53 signaling was essential for the progression of left ventricular dysfunction.[25,52-54] Furthermore, a recent study that implemented a multiomics approach to human data revealed that DNA damage in cardiomyocytes also contributed to the development of heart failure with preserved ejection fraction.[55] This could be a possible explanation of the beneficial effect of sodium glucose cotransporter 2 inhibition on heart failure regardless of ejection fraction, given that sodium glucose cotransporter 2 inhibition has been shown to reduce DNA damage and to improve the phenotypes of cell senescence in a murine kidney disease model.[56] In the present study, DNA damage markers in the endomyocardial biopsy specimens were closely correlated with LVRR and long-term clinical events. Collectively, these results and observations strongly suggest that DNA damage is a common cause of cardiac dysfunction in various etiologies.

Among the different underlying etiologies, doxorubicin-induced cardiotoxicity and dilated-phase hypertrophic cardiomyopathy exhibited the most significant level of DNA damage. Doxorubicin-induced cardiotoxicity has been shown to be

TABLE 2 ORs From Logistic Regression Analysis for LVRR Achievement and Inverse Probability Weighted Analysis

	OR (95% CI)	*P* Value
Crude analysis		
Age, per 10-y increase	1.05 (0.87-1.26)	0.617
BMI, per 5-kg/m^2 increase	1.12 (0.83-1.51)	0.454
Family history of HF	0.30 (0.11-0.83)	0.020
Duration since onset of HF, per 1-y increase	0.92 (0.85-0.99)	0.037
NYHA functional class, per 1 increase in class	1.12 (0.83-1.68)	0.360
Systolic BP, per 10-mm Hg increase	1.12 (0.98-1.29)	0.098
LVEDD, per 5-mm increase	0.78 (0.67-0.91)	0.001
Severe MR	0.33 (0.15-0.76)	0.009
BNP, per 10-pg/mL increase	1.00 (1.00-1.01)	0.250
On ACEI/ARB/ARNI when biopsy was done	0.40 (0.22-0.75)	0.004
On BB when biopsy was done	0.38 (0.21-0.70)	0.002
On CRT when biopsy was done	0.09 (0.01-0.75)	0.025
%PAR, per 10% increase	0.06 (0.02-0.15)	<0.001
%γ-H2A.X, per 10% increase	0.39 (0.28-0.54)	<0.001
Inverse probability weighted analysis		
%PAR, per 10% increase	0.07 (0.03-0.17)	<0.001
%γ-H2A.X, per 10% increase	0.40 (0.29-0.55)	<0.001

PAR = poly(ADP-ribose); other abbreviations as in Table 1.

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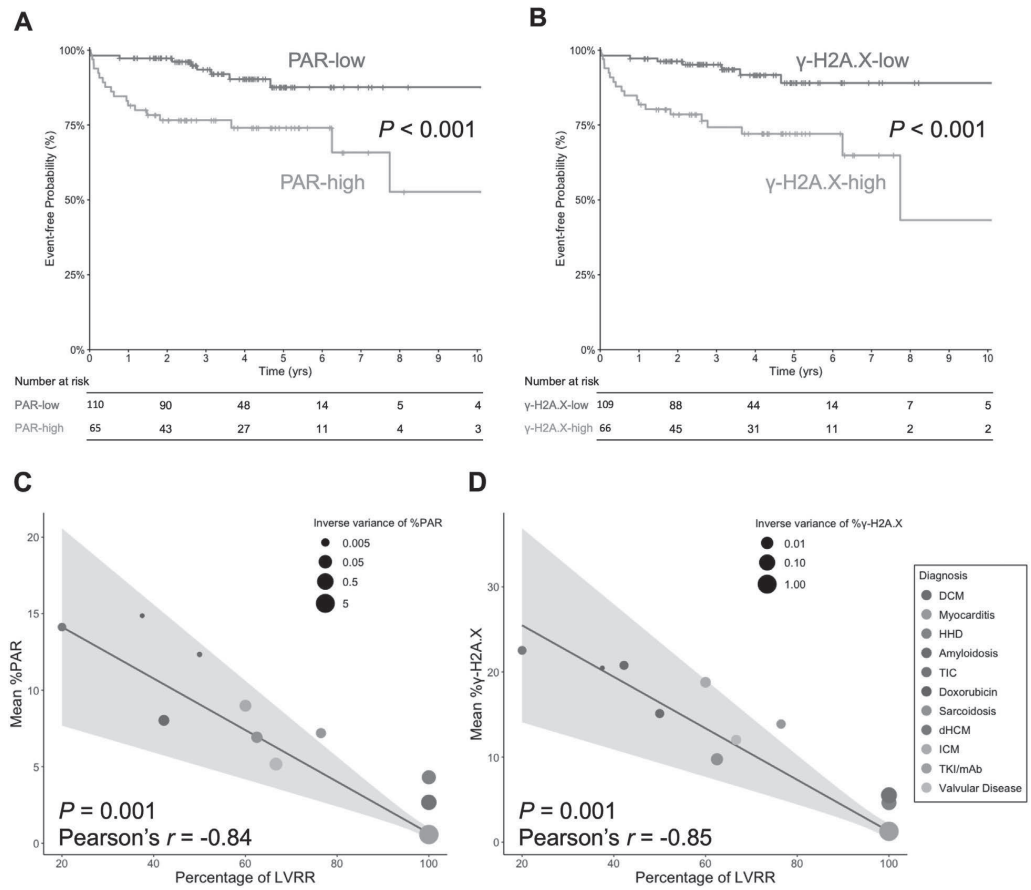
FIGURE 3 Association Between DNA Damage Markers and Outcomes



