Circulation: Heart Failure

RESEARCH LETTER

Myocardial DNA Damage Is Responsible for the Relationship Between Genotype and Reverse Remodeling in Patients With Dilated Cardiomyopathy

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DNA damage response plays a critical role in heart failure, including dilated cardiomyopathy (DCM), and assessment of myocardial DNA damage can predict treatment response and prognosis in patients with DCM.[1] Meanwhile, genotype is also an established predictor of DCM treatment response and prognosis, with nonsarcomere gene variants (*LMNA*, etc.) predicting poorer prognosis compared with sarcomere gene variants (*TTN* truncating variants, etc.).[2] However, the relationship between genotype and myocardial DNA damage remains unclear.

In this study, we assessed both myocardial DNA damage by immunostaining of γ-H2A.X in right ventricular endomyocardial biopsy specimens (Figure [A]) and deleterious variants using whole exome sequencing in patients diagnosed with DCM in 2 previous cohorts.[1,3] Diagnosis of DCM was established based on impaired left ventricular ejection fraction (<40%), following rigorous exclusion of secondary causative etiologies. The study was approved by the institutional research boards of the University of Tokyo (Nos. 11801 and G2249) and Nara Medical University (No. G107). Informed consents were obtained. Evaluation of myocardial DNA damage from biopsy specimens has been previously reported.[1,3] Whole exome sequencing data were mapped to the human reference genome. Rare variants with a minor allele frequency <1% in East Asian databases were extracted and filtered to obtain those predicted to alter protein structure or function, such as nonsynonymous, nonsense and splice site variants, in-frame and frameshift deletions, and insertions, in prespecified cardiomyopathy-related genes. Pathogenic and likely pathogenic (P/LP) variants, classified according to the American College of Medical Genetics and Genomics guidelines, were defined as deleterious mutations. Left ventricular reverse remodeling (LVRR) at 1 year after biopsy was defined by an absolute increase in left ventricular ejection fraction of ≥10% to a final value >35%, accompanied by a decrease in left ventricular end-diastolic diameter >10%, as assessed by echocardiography.[1] Data are available from the corresponding authors upon reasonable request.

Key Words: dilated cardiomyopathy ■ DNA damage ■ genotype ■ heart failure ■ prognosis