Kaplan-Meier curves of the composite endpoint of cardiac death, ventricular assist device, and heart transplantation. Patients were dichotomized by the optimal cutoffs of (A) %PAR (6.26%) and (B) %γ-H2A.X (13.50%). Negative correlations between mean (C) %PAR and (D) %γ-H2A.X and the proportion of LVRR across various etiologies were noted. The blue lines indicate the regression lines fitted with the use of a weighted linear regression model, with their 95% CIs indicated by the gray areas. DCM = idiopathic/familial dilated cardiomyopathy; dHCM = dilated-phase hypertrophic cardiomyopathy; HHD = hypertensive heart disease; ICM = ischemic cardiomyopathy; TIC = tachycardia-induced cardiomyopathy; TKI/mAB = tyrosine kinase inhibitor or monoclonal antibody cardiotoxicity; other abbreviations as in Figure 1.

mediated by the binding of doxorubicin to both DNA and topoisomerase IIB, resulting in DNA cleavage and cell death.[57] A subset of patients with hypertrophic cardiomyopathy develop to dilated phase, which has been proposed to be attributed to cardiomyocyte energy depletion and apoptosis leading to progressive myocyte loss and fibrous substitution.[58,59] In both etiologies, DNA damage is thought to be an essential process in the development of reduced cardiac function, which may explain the present observations.

In the present study, we analyzed DNA damage marker signals in both cardiomyocytes and noncardiomyocytes in myocardial tissue without distinguishing them. Our group and others have reported that DNA damage in noncardiomyocytes also plays an important role in heart failure progression in

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murine heart failure models.[60,61] In a previous human dilated cardiomyopathy pilot study, we demonstrated that more than 90% of DNA damage marker signals in biopsy specimens were located within cardiomyocytes, whereas the rest originated from noncardiomyocytes.[8] Sensing DNA damage marker signals in all cell types in a tissue specimen offers the advantage of simplifying the analysis process and facilitating the clinical application of this approach.

Although %γ-H2A.X and %PAR were significantly correlated, %γ-H2A.X tended to be higher than %PAR in each patient. This could be attributed to the difference in the antibodies' performance or the different mechanisms of γ-H2A.X and PAR accumulation in response to DNA damage, and it may explain why the derived cutoff of %γ-H2A.X was higher than that of %PAR. In addition to DNA damage markers, telomere shortening has also been reported to be associated with impaired reverse remodeling. Telomere shortening as assessed by quantitative fluorescence in situ hybridization was observed to coincide with oxidative DNA damage in murine heart failure and was associated with impaired reverse remodeling in human nonischemic heart failure,[62] which may provide another reliable approach to prediction of treatment response.

Precision medicine has been recently applied to cancer and other diseases,[63,64] whereas precision medicine in heart failure is still under development, which has been accelerated by recent advances in omics approaches.[65-67] Prognostic stratification and decision of treatment intensity guided by the evaluation of DNA damage in heart tissue might be a promising approach. Because DNA damage is a common molecular mechanism of not only heart failure, but also other aging-related diseases,[68] treatments to reduce DNA damage or augment DNA repair may be beneficial to prevent the management of many aging-related diseases, including heart failure.

STUDY LIMITATIONS. First, it was a retrospective observational study and, because the number of patients in each etiologic category was limited, it was underpowered for an etiology-stratified analysis. A future prospective study with a larger sample size in each category is warranted. Second, the participants were recruited from 2 tertiary hospitals, one of which is a leading transplantation center in Japan. The same cutoffs should not be adopted directly in clinical practice before assessing external validity, because patient backgrounds differ from institution to institution. Third, identifying the etiology of HFrEF in clinical practice presents challenges, and the possibility of misclassification and overlapping etiologies cannot be ruled out. This study also lacked information on viral genome tests using biopsy specimens from patients diagnosed as myocarditis, because this test is not yet a standard practice in clinical settings in Japan. Finally, the quantification of DNA damage was based on immunofluorescence staining of tissue sections from endomyocardial biopsy specimens, which was dependent on the quality of the antibodies, the storage of tissue specimens,

TABLE 3 Cox Proportional Hazards Analysis for the Composite Endpoint and Inverse Probability Weighted Analysis

	HR (95% CI)	*P* Value
Crude analysis		
Age, per 10-y increase	0.72 (0.57-0.90)	0.003
BMI, per 5-kg/m^2 increase	0.58 (0.37-0.92)	0.021
Family history of HF	1.88 (0.71-4.97)	0.203
Duration since onset of HF, per 1-y increase	1.09 (1.03-1.16)	0.004
NYHA functional class, per 1 increase in class	2.31 (1.45-3.68)	<0.001
Systolic BP, per 10-mm Hg increase	0.72 (0.58-0.90)	0.004
LVEDD, per 5-mm increase	1.22 (1.05-1.41)	0.007
Severe MR	3.26 (1.44-7.39)	0.005
BNP, per 10-pg/mL increase	1.00 (1.00-1.01)	0.077
On ACEI/ARB/ARNI when biopsy was done	0.91 (0.43-1.92)	0.807
On BB when biopsy was done	2.12 (0.97-4.64)	0.061
On CRT when biopsy was done	6.55 (2.42-17.72)	<0.001
%PAR, per 10% increase	1.71 (1.36-2.16)	<0.001
%γ-H2A.X, per 10% increase	1.42 (1.20-1.67)	<0.001
Inverse probability weighted analysis		
%PAR, per 10% increase	1.63 (1.31-2.01)	<0.001
%γ-H2A.X, per 10% increase	1.48 (1.27-1.72)	<0.001

Abbreviations as in Tables 1 and 2.

TABLE 4 LVRR Achievement and Average Proportions of DNA Damage Marker-Positive Nuclei Across Different Underlying Diseases

	Total	LVRR Achieved	Mean %PAR	Mean %γ-H2A.X
Dilated-phase HCM	5	1 (20.0)	14.1	22.6
Doxorubicin cardiotoxicity	8	3 (37.5)	14.9	20.3
Idiopathic/familial DCM	90	38 (42.2)	8.0	20.4
Cardiac amyloidosis	10	5 (50.0)	12.3	15.1
Ischemic cardiomyopathy	5	3 (60.0)	9.0	19.3
Cardiac sarcoidosis	8	5 (62.5)	6.9	9.5
Valvular disease	3	2 (66.7)	5.2	11.9
Myocarditis	17	13 (76.5)	7.2	13.6
TKI/mAb cardiotoxicity	3	3 (100.0)	0.6	0.9
Tachycardia-induced cardiomyopathy	9	9 (100.0)	2.7	5.1
Hypertensive heart disease	13	13 (100.0)	4.3	4.9

Values are n or n (%), unless otherwise indicated.
Abbreviations as in Tables 1 and 2.