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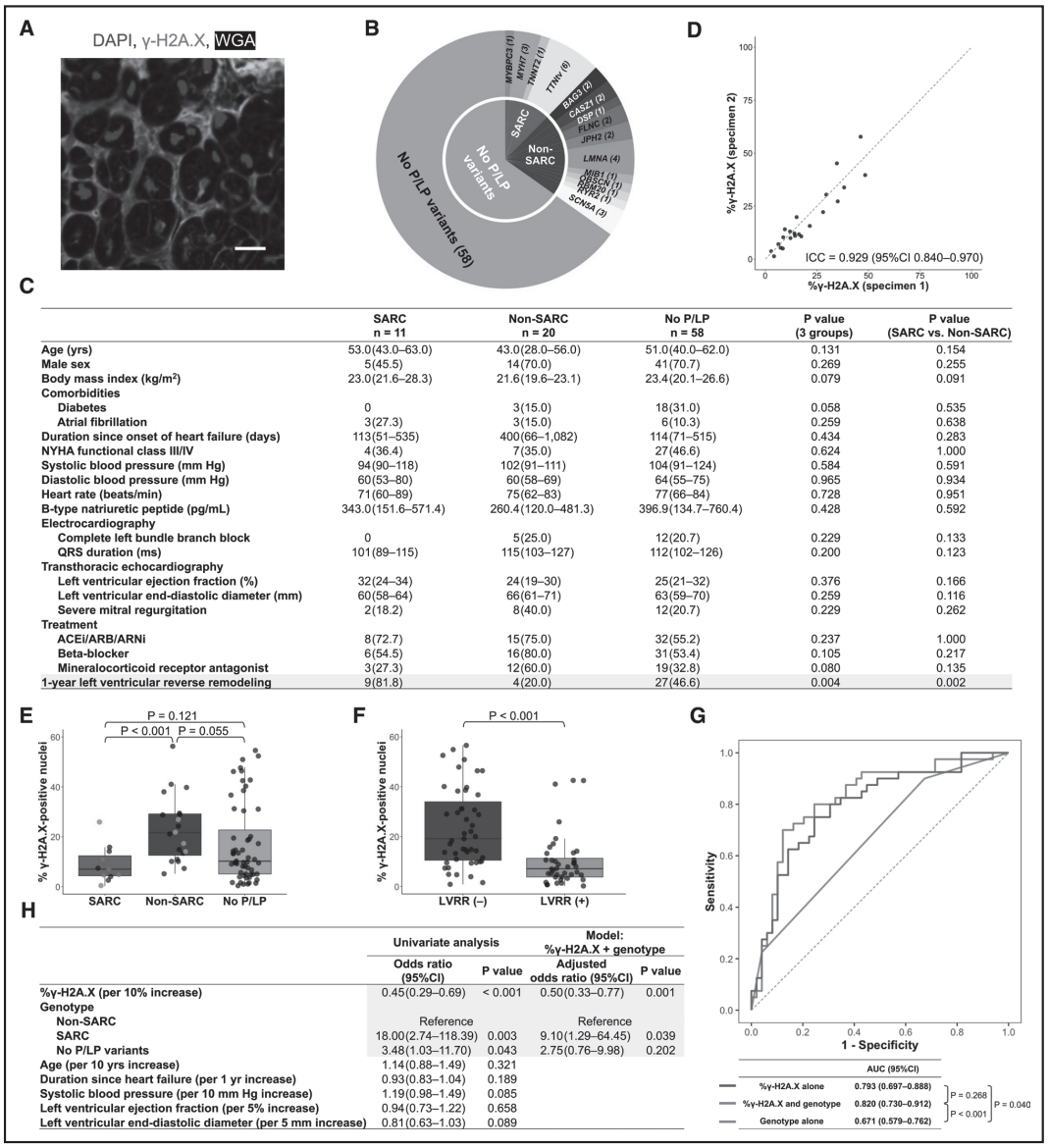

	SARC n = 11	Non-SARC n = 20	No P/LP n = 58	P value (3 groups)	P value (SARC vs. Non-SARC)
Age (yrs)	53.0(43.0–63.0)	43.0(28.0–56.0)	51.0(40.0–62.0)	0.131	0.154
Male sex	5(45.5)	14(70.0)	41(70.7)	0.269	0.255
Body mass index (kg/m²)	23.0(21.6–28.3)	21.6(19.6–23.1)	23.4(20.1–26.6)	0.079	0.091
Comorbidities					
Diabetes	0	3(15.0)	18(31.0)	0.058	0.535
Atrial fibrillation	3(27.3)	3(15.0)	6(10.3)	0.259	0.638
Duration since onset of heart failure (days)	113(51–535)	400(66–1,082)	114(71–515)	0.434	0.283
NYHA functional class III/IV	4(36.4)	7(35.0)	27(46.6)	0.624	1.000
Systolic blood pressure (mm Hg)	94(90–118)	102(91–111)	104(91–124)	0.584	0.591
Diastolic blood pressure (mm Hg)	60(53–80)	60(58–69)	64(55–75)	0.965	0.934
Heart rate (beats/min)	71(60–89)	75(62–83)	77(66–84)	0.728	0.951
B-type natriuretic peptide (pg/mL)	343.0(151.6–571.4)	260.4(120.0–481.3)	396.9(134.7–760.4)	0.428	0.592
Electrocardiography					
Complete left bundle branch block	0	5(25.0)	12(20.7)	0.229	0.133
QRS duration (ms)	101(89–115)	115(103–127)	112(102–126)	0.200	0.123
Transthoracic echocardiography					
Left ventricular ejection fraction (%)	32(24–34)	24(19–30)	25(21–32)	0.376	0.166
Left ventricular end-diastolic diameter (mm)	60(58–64)	66(61–71)	63(59–70)	0.259	0.116
Severe mitral regurgitation	2(18.2)	8(40.0)	12(20.7)	0.229	0.262
Treatment					
ACEi/ARB/ARNi	8(72.7)	15(75.0)	32(55.2)	0.237	1.000
Beta-blocker	6(54.5)	16(80.0)	31(53.4)	0.105	0.217
Mineralocorticoid receptor antagonist	3(27.3)	12(60.0)	19(32.8)	0.080	0.135
1-year left ventricular reverse remodeling	9(81.8)	4(20.0)	27(46.6)	0.004	0.002