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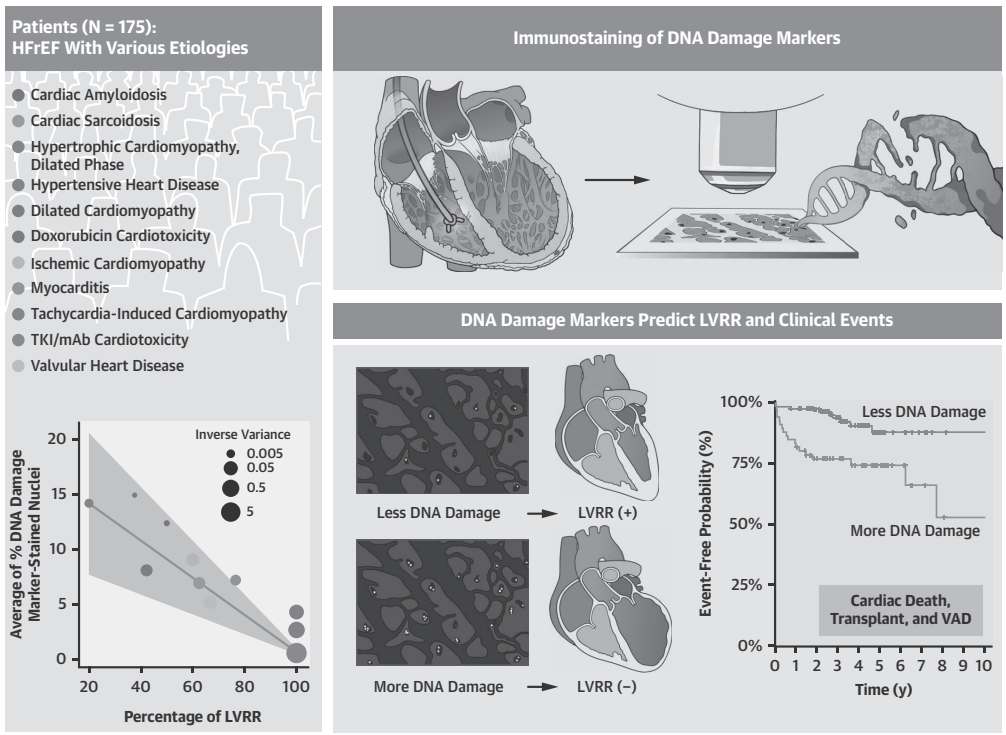
CENTRAL ILLUSTRATION **Immunostaining of DNA Damage Markers Predicts LVRR and Clinical Events**



Dai Z, et al. J Am Coll Cardiol HF. 2024;12(4):648-661.

This study included 175 patients with heart failure with reduced ejection fraction (HFrEF) with various etiologies (left top). We analyzed the immunostaining images of 2 DNA damage markers, poly(ADP-ribose) and γ-H2A.X, in endomyocardial biopsy specimens (right top). Signals of both markers were associated with both 1-year left ventricular reverse remodeling (LVRR) and a composite of cardiac death, heart transplantation, and ventricular assist device (VAD) implantation (right bottom). There was a negative correlation between the average proportion of DNA damage marker–stained nuclei and the probability of LVRR, across different underlying diseases (left bottom). mAb = monoclonal antibody; TKI = tyrosine kinase inhibitor.

and the skills of the laboratory staff. This should be considered when this technique is applied in clinical settings. Our approach to setting a threshold for DNA damage signal-positive nuclei in each image may be instructive because of its low arbitrariness and potential for reproducibility.

CONCLUSIONS

This study suggests that DNA damage determines the consequences of human heart failure and that quantification of DNA damage is useful for predicting treatment response and long-term outcomes of patients with HFrEF regardless of the underlying heart diseases.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: DNA damage in myocardial tissue has been reported to cause the development of heart failure. In this study, the extent of DNA damage in myocardial tissue was demonstrated to determine treatment efficacy of medical therapies and the prognosis of patients with HFrEF regardless of different underlying diseases, suggesting that the extent of DNA damage in myocardial tissue universally determines whether cardiac function can be restored or not.

TRANSLATIONAL OUTLOOK: Assessment of DNA damage by immunostaining of PAR and γ-H2A.X in endomyocardial biopsy specimens from patients with HFrEF is useful to predict the treatment efficacy of medical therapies and prognosis in various underlying etiologies. A large-scale prospective study is needed to further evaluate the impact of DNA damage in myocardial tissues on the progress of heart failure.

REFERENCES

1. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;396:1204-1222.

2. Mohebi R, Chen C, Ibrahim NE, et al. Cardiovascular disease projections in the United States Based on the 2020 Census estimates. *J Am Coll Cardiol*. 2022;80:565-578.

3. Conrad N, Judge A, Tran J, et al. Temporal trends and patterns in heart failure incidence: a population-based study of 4 million individuals. *Lancet*. 2018;391:572-580.

4. Heidenreich PA, Bozkurt B, Aguilar D, et al. 2022 AHA/ACC/HFSA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2022;79(17):e263-e421.

5. Taylor CJ, Ordonez-Mena JM, Roalfe AK, et al. Trends in survival after a diagnosis of heart failure in the United Kingdom 2000-2017: population based cohort study. *BMJ*. 2019;364:l223.

6. Felker GM, Thompson RE, Hare JM, et al. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. *N Engl J Med*. 2000;342:1077-1084.

7. Bragazzi NL, Zhong W, Shu J, et al. Burden of heart failure and underlying causes in 195 countries and territories from 1990 to 2017. *Eur J Prev Cardiol*. 2021;28:1682-1690.

8. Ko T, Fujita K, Nomura S, et al. Quantification of DNA damage in heart tissue as a novel prediction tool for therapeutic prognosis of patients with dilated cardiomyopathy. *J Am Coll Cardiol Basic Trans Science*. 2019;4:670-680.

9. Verdonschot JAJ, Hazebroek MR, Wang P, et al. Clinical phenotype and genotype associations with improvement in left ventricular function in dilated cardiomyopathy. *Circ Heart Fail*. 2018;11:e005220.

10. Tobita T, Nomura S, Fujita T, et al. Genetic basis of cardiomyopathy and the genotypes involved in prognosis and left ventricular reverse remodeling. *Sci Rep*. 2018;8:1998.

11. Subramanian D, Subramanian V, Deswal A, Mann DL. New predictive models of heart failure mortality using time-series measurements and ensemble models. *Circ Heart Fail*. 2011;4:456-462.

12. Khush KK, Zaroff JG, Nguyen J, Menza R, Goldstein BA. National decline in donor heart utilization with regional variability: 1995-2010. *Am J Transplant*. 2015;15:642-649.

13. Nunoda S, Sasaoka T, Sakata Y, et al. Survival of heart transplant candidates in Japan. *Circ J*. 2019;83:681-683.

14. Goldstein BA, Thomas L, Zaroff JG, Nguyen J, Menza R, Khush KK. Assessment of heart transplant waitlist time and pre- and post-transplant failure: a mixed methods approach. *Epidemiology*. 2016;27:469-476.

15. Bulluck H, Carberry J, Carrick D, et al. Redefining adverse and reverse left ventricular remodeling by cardiovascular magnetic resonance following ST-segment-elevation myocardial infarction and their implications on long-term prognosis. *Circ Cardiovasc Imaging*. 2020;13:e009937.

16. Yu CM, Bleeker GB, Fung JW, et al. Left ventricular reverse remodeling but not clinical improvement predicts long-term survival after cardiac resynchronization therapy. *Circulation*. 2005;112:1580-1586.

17. Kim GH, Uriel N, Burkhoff D. Reverse remodelling and myocardial recovery in heart failure. *Nat Rev Cardiol*. 2018;15:83-96.

18. Dal Ferro M, Stolfo D, Altinier A, et al. Association between mutation status and left ventricular reverse remodelling in dilated cardiomyopathy. *Heart*. 2017;103:1704-1710.

19. Kubanek M, Sramko M, Maluskova J, et al. Novel predictors of left ventricular reverse remodeling in individuals with recent-onset dilated cardiomyopathy. *J Am Coll Cardiol*. 2013;61:54-63.

20. Rizzello V, Poldermans D, Boersma E, et al. Opposite patterns of left ventricular remodeling after coronary revascularization in patients with ischemic cardiomyopathy: role of myocardial viability. *Circulation*. 2004;110:2383-2388.

21. Hnat T, Veselka J, Honek J. Left ventricular reverse remodelling and its predictors in non-ischaemic cardiomyopathy. *ESC Heart Fail*. 2022;9:2070-2083.

22. Nakada Y, Nhi Nguyen NU, Xiao F, et al. DNA damage response mediates pressure overload–induced cardiomyocyte hypertrophy. *Circulation*. 2019;139:1237-1239.

23. Higo T, Naito AT, Sumida T, et al. DNA single-strand break-induced DNA damage response causes heart failure. *Nat Commun*. 2017;8:15104.

24. Sato M, Kadomatsu T, Miyata K, et al. The lncRNA Caren antagonizes heart failure by inactivating DNA damage response and activating mitochondrial biogenesis. *Nat Commun*. 2021;12:2529.