	Univariate analysis		Model: %γ-H2A.X + genotype	
	Odds ratio (95%CI)	P value	Adjusted odds ratio (95%CI)	P value
%γ-H2A.X (per 10% increase)	0.45(0.29–0.69)	< 0.001	0.50(0.33–0.77)	0.001
Genotype				
Non-SARC	Reference		Reference	
SARC	18.00(2.74–118.39)	0.003	9.10(1.29–64.45)	0.039
No P/LP variants	3.48(1.03–11.70)	0.043	2.75(0.76–9.98)	0.202
Age (per 10 yrs increase)	1.14(0.88–1.49)	0.321		
Duration since heart failure (per 1 yr increase)	0.93(0.83–1.04)	0.189		
Systolic blood pressure (per 10 mm Hg increase)	1.19(0.98–1.49)	0.085		
Left ventricular ejection fraction (per 5% increase)	0.94(0.73–1.22)	0.658		
Left ventricular end-diastolic diameter (per 5 mm increase)	0.81(0.63–1.03)	0.089		

Figure. Association between genotype and myocardial DNA damage and their effects on the prediction of reverse remodeling. **A**, A representative immunofluorescence staining image. γ-H2A.X (red) staining image is presented with DAPI (blue) indicating nuclei and WGA (white) indicating cell membrane. Scale bar: 20 μm. **B**, Distribution of patients with dilated cardiomyopathy who have pathogenic or likely pathogenic (P/LP) variants in cardiomyopathy-related genes. Each gene is presented along with the number of carriers of P/LP variants in the corresponding gene. **C**, Baseline characteristics, treatments, and 1-year left ventricular reverse remodeling (LVRR) in each category of: carriers of P/LP variants in sarcomere genes, carriers of P/LP variants in nonsarcomere genes, and patients without P/LP variants in cardiomyopathy-related genes. Data are presented as number (percentage) for categorical variables and compared using Fisher's exact test; or as median (interquartile range) for continuous variables and compared using Kruskal-Wallis test among 3 groups and Mann-Whitney *U* test between 2 groups. **D**, Dot plot for %γ-H2A.Xs assessed in 2 separate specimens from the same patients to evaluate reproducibility (n=22). The dashed line indicates the line for Y=X. **E**, Comparisons of the proportion of γ-H2A.X-positive nuclei (%γ-H2A.X) among different genotype categories. The carriers of P/LP variants in *TTN* and *LMNA* are shown as blue and orange dots, respectively. **F**, Comparison of %γ-H2A.X between patients who achieved LVRR [LVRR(+)] and did not achieve LVRR [LVRR(–)] at 1 year. **G**, Receiver operating characteristic curves of %γ-H2A.X alone (blue), genotype alone (green), and the combination of %γ-H2A.X and genotype (red) for predicting 1-year LVRR. (*Continued*)

Figure Continued. **H**, Odds ratios for achieving LVRR at 1 year developed from logistic regression models, and adjusted odds ratios developed from a multivariate logistic regression model that included both %γ-H2A.X and genotype as covariables. ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNi angiotensin receptor-neprilysin inhibitor; AUC, area under the curve; DAPI, 4,6-diamidino-2-phenylindole; ICC, intraclass correlation coefficient; Non-SARC, pathogenic or likely pathogenic variants in nonsarcomere genes; NYHA, New York Heart Association; SARC, pathogenic or likely pathogenic variants in sarcomere genes; *TTN*tv, *TTN* truncating variants; and WGA, wheat germ agglutinin.