25. Nomura S, Satoh M, Fujita T, et al. Cardiomyocyte gene programs encoding morphological and functional signatures in cardiac hypertrophy and failure. *Nat Commun*. 2018;9:4435.

26. Choudhury S, Huang AY, Kim J, et al. Somatic mutations in single human cardiomyocytes reveal age-associated DNA damage and widespread oxidative genotoxicity. *Nat Aging*. 2022;2:714–725.

27. Canseco DC, Kimura W, Garg S, et al. Human ventricular unloading induces cardiomyocyte proliferation. *J Am Coll Cardiol*. 2015;65:892–900.

28. Jimenez J, Rentschler SL. DNA damage prediction tool in dilated cardiomyopathy: don't go breaking my heart. *J Am Coll Cardiol Basic Trans Science*. 2019;4:681–683.

29. Alemasova EE, Lavrik OI. Poly(ADP-ribosyl)ation by PARP1: reaction mechanism and regulatory proteins. *Nucleic Acids Res*. 2019;47:3811–3827.

30. Kinner A, Wu W, Staudt C, Iliakis G. Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. *Nucleic Acids Res*. 2008;36:5678–5694.

31. Hunt SA, Abraham WT, Chin MH, et al. ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. *J Am Coll Cardiol*. 2005;46(6):e1–82.

32. McDonagh TA, Metra M, Adamo M, et al. 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2021;42:3599–3726.

33. Drazner MH. The progression of hypertensive heart disease. *Circulation*. 2011;123:327–334.

34. JCS Joint Working Group. Guidelines for diagnosis and treatment of myocarditis (JCS 2009): digest version. *Circ J*. 2011;75:734–743.

35. Bozkurt B, Colvin M, Cook J, et al. Current diagnostic and treatment strategies for specific dilated cardiomyopathies: a scientific statement from the American Heart Association. *Circulation*. 2016;134:e579–646.

36. Japp AG, Gulati A, Cook SA, Cowie MR, Prasad SK. The diagnosis and evaluation of dilated cardiomyopathy. *J Am Coll Cardiol*. 2016;67:2996–3010.

37. Gronich N, Lavi I, Barnett-Griness O, Saliba W, Abernethy DR, Rennert G. Tyrosine kinase-targeting drugs-associated heart failure. *Br J Cancer*. 2017;116:1366–1373.

38. Huizar JF, Ellenbogen KA, Tan AY, Kaszala K. Arrhythmia-induced cardiomyopathy: JACC state-of-the-art review. *J Am Coll Cardiol*. 2019;73:2328–2344.

39. Ribeiro Neto ML, Jellis CL, Joyce E, Callahan TD, Hachamovitch R, Culver DA. Update in cardiac sarcoidosis. *Ann Am Thorac Soc*. 2019;16:1341–1350.

40. Alexandre J, Cautela J, Ederhy S, et al. Cardiovascular toxicity related to cancer treatment: a pragmatic approach to the American and European cardio-oncology guidelines. *J Am Heart Assoc*. 2020;9:e018403.

41. Ommen SR, Mital S, Burke MA, et al. 2020 AHA/ACC guideline for the diagnosis and treatment of patients with hypertrophic cardiomyopathy: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2020;76(25):e159–e240.

42. Haas GJ, Zareba KM, Ni H, et al. Validating an idiopathic dilated cardiomyopathy diagnosis using cardiovascular magnetic resonance: the Dilated Cardiomyopathy Precision Medicine Study. *Circ Heart Fail*. 2022;15:e008877.

43. Caforio AL, Pankuweit S, Arbustini E, et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J*. 2013;34:2636–2648.a-d.

44. Cohen-Solal A, Jacobson AF, Pina IL. Beta blocker dose and markers of sympathetic activation in heart failure patients: interrelationships and prognostic significance. *ESC Heart Fail*. 2017;4:499–506.

45. Escobar-Lopez L, Ochoa JP, Mirelis JG, et al. Association of genetic variants with outcomes in patients with nonischemic dilated cardiomyopathy. *J Am Coll Cardiol*. 2021;78:1682–1699.

46. Youden WJ. Index for rating diagnostic tests. *Cancer*. 1950;3:32–35.

47. Naimi AI, Moodie EE, Auger N, Kaufman JS. Constructing inverse probability weights for continuous exposures: a comparison of methods. *Epidemiology*. 2014;25:292–299.

48. Shieh G. A comparison of two indices for the intraclass correlation coefficient. *Behav Res Methods*. 2012;44:1212–1223.

49. Austin PC. Assessing covariate balance when using the generalized propensity score with quantitative or continuous exposures. *Stat Methods Med Res*. 2019;28:1365–1377.

50. Struber M, Meyer AL, Malehsa D, Kugler C, Simon AR, Haverich A. The current status of heart transplantation and the development of "artificial heart systems.". *Dtsch Arztebl Int*. 2009;106:471–477.

51. McNamara DM, Starling RC, Cooper LT, et al. Clinical and demographic predictors of outcomes in recent onset dilated cardiomyopathy: results of the IMAC (Intervention in Myocarditis and Acute Cardiomyopathy)-2 study. *J Am Coll Cardiol*. 2011;58:1112–1118.

52. Chen SN, Lombardi R, Karmouch J, et al. DNA damage response/TP53 pathway is activated and contributes to the pathogenesis of dilated cardiomyopathy associated with LMNA (lamin A/C) mutations. *Circ Res*. 2019;124:856–873.

53. Sano M, Minamino T, Toko H, et al. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature*. 2007;446:444–448.

54. Kehat I, Molkentin JD. Molecular pathways underlying cardiac remodeling during pathophysiological stimulation. *Circulation*. 2010;122:2727–2735.

55. Cao Y, Pan C, Wang YC, et al. Identification of DNA damage repair enzyme Ascc2 as causal for heart failure with preserved ejection fraction. *Circulation*. 2022;145:1102–1104.

56. Kim MN, Moon JH, Cho YM. Sodium-glucose cotransporter-2 inhibition reduces cellular senescence in the diabetic kidney by promoting ketone body-induced NRF2 activation. *Diabetes Obes Metab*. 2021;23:2561–2571.

57. Zhang S, Liu X, Bawa-Khalfe T, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med*. 2012;18:1639–1642.

58. Ino T, Nishimoto K, Okubo M, et al. Apoptosis as a possible cause of wall thinning in end-stage hypertrophic cardiomyopathy. *Am J Cardiol*. 1997;79:1137–1141.

59. Olivotto I, Cecchi F, Poggesi C, Yacoub MH. Patterns of disease progression in hypertrophic cardiomyopathy: an individualized approach to clinical staging. *Circ Heart Fail*. 2012;5:535–546.

60. Wu L, Sowers JR, Zhang Y, Ren J. Targeting DNA damage response in cardiovascular diseases: from pathophysiology to therapeutic implications. *Cardiovasc Res*. 2023;119:691–709.

61. Ko T, Nomura S, Yamada S, et al. Cardiac fibroblasts regulate the development of heart failure via Htra3-TGF-β-IGFBP7 axis. *Nat Commun*. 2022;13:3275.

62. Brandt M, Dorschmann H, Khraisat S, et al. Telomere shortening in hypertensive heart disease depends on oxidative DNA damage and predicts impaired recovery of cardiac function in heart failure. *Hypertension*. 2022;79:2173–2184.

63. Mateo J, Steuten L, Aftimos P, et al. Delivering precision oncology to patients with cancer. *Nat Med*. 2022;28:658–665.

64. Guthridge JM, Wagner CA, James JA. The promise of precision medicine in rheumatology. *Nat Med*. 2022;28:1363–1371.

JACC: HEART FAILURE VOL. 12, NO. 4, 2024
APRIL 2024:648–661

65. Koenig AL, Shchukina I, Amrute J, et al. Single-cell transcriptomics reveals cell-type-specific diversification in human heart failure. *Nat Cardiovasc Res*. 2022;1:263-280.

66. Papait R, Cattaneo P, Kunderfranco P, et al. Genome-wide analysis of histone marks identifying an epigenetic signature of promoters and enhancers underlying cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 2013;110:20164-20169.

67. Adamo L, Yu J, Rocha-Resende C, Javaheri A, Head RD, Mann DL. Proteomic signatures of heart failure in relation to left ventricular ejection fraction. *J Am Coll Cardiol*. 2020;76:1982-1994.

68. Schumacher B, Pothof J, Vijg J, Hoeijmakers JHJ. The central role of DNA damage in the ageing process. *Nature*. 2021;592:695-703.

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