Nonstandard Abbreviations and Acronyms

DCM	dilated cardiomyopathy
LVRR	left ventricular reverse remodeling
P/LP	pathogenic or likely pathogenic

We screened 148 patients from the 2 previous cohorts, among whom 89 eligible patients consented to whole exome sequencing and were included in this analysis, and 31 of them had P/LP variants in cardiomyopathy-related genes. We further categorized the P/LP variants into sarcomere and nonsarcomere gene variants as previously reported (Figure [B]).[2] Among patients with P/LP variants in sarcomere genes such as *TTN*, 82% achieved LVRR at 1 year, which was significantly higher compared with 20% of patients with P/LP variants in nonsarcomere genes such as *LMNA* (P=0.002), although baseline characteristics were comparable (Figure [C]). The assessment of the percentage of nuclei stained with γ-H2A.X (%γ-H2A.X) was reproducible in different specimens from the same patients (Figure [D]). %γ-H2A.X was significantly higher in nonsarcomere gene P/LP variant carriers than in sarcomere gene P/LP variant carriers (21.6 [12.6–29.2]% versus 7.0 [4.3–12.4]%; P<0.001; Figure [E]). Patients who achieved LVRR at 1 year presented with lower %γ-H2A.X regardless of genotype (Figure [F]). %γ-H2A.X alone was able to predict 1-year LVRR with the area under the receiver operating characteristic curve being 0.793 (95% CI, 0.697–0.888). The addition of genotype information (P/LP variants in sarcomere genes; P/LP variants in nonsarcomere genes; no P/LP variants in cardiomyopathy-related genes) resulted in similar prediction performance (area under the receiver operating characteristic curve, 0.820 [95% CI, 0.730–0.912]; DeLong's P=0.268; Figure [G]). Performance of either model may be superior to that of previously reported LVRR predictors such as late gadolinium enhancement. The odds ratio for carriers of P/LP variants in sarcomere genes to achieve LVRR was 18.00 (95% CI, 2.74–118.39) compared with that for carriers of P/LP variants in nonsarcomere genes. However, this effect was largely attenuated after adjusting for %γ-H2A.X (odds ratio, 9.10 [95% CI, 1.29–64.45]; Figure [H]). These results suggest that myocardial DNA damage might mediate the link between genotype and LVRR in DCM. Different levels of myocardial DNA damage among different genotypes may be attributed to (1) variant-specific contribution to DNA damage (such as *LMNA* variants via nuclear envelope abnormality)[4] and/or (2) different extents of longstanding mechanical stress secondary to the variants, which warrants further clarification.[5]

This study has several limitations. First, this is a retrospective cohort study with a limited number of patients. The effect of genotype was assessed according to the categories of variant genes rather than based on individual genes. The limited number also precluded the analysis of the association between LVRR and %γ-H2A.X stratified by genotype. Second, there were few clinical events, including death and implantation of ventricular assist device. Third, %γ-H2A.X was assessed in right ventricular biopsy specimens, which might not be equivalent to those from left ventricles. Finally, the invasive nature of endomyocardial biopsy must be carefully considered when applying this approach in clinical practice.

In conclusion, among patients with DCM, carriers of P/LP variants in sarcomere genes showed less DNA damage and higher probability of achieving LVRR, when compared with carriers of P/LP variants in nonsarcomere genes. Quantification of myocardial DNA damage may be useful to predict reverse remodeling, regardless of the availability of genotype information.

ARTICLE INFORMATION

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Disclosures

None.

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